INTESTINAL DRUG ABSORPTION, CYTOCHROME P450 3A-MEDIATED

METABOLISM, AND TRANSPORT AFTER SMALL BOWEL TRANSPLANTATION

by

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Soli Deo honor et gloria

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ABSTRACT

Small bowel allograft recipients require multiple medications after transplant, many of which are orally administered cytochrome P450 3A (CYP3A) and/or p-glycoprotein (p-gp) substrates. A previous study in dogs has shown that surgical manipulation of the intestine and ischemia-reperfusion injury lead to suppression of CYP3A and p-gp function in the early post-transplant period, presumably due to release of pro-inflammatory cytokines, a suppression that diminished over time. The work presented in this dissertation compares intestinal CYP3A (using midazolam) and p-gp (using fexofenadine) expression and function in small bowel transplant recipients in the early post-transplant period (the first 40 days after surgery, n=16) and later (four to 12 months) post transplant (n=10) as well as with age- and gender-matched (n=16) healthy control subjects.

Oral AUC and oral bioavailability of midazolam were significantly higher in transplant subjects early post-transplant, but not different from controls at four to 12 months post-transplant. The oral AUC ratio of 1'hydroxymidazolam to midazolam, a measure of the extent of CYP3A-mediated metabolism, was significantly lower in the early post-transplant period compared with controls, but at the later period no difference was observed. No difference in

fexofenadine AUC was observed between subject groups, and although Tmax of fexofenadine was significantly higher in transplant subjects at both time periods compared with healthy controls, AUC and Cmax were more influenced by route of administration (jejunostomy tube vs. oral) and transplant subtype (modified multivisceral vs. isolated intestine) than by ileal *ABCB1* expression. Dose-normalized AUC_{0-7} and Cmax of oral tacrolimus (a CYP3A/p-gp substrate) were significantly higher early post transplant compared with later.

Overall this work presents strong evidence for an early immune-mediated suppression of intestinal CYP3A that eventually returns to normal in stable intestinal transplant patients, indicating that bioavailability of highly soluble, highly permeable CYP3A substrates such as midazolam will be significantly higher early post-transplant, requiring caution in their dosing during this time and by extrapolation, during other times of high immune activation, such as acute rejection. These findings have clinical relevance for appropriate medication use in small bowel transplant recipients.

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1.0   INTRODUCTION

1.1   FUNCTIONAL ANATOMY AND PHYSIOLOGY OF THE SMALL INTESTINE

1.1.1   Structure

The small intestine is the major site of drug absorption after oral administration. Therefore, an understanding of factors that affect its function is critical to comprehending changes in drug absorption after small bowel transplantation. The small intestine is an organ approximately six meters in length that extends from the pyloric sphincter of the stomach to the ileocecal valve that marks the beginning of the colon. It is composed of three parts: the duodenum, approximately 12 cm in length; the jejunum, approximately 2.5 m long, which is the major site of most drug and nutrient absorption due to the maximization of absorptive surface area resulting from the large number of folds and small, fingerlike structures called villi; and the ileum, approximately two to four meters long, narrower and containing fewer folds and villi(Rubin, 2009).

The small intestine is composed of four layers: the serosa, the muscularis propria, the submucosa, and the mucosa (See Figure 1). The serosa is the thin outermost layer, containing connective tissue and covered with a layer of mesothelial cells. The next layer, the muscularis propria, contains two separate layers of smooth muscle cells—the outermost layer composed of cells arranged longitudinally and the thicker inner layer containing circular muscle cells.

Between the two sublayers lies the myenteric or Auerbach's plexus, a nerve plexus composed of ganglia and connecting nerve fibers(Furness et al., 2009). The muscularis propria is the layer mainly responsible for intestinal contractility(Kahn and Daum, 2010). The next layer, the submucosa, is a dense layer that is highly vascularized and contains the Meissner plexus, a plexus of autonomic nerve fibers that communicates with the myenteric plexus to coordinate peristalsis(Rubin, 2009).

The innermost layer of small intestine is the mucosa, which consists of a base layer of smooth muscle cells called the muscularis mucosae, a connective tissue layer called the lamina propria that contains many blood vessels, lymphoid tissue, lymphatic channels, additional nerve and muscle fibers, and fibroblasts, and lastly the single-layered epithelium, which is directly exposed to the lumen. The lamina propria is the main site of the mucosa-associated lymphoid tissue (MALT), and is the location of T and B lymphocytes involved in intestinal immune responses. Lining the lamina propria is a single layer of epithelial cells, mainly the absorptive, columnar cells called enterocytes but also the mucus-producing goblet cells and the microfold (M) cells that are located over lymphoid aggregates(Standring et al., 2008).

The mucosa of the intestine is designed to maximize the surface area exposed to the lumen. Ridges in the submucosa force the mucosa into circular folds, and the entire mucosal surface is covered with small projections called villi, each of which contains a lymphatic vessel called a lacteal as well as blood vessels, nerve cells, and smooth muscle cells. Between and at the bases of the villi are indentations called crypts. The crypts are the site of development of the enterocytes through stem cell mitosis. The enterocytes mature as they migrate up the side of the villus and reach full maturity at the tip, where they undergo apoptosis and are shed into the

lumen. The total lifespan of an enterocyte is approximately five days(Standring et al., 2008).

The enterocyte is the key cell for intestinal drug absorption and metabolism.

Figure 1. The architecture of an intestinal villus

Direction of cell migration

Absorptive enterocyte

Goblet cell

Central lymphatic vessel (lacteal)

Orifices of crypts of Lieberkühn

Fibroblasts

Lymphoid follicle

Epithelium

Lamina propria

Mucosa

Muscularis mucosae

Submucosa

Circular muscle

Muscularis externa

Longitudinal muscle

Serosa

Fig. 66.16 The architecture of an intestinal villus. The various layers and cells are not drawn to scale.

Reprinted from Healy et al. (Healy et al., 2005) with permission of Elsevier, Inc.

1.1.2 Gastrointestinal motility

The distal portion of the stomach is responsible for the grinding of foods and regulation of the emptying of solids. During times of fasting, a pattern of motor activity called the migrating motor complex (MMC) works to clear the stomach and upper intestine of any food residue and/or sloughed cells and mucus. In a healthy person, the entire MMC cycle lasts approximately 84-112 minutes and is comprised of three phases. The first phase is a quiet period of slow pressure waves in which high-pressure contractions are entirely absent. The second phase is marked by irregular contractions that increase in frequency. The third and final phase, which lasts five to ten minutes, consists of intense contractions that typically start in the stomach and propagate sequentially down the gastrointestinal tract from the stomach to the colon. Five to ten minutes after a meal, intermittent contractions of the stomach, similar to the second phase of the MMC, lead to emptying of the gastric contents(Hasler, 2009b). It is important to note that the rate of gastric emptying depends strongly on the type of "meal" ingested. Solids are emptied most slowly from the stomach, with a lag time of up to one hour. Gastric emptying rates of liquids depend on the properties of the liquid. Inert aqueous solutions are emptied most rapidly, with a half-life of approximately 8 to 18 minutes, in a process conforming to the principles of first order kinetics. Particulate suspensions, high-fat, high-protein, and high-calorie liquids empty significantly more slowly, with an initial rapid phase of emptying followed by a slower phase exhibiting zero-order kinetics(Barrett, 2006; Hasler, 2009b).

Figure 2. Gastric emptying times by content
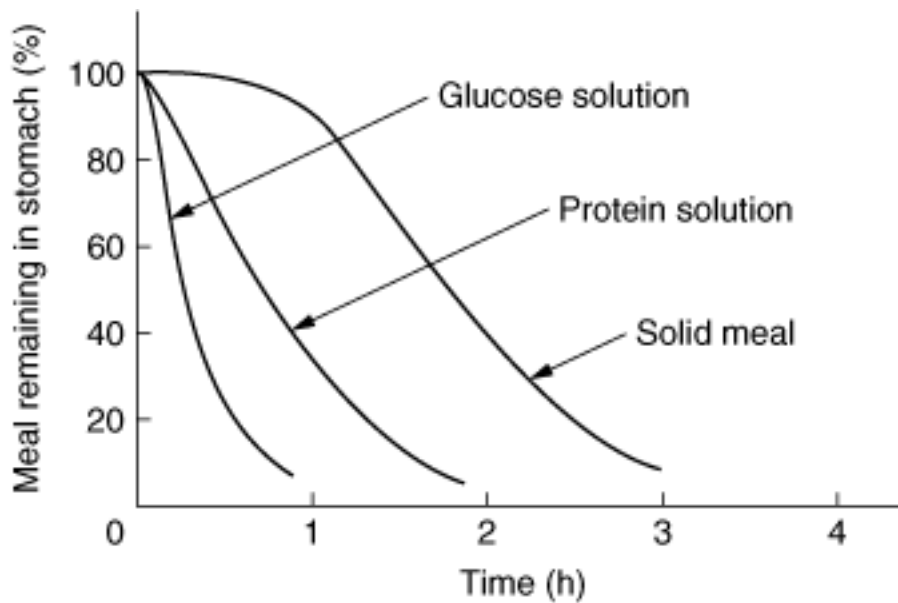
Gastric emptying depends on the nature of the gastric
contents. Note that emptying of a solid meal does not begin
immediately, but only after a lag of approximately one hour.

Reprinted from Barrett (Barrett, 2006) by permission of McGraw-Hill.

After emptying into the small intestine, the stomach contents are mixed with bile and pancreatic enzymes and propelled through the gastrointestinal tract, largely by the circular muscle of the muscularis externa. In a healthy person, the time of transit through the entire length of the small intestine is typically between 78 and 264 minutes(Hasler, 2009a; McConnell et al., 2008). During this time the intestinal contents in the lumen come into contact with the enterocytes and absorption into the systemic circulation may occur.

1.1.3 Gastrointestinal transit of pharmaceutical dosage forms

Gastric emptying rates for pharmaceutical dosage forms follow a pattern similar to those shown above for various meals. Drugs in solution form and those containing small drug pellets (less than 2 mm) have been shown to empty from the stomach rapidly and largely unaffected by whether or not the subject is in the fasted or fed state. Large, single unit dosage forms such as matrix tablets and osmotic pump tablets often exhibit rapid gastric emptying in the fasted state, but in the postprandial state emptying is delayed. A light meal will delay emptying less than a heavy meal(Davis et al., 1986).

Once the formulation has emptied from the stomach into the small intestine, however, there appears to be no difference in small intestinal transit times between solutions, small pellets, and large single unit dosage forms in healthy individuals. In addition, the presence of food in the stomach does not affect small intestinal transit of drugs(Davis et al., 1986; McConnell et al., 2008). There is nevertheless considerable inter-individual and intraday variability in transit times, with usual estimates ranging from approximately one to six hours. The correlation between the MMC and intestinal transit times through the jejunum and ileum has been shown to be high, with mean speeds of 4.2 to 5.6 cm per minute for a non-disintegrating capsule very similar to the recorded MMC velocity of 4.7 cm per minute. Transit times are not uniform throughout the three segments of the small intestine. Passage through the duodenum is the most rapid, with transit times of a few seconds to a few minutes. Colonic transit times show the most variability, with estimates typically ranging between 6 and 48 hours(McConnell et al., 2008).

1.1.4   Drug absorption via the enterocyte

1.1.4.1 Solubility

A drug must be dissolved before absorption can occur. Dissolution of solid drug depends mostly on the solubility properties of the drug molecule although the amount of fluid in the gastrointestinal tract also plays a role, especially for poorly water-soluble compounds. Measurements of the total amount of fluid in the small intestine of healthy volunteers at any given time have ranged from 10 mL to 350 mL, with average values of 90, 105, and 165 mL reported in three separate small studies. In addition, discrete pockets of fluid have been found in separate areas throughout the small intestine, indicating that fluid volumes are not consistent throughout the lumen. Poorly soluble drugs consequently have the propensity to undergo dissolution in some areas and re-precipitation in others, leading to erratic patterns of absorption(Sutton, 2009).

1.1.4.2 Paracellular absorption

Intestinal absorption of drugs through the epithelium can occur by different pathways. The enterocytes of the small intestine are epithelial cells of the simple columnar type, each containing approximately 1000 luminal protrusions called microvilli made primarily of actin filaments(Washington et al., 2003b). This single layer of epithelium is designed to protect the deeper layers of the intestine as well as the bloodstream from direct contact with the contents of the intestinal lumen. Therefore, the enterocytes and other cells of the small intestinal epithelium are bonded closely together by several different types of junctions. The most prominent of these is the tight junction, a structure formed by proteins from adjacent cell membranes wrapping around the cells such as to effectively eliminate intercellular space (the cell membranes are

within two Å of each other), and sealing off the layers of the intestine below the epithelial cell layer. Intact tight junctions are impermeable to most substances, including most drug molecules. The paracellular space left by tight junctions has been estimated at 0.8 nm in the jejunum and 0.3 nm in the ileum(Washington et al., 2003a). Despite this, a few drugs are absorbed via the paracellular pathway, through aqueous pores in tight junctions. In general, these are hydrophilic molecules with a molecular weight less than 250(Reynolds et al., 2009).

1.1.4.3 Transcellular absorption and permeability

Most drugs are absorbed into the systemic circulation via the transcellular route (i.e. by entering the enterocyte via the plasma membrane on the luminal side and exiting via the basolateral). The plasma membrane, which is designed to hold the contents of the cell and act as a barrier to permeability, is essentially a bilayer of phospholipid surfactants in which various integral proteins are embedded. Drug molecules and other compounds may enter and exit through the membrane via diffusion or transport(Washington et al., 2003a). In order to be absorbed, a drug must not only be dissolved in the fluid of the intestine, but must also be permeable through the enterocyte membrane.

The pH of the intestinal milieu and the pKa will both affect the solubility and permeability of a drug. The generally accepted pH partition hypothesis states that for organic molecules only the un-ionized form of the compound can penetrate the cell membrane and be absorbed. According to this theory, the membrane permeation of a drug in any segment of the GI tract depends not only on the pH of the intestinal segment, but also on the pKa of the drug. Every drug will have a distribution coefficient, D, defined as:

$$\log D = \log P + \log f_U = \log P + \log(1\text{-}f_I)$$

where f_U is the un-ionized fraction of the drug and f_I the ionized fraction(Granero et al., 2003). The pH of the GI tract varies greatly between intestinal segments and between individuals, and also between health and disease. However, in healthy people it is generally assumed that while the pH of gastric fluid is 1-2.5, the pH increases distally to approximately 5.5 in the duodenum, 6.6 in the jejunum and 7.5 in the ileum(McConnell et al., 2008).

Although the cell membrane is phospholipid-based and thus most accessible to hydrophobic and un-ionized molecules, in order to be absorbed a drug must also diffuse across the unstirred water layer (UWL), a stagnant coating approximately 30-100 μm in thickness and composed of water, mucus, and glycocalyx, that exists at the epithelial barrier(Lennernas, 1998; Reynolds et al., 2009). Since solubility and permeability are the two driving forces behind drug absorption, a drug compound must be hydrophilic enough to be reasonably soluble in the relatively aqueous medium of the luminal contents (including the UWL), as well as lipophilic enough to cross the cell membrane. Therefore, drugs with very high or very low octanol:water partition coefficient (LogP) values are not likely to be well absorbed. In addition, the molecular weight of a drug will also affect its absorbability, since larger molecules diffuse more slowly across the membrane(Washington et al., 2003a).

Lipinski proposed the "rule of 5" to assess a drug's likelihood of poor membrane permeability and thus poor oral absorption. The rule states that low permeability is likely when the drug compound has a molecular weight greater than 500; contains more than five hydrogen-bond donors (the sum of –OHs and -NHs); contains more than 10 hydrogen-bond acceptors (the sum of Ns and Os); and the calculated LogP is greater than five. Although the rule was developed to aid in weeding out poor drug candidates in the drug development process, a few drugs that meet all of the rule of five criteria are still orally active and in use today, including

certain vitamins, cardiac glycosides, antibiotics and antifungals whose absorption is aided by transporter proteins. Examples of currently-marketed drugs that meet the rule of five criteria for low permeability are azithromycin, cyclosporine, erythromycin, itraconazole, and methotrexate(Lipinski et al., 2001).

1.1.4.4 The Biopharmaceutic Classification System

With the understanding that the rate and extent of drug absorption depend largely on a drug molecule's dissolution in intestinal fluid and permeability into the enterocyte, Amidon et al. extended this concept by the creation of the Biopharmaceutic Classification System (BCS), which assigns drug compounds to one of four classes based on the compound's solubility and permeability. The central premise of the BCS is based on Fick's law of diffusion, $J_W = P_W \, x \, C_W$, where J_W is the drug flux through the intestinal wall, P_W is the permeability of the membrane, and C_W is the drug concentration at the membrane(Amidon et al., 1995). Solubility of a drug is calculated in aqueous media at 37°C at several points in the pH range of 1 to 7.5, chosen depending on the pKa of the drug(FDA, 2000). The four BCS classes are shown in figure 3.

BCS class I compounds (high solubility, high permeability) are well absorbed through the enterocyte membrane. High solubility is defined as the highest dose strength of the compound that is soluble in less than or equal to 250 mL of aqueous media over pH of 1 to 7.5. Class I compounds dissolve rapidly in gastrointestinal fluid, and if contained in an immediate-release formulation, their absorption rate will be controlled primarily by the rate of gastric emptying. In the fasted state in healthy people, the gastric emptying half life is 22 minutes for an administered volume of 200 mL(Amidon et al., 1995). Examples of orally-administered BCS class I drugs include metoprolol (often used as a reference drug for permeability), midazolam, diltiazem, diphenhydramine, and valproic acid(Takagi et al., 2006).

Figure 3. The original Biopharmaceutic Classification Stystem as proposed by Amidon et al.

Class I	**Class II**
High solubility	Low solubility
High permeability	High permeability
Class III	**Class IV**
High solubility	Low solubility
Low permeability	Low permeability

For BCS class II compounds (low solubility, high permeability), drug dissolution throughout the segments of the intestine is the rate-limiting step for the absorption process, which tends to be slower and more variable than for class I compounds, as the compounds move through the varied pH, fluid, and absorptive conditions in the different sections of the GI tract(Amidon et al., 1995). As noted previously, their low solubility may lead to pockets of drug precipitation and re-dissolution in various segments of the intestine according to fluid volume and composition, leading to erratic absorption profiles(Sutton, 2009). Orally-administered members of BCS class II include tacrolimus, cyclosporine, phenytoin, sirolimus, voriconazole, and warfarin(Takagi et al., 2006).

Permeability through the cell membrane is the rate-limiting step in the absorption process for drugs in BCS class III (high solubility, low permeability). Rate and extent of absorption may be quite variable for compounds in this category. In the case of immediate-release dosage forms, however, if dissolution is fast, variability in absorption will depend upon variations in intestinal transit times, composition of the luminal contents, and the ability of the drug to permeate the enterocyte membrane(Amidon et al., 1995). Typical class III drugs include amoxicillin, doxycycline, fexofenadine, fluconazole, ganciclovir, lisinopril, and metformin(Takagi et al., 2006; Tannergren et al., 2002).

BCS class IV drugs (low solubility, low permeability) are problematic in terms of oral absorption, and this class has the smallest number of members amongst currently marketed

drugs. However, some examples include nystatin, neomycin, amphotericin B, and colistin(Takagi et al., 2006), all of which are used clinically for gut decontamination prior to surgery, and in the case of nystatin and amphotericin B, for treatment or prevention of candida esophagitis in immunosuppressed patients. In both these situations, the medications are used for their local effects after oral administration and absorption into the systemic circulation is not desired.

1.1.5   Absorption via uptake transporters

For many drugs, reaching the systemic circulation depends on more than solubility and permeability. Many exogenous substances are actively transported across the enterocyte membrane by means of uptake transporter proteins. Figure 2 is a simplified drawing of transporter locations at both the apical (luminal) and basolateral (blood) side of the enterocyte. Uptake transporters playing a significant role in drug disposition include the organic anion transporting polypeptides 1A2 and 2B1 (OATP1A2 and OATP2B1) and the peptide transport proteins PEPT1 and PEPT2. Uptake transporters expressed on the apical side of the enterocyte membrane include the monocarboxylate transporter protein 1 (MCT1), the organic cation transporter 3 (OCT3), the apical sodium-dependent bile acid transporter (ASBT), the electroneutral organic cation transporters OCTN1 and OCTN2, and the concentrative nucleotide transporters CNT1 and CTN2(Klaasen and Aleksunes, 2010; Shugarts and Benet, 2009), while those expressed on the basolateral side include OATP3A1 and OATP4A1 and OCT1 and OCT2(Shugarts and Benet, 2009).

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1.1.5.1 Organic anion transporting polypeptides

The organic anion transporting polypeptides (OATPs) are a superfamily of uptake transporters expressed in many tissues, including the sinusoidal membrane of hepatocytes, the blood-brain barrier, and the apical and basolateral membranes of enterocytes. The OATPs are encoded by *SLCO* gene family members located on chromosome 12(Hagenbuch and Gui, 2008). In general, OATPs assist in cellular uptake of many endogenous and exogenous compounds, including bile acids, thyroid hormones, HMG CoA-reductase inhibitors (statins) and angiotensin II receptor antagonists. OATP1A2, OATP2A1, and OATP2B1 are expressed on the apical side of the enterocyte membrane(Kalliokoski and Niemi, 2009) and OATP3A1 and OATP4A1 on the basolateral side(Shugarts and Benet, 2009). A study in healthy volunteers found no significant differences in OATP2B1 mRNA expression between duodenum, ileum, and the three segments of the colon in biopsies obtained from normal human intestine(Meier et al., 2007).

1.1.5.2 Other uptake transporters

The peptide uptake transporter PEPT1 is expressed in equal amounts in the three regions of the small intestine, but at significantly lower levels in the colon. PEPT1 substrates include oseltamivir(Ogihara et al., 2009), ganciclovir(Sugawara et al., 2000), and some beta-lactam and cephalosporin antibiotics(Balimane et al., 1998). The electroneutral organic cation uptake transporters OCTN1 and OCTN2 are expressed throughout the small intestine and colon, but with higher levels in the colon than the intestine. The high affinity concentrative nucleoside transporters CNT1 and CNT2 were detectable in duodenum and ileum, but not in any colonic segment, whereas the equilibrative nucleoside transporters ENT1 and ENT2 were only detected in the colon and not in the small intestine. The apical sodium-dependent bile acid transporter

(ASBT) has been found at higher levels in the ileum than in the duodenum, and has not been detected in the colon.

Figure 4. Simplified drawing of enterocytes with membrane transporters
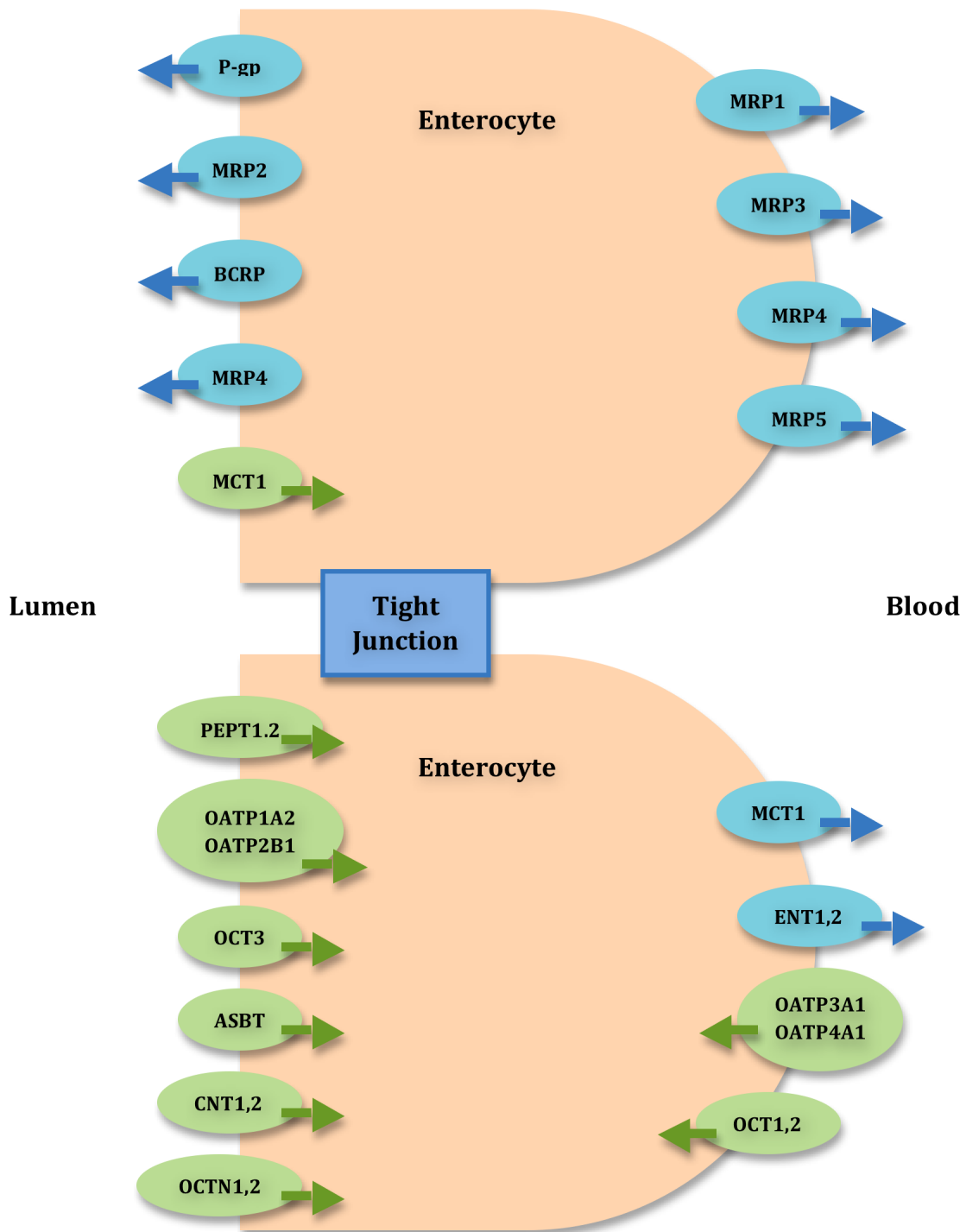
Adapted from Shugarts and Benet(Shugarts and Benet, 2009). Efflux transporters are in blue and uptake transporters in green.

1.1.6 Enterocyte barriers to absorption: Efflux transport and intestinal first-pass metabolism

The oral bioavailability of a drug is defined as the amount of drug that reaches the systemic circulation after oral administration. Substances absorbed via the intestine may undergo first-pass metabolism in the enterocytes of the intestine or the hepatocytes of the liver, since the intestinal blood supply drains ultimately to the portal vein and liver before reaching the systemic circulation. For drugs absorbed by the transcellular route, bioavailability is mainly dependent on the fraction of dose absorbed through the enterocyte membrane (Fa), the fraction of drug that escapes from intestinal first-pass metabolism (Fg), and the fraction of drug that escapes from hepatic first-pass metabolism (Fh)(Dahan and Amidon, 2009). Once absorbed, some drugs will also be pumped out of the enterocyte back into the intestinal lumen by efflux transporters.

Efflux transporters expressed on the apical side of the enterocyte include p-glycoprotein (p-gp), multidrug resistance associated protein 2 (MRP2), breast cancer resistance protein (BCRP), and multidrug resistance associated protein 4 (MRP4). MRP4 is also expressed on the basolateral side of the membrane, along with multidrug resistance associated proteins 1, 3 and 5 (MRP1, MRP3, MRP5). Other efflux transporters on the basolateral side are MCT1 and equilibrative nucleoside transporters 1 and 23 (ENT1 and ENT2)(Klaasen and Aleksunes, 2010; Shugarts and Benet, 2009).

Drug-metabolizing enzymes such as cytochrome P450s, UDP-glucuronosyltransferases, glutathione-S-transferases, sulfotransferases, and esterases located within the enterocyte may also reduce the amount of drug reaching the systemic circulation. Many drugs are substrates for both the efflux pump p-glycoprotein and the drug metabolizing enzyme cytochrome P450 3A (CYP3A). P-gp and CYP3A are proposed to work synergistically in the gut to prevent systemic

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absorption of xenobiotics, with p-gp effluxing substrates back into the intestinal lumen to keep CYP3A metabolism from becoming saturated(Christians et al., 2005).

1.1.6.1 P-glycoprotein

Of the efflux transporters known to be expressed on the apical side of the enterocyte, the best studied and most relevant for drug disposition is p-glycoprotein. P-glycoprotein (p-gp) is an ATP-dependent efflux transporter with a wide distribution in the human body, including the brush border of the apical membrane of enterocytes, the bile canalicular membrane of hepatocytes, the apical membrane of renal proximal tubular cells, blood cells, and capillary endothelial cells of the blood-brain barrier(Canaparo et al., 2007). P-gp is encoded by the *ABCB1* gene located on chromosome 7 in humans and its expression is regulated by the pregnane X receptor (PXR), a nuclear receptor that acts as a ligand-activated transcription factor(Watkins et al., 2001).

P-gp transports a wide variety of xenobiotics, including tacrolimus, cyclosporine, sirolimus, fexofenadine, digoxin, loperamide, ketoconazole, levofloxacin, and numerous others(Klaasen and Aleksunes, 2010). The expression pattern of p-gp in the intestine is generally assumed to be lowest in the proximal small intestine, with expression levels increasing throughout the GI tract and peaking in the colon(Thörn et al., 2005), although one study found the highest expression in the jejunum as compared with the ileum and colon(Berggren et al., 2006). Small amounts of p-gp have also been found in gastric tissue(Canaparo et al., 2007; Thörn et al., 2005).

1.1.6.2 Breast cancer resistance protein (ABCG2)

Breast cancer resistance protein (BCRP) is an efflux transporter expressed on the apical membrane of enterocytes as well as in breast tissue, the liver, kidney, and blood-brain barrier(Giacomini et al., 2010). It is encoded by the *ABCG2* gene, whose regulation has not been fully elucidated, although dexamethasone(Narang et al., 2008) and 2-acetylaminofluorine(Anapolsky et al., 2006) have induced its upregulation, indicating roles for the glucocorticoid receptor and PXR, respectively. Ciprofloxacin(Haslam et al., 2011), nitrofurantoin(Wright et al., 2011), imatinib(Krishnamurthy and Schuetz, 2006), and acyclovir(Gunness et al., 2011) are substrates of BCRP, among others.

1.1.6.3 Multidrug resistance proteins

Multidrug resistance protein 2 (MRP2) is an efflux protein located on the apical membrane of enterocytes as well as in the bile canalicular membrane of hepatocytes and the kidney proximal tubule(Takano et al., 2006). It is encoded by the *ABCC2* gene, which is regulated by the nuclear receptors PXR, constitutive androstane receptor (CAR) and farnesoid X receptor (FXR)(Choudhuri and Klaasen, 2006). Substrates of MRP2 include methotrexate, various prostaglandins, and glucuronide conjugates such as conjugated bilirubin, estradiol 17-β-D-glucuronide, acetaminophen-glucuronide, pravastatin, and ampicillin(Choudhuri and Klaasen, 2006). Its expression levels have been shown to be highest in the jejunum, decreasing to lowest levels in the colon(Berggren et al., 2006). MRP3 (*ABCC3*) has much overlapping substrate specificity with MRP2, and has been shown to be regulated by PXR(Anapolsky et al., 2006) and CAR(Choudhuri and Klaasen, 2006).

1.1.6.4 Cytochrome P450 enzymes

The cytochrome P450 (CYP) enzymes are a superfamily of heme-containing enzymes responsible for metabolism of many endogenous compounds as well as for the oxidative metabolism of as many as 80% of commonly used drugs(Wilkinson, 2005). The CYP3A family accounts for approximately 50% of CYP content in the liver(Wilkinson, 2005) and approximately 82% of jejunal CYP content(Paine et al., 2006). Of the other CYP enzymes expressed in the small intestine, CYP2C9 accounts for at most approximately 14%, and very small amounts of CYP2C19 (2%), CYP2D6 (0.7%), and CYP2J2 (approximately 1.4%) protein have also been detected(Paine et al., 2006). Although expression levels of CYP3A in the intestine are approximately 1% of those in the liver(Paine et al., 2006), intestinal CYP3A plays a vital role in intestinal first-pass metabolism of substrates after oral administration. Examples of medications whose bioavailabilities are strongly affected by intestinal CYP3A content include the immunosuppressive medications tacrolimus and cyclosporine, calcium channel blocker antihypertensives, HIV protease inhibitors, and HMG-CoA reductase inhibitors. The benzodiazepine sedative-hypnotic drug midazolam is metabolized nearly exclusively by CYP3A, and therefore is often used as a probe to assess CYP3A phenotype.

There are two major CYP3A isoforms expressed in human adults, CYP3A4 and CYP3A5, and expression is regulated by PXR(Bertilsson et al., 1998; Lehmann et al., 1998). Although CYP3A is expressed throughout the small intestine, it is generally agreed that within the GI tract, CYP3A expression is highest in the duodenum and jejunum and gradually declines further along through the ileum, reaching its lowest levels in the colon(Berggren et al., 2006; Paine et al., 1997). Small amounts of CYP3A4 and CYP3A5 mRNA and protein have also been detected in gastric tissue(Canaparo et al., 2007; Thörn et al., 2005).

1.1.6.5 UDP-glucuronosyltransferases

The UDP-glucuronosyltransferases (UGTs) are a superfamily of conjugating enzymes widely distributed in the body, with several isoforms found in the small intestine, located on the endoplasmic reticulum of the enterocyte. The UGTs use UDP-glucuronic acid as a co-substrate in glucuronidation reactions that make compounds more water soluble to facilitate renal excretion. The UGTs form glucuronide conjugates of many drug metabolites created by CYP enzymatic reactions, but some endogenous and exogenous compounds undergo direct glucuronidation, including bilirubin, steroid hormones, morphine, and mycophenolic acid(Kiang et al., 2005). The UGT isoenzymes known to be expressed in the GI tract are UGT 1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A10, 2B4, 2B7, 2B10, 2B15, and 2B28(Finel et al., 2005; Gregory et al., 2004). Of these, UGT1A7, 1A8, and 1A10 are exclusively found in the GI tract, where their expression is regulated by cooperation of the transcription factors hepatocyte nuclear factor 1 alpha (HNF1α) and caudal related homeodomain protein 2 (Cdx2)(Gregory et al., 2004).

1.1.6.6 Other drug-metabolizing enzymes expressed in the enterocyte

Other drug-metabolizing enzymes expressed in the enterocytes of the small intestine include glutathione-S-transferases (GSTs), sulfotransferases, and esterases. GSTs are a family of enzymes that conjugate compounds with glutathione thus making them more water soluble and easily excreted, and for which azathioprine is a substrate. GSTs of the alpha class have been found in the jejunum, ileum, and colon(Peters et al., 1989). Sulfotransferases catalzye sulfation reactions that add sulfate to phenolic and hydroxyl functional groups and amines, including acetaminophen and terbutaline(Beaumont, 2003). Lastly, esterases are responsible for catalyzing the hydrolysis of ester bonds. Many prodrugs, including oseltamivir phosphate, valganciclovir,

olmesartan medoxomil, benazapril, and enalapril, are rapidly hydrolyzed to their active metabolites by esterases inside the enterocyte(Beaumont, 2003; Benet et al., 2011).

1.1.6.7 The Biopharmaceutics Drug Disposition Classification System

Further insight into bioavailability can be derived from consideration of the Biopharmaceutics Drug Disposition Classification System (BDDCS), which was developed by Wu and Benet to build upon the BCS. Wu and Benet noted that the majority of BCS class I and II drugs (highly permeable) were extensively metabolized (many by CYP3A) and that the majority of class III and IV drugs were poorly metabolized with mostly renal and/or biliary excretion. The BDDCS reflects the fact that although drug compound solubility is easy to measure *in vitro*, permeability is less so and values may vary depending on the system used.

For drugs for which intestinal permeability is known from *in vivo* studies, a high correlation between jejunal permeability and metabolism is observed, which is higher than the observed correlation between jejunal permeability and LogP. The fact that the BCS fails to take transporter effects into account is also an important consideration, for while *in vitro-in vivo* permeability measurements are highly correlated for drugs absorbed via passive diffusion, drugs absorbed via uptake transporters may deviate significantly from this relationship. Some drugs, such as cefadroxil, levofloxacin, pregabalin, and sotalol, have shown low permeability but are well absorbed, probably due to uptake transporter effects(Lennernas and Abrahamsson, 2005). Given that most drugs on the market are substrates for enzymes and/or transporters, Benet et al. have now classified more than 900 drugs using the BDDCS (see figure 5)(Benet et al., 2011). Medications relevant to small bowel transplant recipients listed by BDDCS class appear in Table 1 of this chapter and will be discussed in a future section.

Figure 5. The Biopharmaceutics Drug Disposition Classification System as proposed by Wu and Benet (Benet, 2009)

Class I	Class II
High solubility	Low solubility
Extensive metabolism	Extensive metabolism
Transporter effects minimal in intestine and liver	*Efflux transporter effects predominate in intestine, but both uptake and efflux effects play a role in liver*
Class III	**Class IV**
High solubility	Low solubility
Poor metabolism	Poor metabolism
Uptake transporter effects predominate, but effects may be modulated by efflux transporters	*Uptake and efflux transporters may play role*

BDDCS class I drugs exhibit high solubility and are extensively (greater than 70%) metabolized. Transporter effects are minimal for members of this class of compounds, even if a drug is a transporter substrate *in vitro*. Class I includes many of the same compounds as class I of the BCS, including midazolam and metoprolol, as well as the prodrugs activated by intestinal esterases discussed previously. These prodrugs such as enalapril are designed to reduce hydrophilicity of the active moiety and increase permeability across the intestinal wall. Members of BDDCS class II exhibit low solubility but are extensively (greater than 70%) metabolized. If a class II compound is a transporter substrate, the effects of efflux transporters such as p-gp will predominate in the intestine, although both uptake and efflux transporters will affect its disposition in the liver.

BDDCS class III drugs are highly soluble and poorly (less than 30%) metabolized. Many members of this class depend on uptake transporters such as PEPT1 and PEPT2 and the OATPs

for absorption. The active metabolites of the prodrugs mentioned in the previous section, including oseltamivir, ganciclovir, benazeprilat, enalaprilat, and olmesartan, all belong to class III of the BDDCS, as do fexofenadine, many of the cephalosporin antibiotics, digoxin, and famotidine. Finally, BDDCS class IV compounds are poorly soluble and poorly metabolized, but their disposition may be affected by either uptake or efflux transporters to some extent. Examples of class IV compounds include acyclovir, atovaquone, ciprofloxacin, furosemide and valsartan(Benet, 2009; Benet et al., 2007; Benet et al., 2011).

1.2 INTESTINAL FAILURE

Intestinal failure is a serious condition in which the absorptive capacity of the intestine is severely impaired. "Intestinal failure," O'Keefe et al. state in their consensus document on short bowel syndrome and intestinal failure, "results from obstruction, dysmotility, surgical resection, congenital defect, or disease-associated loss of absorption and is characterized by the inability to maintain protein-energy, fluid, electrolyte, or micronutrient balance."(O'Keefe et al., 2006). Some of the more common causes of intestinal failure include resections due to Crohn's disease, intestinal trauma, or thrombosis of a superior mesenteric vein or artery, and conditions in which the full length of the non-functioning bowel often remains, including intestinal pseudo-obstruction syndrome, radiation enteritis, or congenital villus atrophy(Buchman et al., 2003). Patients with intestinal failure are dependent on intravenous fluids and nutrition to sustain life. Daily dependence on total parenteral nutrition (TPN) is associated with significant health risks over time, including liver disease and sepsis due to line infection(Cavicchi et al., 2000; O'Keefe

et al., 1994). Currently, intestinal transplantation is the only permanent treatment for irreversible intestinal failure.

1.3   INTESTINAL TRANSPLANTATION

The three main types of transplant that include a small intestine are the isolated intestinal transplant (Figure 6), in which the jejunoileum is transplanted; the combined liver-intestine transplant; and the multivisceral transplant, which includes the liver, stomach, duodenum, pancreas, and jejunoileum(Fishbein, 2009). A subtype of multivisceral transplant, the modified multivisceral (Figure 7), includes all of the preceding except the liver. In some cases of modified multivisceral transplantation, the native splenopancreaticoduodenal complex (native spleen, pancreas, and a portion of native duodenum) is retained, with the native segment of duodenum anastomosed to the transplanted duodenum in a side to side fashion(Cruz Jr et al., 2010), while in others the spleen is preserved but the native pancreaticoduodenal complex is removed(Cruz Jr et al., 2011). In the latter case and in cases of modified multivisceral transplantation in which no native spleen or pancreaticoduodenal complex is retained, a T-tube is placed to connect the native and transplanted portions of the bile duct. In addition, during multivisceral transplantation a pyloroplasty is performed on the transplanted stomach to facilitate gastric emptying(Cruz Jr et al., 2010).

1.3.1 Isolated intestinal transplant graft drainage

In isolated intestinal transplant recipients, the venous outflow from the graft is sometimes arranged to drain directly into the systemic circulation via the vena cava rather than via the portal vein(Abu-Elmagd et al., 2009; Fishbein, 2009). This is illustrated in the upper insert of Figure 6. This has occurred in 52% of the isolated intestinal transplants performed at the University of Pittsburgh Medical Center between July 2001 and November 2008(Abu-Elmagd et al., 2009). The presence of the caval graft drainage arrangement is an important consideration for drug dosing, since hepatic first-pass metabolism of orally administered highly metabolized drugs will be bypassed, leading to greater bioavailability. In a study comparing the effects of portal systemic drainage (n=19 patients) with caval systemic drainage (n=18 patients), mean oral tacrolimus dosage requirements were nearly double in the patients with portal drainage as compared with the patients with systemic drainage (0.55 ± 0.09 vs. 0.23 ± 0.05 mg/kg/day, p=0.02), despite no significant difference in mean tacrolimus trough levels between the two groups (12.6 ±1.6 for portal vs. 12.0±1.3 ng/mL for systemic)(Berney et al., 2002). This shows that the bioavailability and therefore the dosing requirements of orally-administered drugs in which first-pass metabolism plays an important role will be significantly increased in patients for whom the transplanted graft drains into the vena cava rather than the portal venous system.

Figure 6. Isolated intestinal transplantation
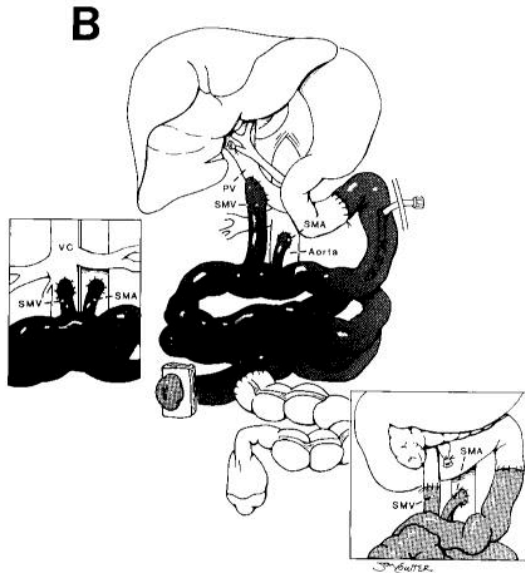
Reprinted from Kocoshis et al. (Kocoshis et al., 1997) with permission of Springer Publishing.
Isolated small bowel transplantation: (B) Recipient operation. Anastomosis of the full length of SMA to the aorta and the angled end of the SMV to the portal vein. In an alternative method the SMV is anastomosed to the recipient SMV inferior to the pancreas (lower insert). Option of SMV drainage into the inferior vena cava is shown in upper insert.

Figure 7. Modified multivisceral transplantation
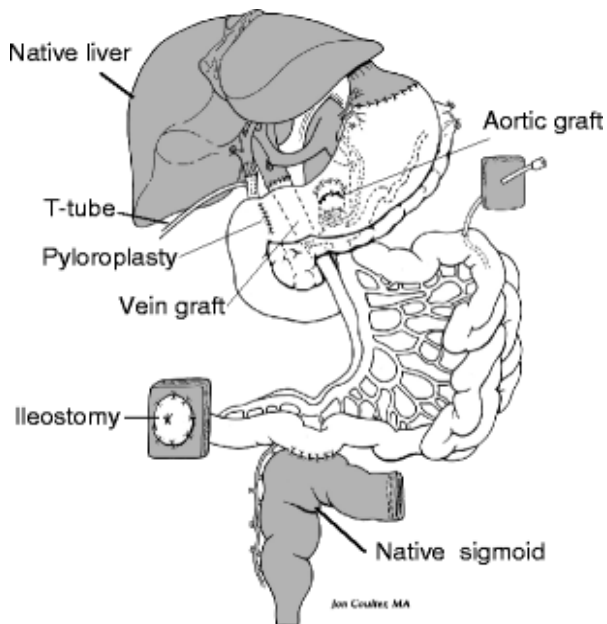
Reprinted from Cruz et al. (Cruz Jr et al., 2010) with permission of Springer Publishing.

1.3.2 Medication use after small bowel transplantation

Small bowel transplant recipients require numerous medications after transplant. In the first three or four days post-transplant, the recipient receives all medications intravenously, including the primary immunosuppressive drug tacrolimus. After stabilization of the blood concentrations of tacrolimus at 10 to 15 ng/mL, tacrolimus and some other enteral medications are given via the jejunostomy tube (J-tube) placed during surgery. These are mostly liquid formulations (tacrolimus suspension, sulfamethoxazole-trimethoprim solution) but in some cases tablets must be crushed and mixed with water before being administered via the tube. At this point, the patient still has a nasogastric tube (NG tube) in place draining liquid from the stomach in order to prevent aspiration due to post-surgical gastroparesis. Medication administration via J-tube

allows delivery to the major site of intestinal medication absorption while bypassing the effects of impaired gastric emptying.

When the NG tube is no longer draining significant amounts of liquid (usually within 14 days post-transplant) it is removed and patients begin transitioning to taking medications by mouth. These are usually solid dosage forms (tables or capsules) to allow the patient to learn about and recognize the oral medications and dosage forms they will be self-administering at home. A typical outpatient oral medication regimen for a small bowel transplant patient includes the immunosuppressive drug tacrolimus, an oral corticosteroid such as hydrocortisone or prednisone, sulfamethoxazole-trimethoprim or dapsone for *Pneumocystis carinii* pneumonia prophylaxis, ganciclovir for cytomegalovirus prevention, and magnesium, calcium, vitamin B6, and vitamin D supplements. In addition, many patients require oral psychotropic medications such as sertraline, haloperidol, mirtazepine, and trazodone, and perhaps zolpidem for sleep or hydromorphone or methadone for pain control. Many also take oral antihypertensives such as calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors, and angiotensin II receptor antagonists. Most of these medications are substrates for CYP3A and/or transporters. Oral medications commonly used in small bowel transplant recipients, arranged by BDDCS class, are listed in Table 1.

Table 1. Oral medications relevant to small bowel transplant recipients by BDDCS class

Class I High Solubility High permeability/ metabolism If a substrate, transporter effects minimal	Class II Low Solubility High permeability/ metabolism If a substrate, efflux transporter effects predominate in intestine	Class III High Solubility Low permeability/ metabolism If a substrate, uptake transporter effects predominate over efflux	Class IV Low Solubility Low permeability/ metabolism If a substrate, efflux and uptake transporters may play role
Acetaminophen (UGT1A1, 1A6)	Acitretin (p-gp)	Adefovir (MRP1)	Acyclovir (OCT1, BCRP)
Amlodipine (CYP3A)	Allopurinol	Amoxicillin (PEPT1, 2)	Atovaquone
Azathioprine (Glutathione-S-transferase)	Amiodarone HCl (CYP2C8, 3A, p-gp)	Ampicillin (MRP2, PEPT1, 2)	Candesartan
Benazepril (prodrug-hydrolysis to benazeprilat-see Class III)	Calcitriol	Atenolol (CYP2D6)	Cefdinir
Bupropion	Carvedilol (CYP2D6, UGT1A1, 2B4, 2B7, p-gp)	Azithromycin (MRP2)	Cefixime (PEPT2)
Cholecalciferol (Vit. D3) (hydroxylation)	Citalopram (CYP2C19)	Benazaprilat (active metabolite)	Cefpodoxime
Clonazepam (CYP3A)	Clofibrate (UGT1A3)	Biotin	Cefprozil
Cyanocobalamin (Vit. B12)	Clopidogrel busulfate (CYP2C19)	Bumetanide	Ceftibuten (PEPT1, 2)
Desogestrel (CYP2C9)	Clotrimazole	Captopril (PEPT1, 2)	Ciprofloxacin (OATP1A2, BCRP)
Dextromethorphan HBr (CYP2D6, 3A)	Cyclosporine (CYP3A, p-gp)	Cefaclor (PEPT1, 2)	Furosemide
Diltiazem (CYP3A, p-gp)	Dapsone (CYP3A)	Cefadroxil (PEPT1, 2)	Levonorgestrel
Diphenhydramine (CYP2D6, UGT1A4)	Ergocalciferol (Vit. D2)	Cefamandole (PEPT1, 2)	Nitrofurantoin (BCRP)
Enalapril (prodrug—converted to enalaprilat-see Class III)	Ezetimibe (UGT1A1, 2B7)	Cefazolin	Penicillin V
Escitalopram (CYP2C19, 3A)	Felodipine (CYP3A)	Cefuroxime	Riboflavin (Vit. B2)
Esomeprazole magnesium (CYP2C19, 3A)	Folic acid (MRP1)	Cephalexin (PEPT1, 2)	Rifaximin
Estradiol (CYP3A, UGT2B28)	Gemfibrozil (UGT2B7)	Cetirizine	Valsartan (MRP2)
Ethinyl Estradiol	Glipizide (CYP2C9)	Clavulanic acid	

(UGT1A1)

Fludrocortisone acetate

Fluoxetine
(CYP2D6, p-gp)

Hydralazine HCl
(acetylation – liver)

Hydrocodone
(CYP2D6)

Hydrocortisone
(CYP3A, 2D6)

Hydromorphone
(UGT1A3, 2B7)

Labetalol
(CYP2D6, UGT1A1, 2B7)

Linezolid
(oxidation-liver)

Methadone
(CYP2D6, 3A, p-gp)

Methylphenidate

Metoprolol
(CYP2D6)

Metronidazole
(CYP2C9)*

**Midazolam HCl
(CYP3A)**

Mirtazapine
(CYP2D6, 3A, 2C19)

Niacin, nicotinic acid

Niacinamide, nicotinamide

Nicardipine
(CYP3A, p-gp)

Norethindrone
(CYP3A)

Norgestimate

Norgestrel

Olmesartan medoxomil
(prodrug—converted to
olmesartan-see Class III)

Glyburide
(CYP2C9, OATP2B1)

Haloperidol
(CYP2D6, 3A)

Irbesartan
(CYP2C9)

Isradipine
(CYP3A)

Itraconazole
(CYP3A, p-gp)

Lansoprazole
(CYP2C19, p-gp)

Leflunomide

Levothyroxine
(OATP1A2, 3A1, 4A1)

Loratadine
(CYP2D6, 3A)

Losartan potassium
(CYP2C9, p-gp)

Nifedipine
(CYP3A)

Nimodipine
(CYP3A)

Nitrendipine
(CYP3A)

Norethindrone acetate

Olanzapine
(UGT1A4)

Paricalcitol
(CYP24, 3A, UGT1A4)

Phenytoin sodium
(CYP2C9, 2C19, 3A, p-gp)

Prasugrel
(GI hydrolysis, then CYP3A)

Prednisone
(CYP3A, p-gp)

Progesterone
(CYP2C19, 3A, p-gp)

Quinapril
(prodrug – hydrolysis to
quinaprilat-see Class III)

Clonidine
(CYP2D6)

Digoxin
(p-gp)

Doxycycline
(p-gp)

Enalaprilat (active metabolite)
(PEPT1)

Famotidine
(p-gp, OCT2)

Ferrous sulfate

**Fexofenadine
(p-gp, OATP1A2, 2B1)**

Fluconazole
(p-gp)

Gabapentin

Ganciclovir sodium
(PEPT1, OCT1)

Levetiracetam

Levocetirizine

Levofloxacin
(p-gp, OATP1A2)

Lisinopril
(PEPT1)

Loperamide (p-gp)

Metformin
(OCT1, OCT2)

Metoclopramide (CYP2D6)

Moxifloxacin HCl (p-gp)

Nystatin

Olmesartan (active metabolite)

Oseltamivir (active metabolite)
(PEPT1)

Omeprazole
(CYP2C19)

Sirolimus
(CYP3A, p-gp)

Potassium chloride

Ondansetron
(CYP2D6, 3A, p-gp)

Sulfamethoxazole
(N-acetyltransferase 1)

Pravastatin
(MRP2, OATP2B1)

Oseltamivir phosphate
(prodrug—converted to
oseltamivir-see Class III)

**Tacrolimus
(CYP3A, p-gp)**

Pregabalin
(LAT-1)

Oxybutynin
(CYP3A)

Telmisartan

Quinaprilat (active metabolite)

Oxycodone
(CYP2D6)

Trazodone
(CYP3A)

Sotalol

Pantoprazole sodium
(CYP2C19, BCRP)

Ursodiol

Tetracycline
(p-gp)

Propranolol HCl
(CYP2C19, 2D6, 3A)

Vitamin A (retinol)
(UGT2B7)

Thiamine (Vit. B1)

Pyridoxine

Voriconazole
(CYP2C19, 2C9, 3A4)

Topiramate
(p-gp)

Quetiapine fumarate
(CYP3A)

Warfarin
(CYP1A2, 2C9, 2C19)

Trimethoprim

Sertraline HCl
(CYP2C19, 2D6, p-gp)

Zaleplon
(CYP3A)

Sildenafil
(CYP3A)

Ziprasidone HCl
(CYP3A)

Sumatriptan succinate
(MAO-A)
Tamoxifen
(CYP2C9, 2D6, 3A, UGT1A4)

Tamsulosin
(CYP2D6, 3A)

Valganciclovir
(prodrug--converted to
ganciclovir-see Class III)

Valproic acid
(UGT1A3, 1A6)

Venlafaxine HCl
(CYP2D6)

Zolpidem tartrate
(CYP3A, 2D6)

Table adapted from Benet et al. (Benet et al., 2011). Relevant intestinal enzymes and transporters in parentheses.
Liver/kidney/other non-intestinal transporters not included.
Medications in **bold** are those examined in this work.

1.4 EFFECTS OF SMALL BOWEL TRANSPLANTATION ON INTESTINAL FUNCTION AND ORAL DRUG DISPOSITION

Transplantation of a small intestine into a recipient involves a number of processes that may affect intestinal function as well as drug absorption, metabolism, and transport. Anatomical changes include extrinsic denervation of the intestine, which affects motility, and severing of lymphatic channels, which affects fat absorption. The intestinal microbiome after transplant is different than that of healthy persons in terms of relative populations of bacterial species and increased colony count. Events causing inflammatory changes include intestinal manipulation during surgery, ischemia-reperfusion injury, and activation of cell-mediated and humoral immunity as donor immune cells come into contact with those of the recipient. This immune activation and the associated inflammatory mediator production may suppress drug-metabolizing enzyme and transporter expression and function.

1.4.1 Extrinsic denervation of the intestine

Unless the recipient already has an ultra short intestine due to multiple resections, removal of the native intestine during transplant surgery severs the existing extrinsic nervous connections between the small intestine and the spinal cord. During the multivisceral and modified multivisceral transplant procedure, the stomach also undergoes complete denervation, requiring a pyloroplasty to enable gastric emptying(Cruz Jr et al., 2010).

Under normal conditions, the jejunum and the ileum are innervated by both sympathetic and parasympathetic nerve fibers from the superior mesenteric plexus. Movement of the small intestine results from a combination of contractions and relaxations of the circular and longitudinal muscles of the muscularis externa and of the muscularis mucosae(Furness et al., 2009). The sympathetic nerve fibers are inhibitory towards the muscle tissue of the jejunum and ileum and cause vasoconstriction of the surrounding blood vessels when activated(Standring et al., 2008), but they have little effect on intestinal motility under resting conditions(Furness et al., 2009) The parasympathetic nerve fibers travel via the vagus nerve and act as motor neurons in the muscularis externa and as secretomotor neurons in the mucosa(Standring et al., 2008). Normal activity of the enteric nervous system is modified by the central nervous system via extrinsic pathways, although it is capable of functioning on its own without external control(Furness et al., 2009).

Denervation may cause impairments in gastric motility but increases in small intestinal motility and dysregulation of the MMC between segments. In a study of denervation of the entire upper gastrointestinal tract in dogs, including stomach and small intestine, gastric motility during phase III of the MMC was decreased, and the duration between contractions increased(Tanaka et al., 2001). In a dog model of selective neural isolation of the jejunoileum, a lack of coordination between phase III of the MMC between the duodenum and jejunum was observed, as was a decreased jejunal MMC period, and increased measures of motility through the jejunum(Behrns et al., 1996). Similar patterns have been seen in a dog model of jejunoileal autotransplantation, which found recovery of MMC coordination between segments within one month in most dogs, but a persistent shortened intestinal transit time up to six months post-surgery(Nakada et al., 1994).

Eventual reinnervation of the transplanted intestine has been shown to occur by one year post-transplant in rat models of syngeneic intestinal transplantation, with spontaneous contractile activity of longitudinal muscle from the transplanted jejunum no different from that of nontransplanted control rats of the same age. In addition, immunohistofluorescence staining for tyrosine hydroxylase, a substance found primarily in sympathetic neurons, was found to be identical in both duodenum and jejunum of transplanted and control rats, indicating intestinal reinnervation(Kasparek et al., 2008).

1.4.2   Severing of lymphatics

The lymphatic system of the small intestine is complex. Each villus in the mucosa contains a fingerlike lymph channel projection called a lacteal. These lacteals drain into a plexus of lymphatic channels in the submucosa, which in turn drain into larger mesenteric lymph nodes that often run alongside major arteries. The larger mesenteric lymph nodes of the jejunum and ileum drain into superior mesenteric nodes that are located near the base of the superior mesenteric artery(Healy et al., 2005). Dietary fats are normally absorbed as chylomicrons and very low density lipoproteins (VLDLs), which enter the lacteals inside the villi by diffusion and are transported through the branches of the intestinal lymphatic system into the circulatory system(Porter and Charman, 2001). Small bowel graft harvesting necessitates severing of the lymphatics that drain the jejunum and ileum, and also − in the case of modified multivisceral transplantation − those that drain the stomach and duodenum. This process may lead to malabsorption of fats and the condition of chylous ascites, in which chyle (a combination of lymphatic fluid and fats produced by the intestine) enters peritoneal fluid(Weseman, 2007), as well as the disruption of lymphatic transport and decreased absorption of lipid drug formulations

into the circulation. Some examples of lipid drug formulations now on the market include cyclosporine microemulsion (Neoral®)(Novartis, 2009), ritonavir (Norvir®)(Abbott, 2010), and saquinavir (Fortovase®)(Roche, 2003). In a dog model of jejunoileal autotransplantation, cyclosporine absorption was severely diminished on post-transplant day 1, with a significantly lower AUC and Cmax and higher Tmax compared with normal control dogs. On day 7, this difference remained, but was less pronounced. By post-operative days 14 and 28, cyclosporine profiles were no different from control dogs(Ishikawa et al., 2003), indicating complete recovery of ability to absorb fats and fat-soluble compounds.

In animal models, new lymphatic connections from the graft to outside lymphatic channels begin to be created by day 2 or 3 post-transplant, and are complete by post-operative days 8 to 21(Schmid et al., 1990; Üner et al., 2001), although regrowth patterns may be different than those prior to transplant. Studies using dye have shown increased lymph channels around the vascular anastomosis extending into the retroperitoneal lymphatics(Winkelaar et al., 1997) as well as lymphatic vessels along the wall of the superior mesenteric artery draining via the testicular lymphatic channels to the thoracic lymph duct(Üner et al., 2001).   After lymphangiogenesis, fat absorption improves significantly. Systemic absorption of a fatty acid emulsion containing medium and long chain fatty acids in rats six weeks after small bowel transplantation was similar to healthy control animals(Winkelaar et al., 1997). Another study in dogs found no differences in the pharmacokinetic parameters of cyclosporine microemulsion between stable allotransplanted dogs and autotransplanted and control dogs after intensive blood sampling between post-operative days 110 and 196(Iwanami et al., 2003).

1.4.3   Changes in intestinal microflora

The microbiome of the transplanted intestine is different from that observed in the healthy native intestine. In the healthy gastrointestinal tract, bacterial colony numbers are low in the stomach and small intestine as compared with the colon. Typical levels (expressed as colony forming units per gram of contents) are 1×10^3 in the stomach, 1×10^4 in the duodenum and jejunum, and 1×10^6-10^7 in the ileum, whereas levels in the colon are reported as 1×10^{11} - 10^{12}(McConnell et al., 2008). In small bowel transplant recipients, impaired gastrointestinal motility and long-term use of proton pump inhibitors may lead to bacterial overgrowth, which carries a risk of bacterial translocation and systemic infection, and the bacterial species composition of the intestinal lumen is different due to gut decontamination prior to surgery, heavy antibiotic use, and presence of an ileostomy(Abu-Elmagd et al., 1994). In subjects with an open ileostomy, Lactobacilli and Enterobacteria (facultative anaerobes) are more numerous than the obligate anaerobes of the Clostridia and Bacteroides families, a pattern opposite to that seen in most normal intestinal flora and similar to non-transplanted patients with ileostomies. After ostomy closure, the microbiological pattern reverts back to a predominance of obligate anaerobes(Amarri et al., 2002; Hartman et al., 2009). Examples of drugs that undergo some degree of bacterial metabolism in the environment of the healthy intestine include clonazepam, metronidazole, omeprazole, and digoxin(Sousa et al., 2008). The effects of altered microflora on intestinal drug metabolism in a small bowel transplant context are not understood at this time.

1.4.4   Effects of intestinal manipulation

Surgical manipulation of the intestine impairs intestinal motility and gastric emptying even outside of a transplant context. In a rat model of selective jejunal manipulation, GI transit of orally-administered FITC-labeled dextran over 30 minutes was significantly slowed in the manipulated animals 24 hours after manipulation, with fluorescent label peaks seen in the proximal half of the small intestine, with delays in the stomach. In contrast, in control animals, 30-minute label peaks occurred in the ileum. In addition, jejunal circular muscle strips from manipulated animals showed a significant reduction in bethanechol-induced contractility compared with controls, and increases in leukocyte extravasation into the intestinal muscularis were seen. These effects were partially, but not completely, ameliorated by pretreatment with dexamethasone (Schwarz et al., 2004).

1.4.5   Ischemia-reperfusion injury

After removal from the donor, the intestinal graft is placed in a preservation solution, typically University of Wisconsin (UW) solution, where it undergoes a period of cold ischemia time while it is in transit to the recipient. Cold ischemia times vary, but Abu-Elmagd et al. report a mean of 7.8 ±1.5 hours for transplants done at the University of Pittsburgh between July 2001 and November 2008(Abu-Elmagd et al., 2009). Lack of oxygen during this time leads to tissue ischemia, cellular dysfunction, and cell death. Restoration of blood flow to the organ during surgery and subsequent reoxygenation leads to the formation of reactive and cytotoxic oxygen species. These cause leukocyte activation and adhesion to the endothelial lining of blood vessels. Subsequent dysfunction of microcirculation and parenchymal cell damage ensues. In

addition, CD4+ and CD8+ T cells are also recruited during ischemia-reperfusion, and lead to tissue infiltration by neutrophils and albumin extravasation(Vollmar and Menger, 2011).

Intestinal ischemia-reperfusion results in immediate significant increases in plasma IL-6, IL-8, and TNF-α as well as elevated plasma levels of intestinal fatty acid binding protein (I-FABP), a marker of enterocyte loss, and loss of tight junctions(Grootjans et al., 2010). A case study of a human syngeneic small bowel transplant recipient (a situation that involves ischemia-reperfusion without the additional immune factors of donor vs. recipient) showed peaks in serum I-FABP and IL-6 at 60 minutes after reperfusion but returning to baseline levels by 270 minutes after reperfusion. In addition, IL-8 and IL-10 levels continuously rose for several days after surgery(Kadry et al., 2000).

Intestinal ischemia-reperfusion has also been shown to suppress transporter expression and function. Thirty minutes of induced intestinal ischemia in rats led to a significant reduction in p-gp and MRP2 mRNA expression in rat jejunum, six hours after reperfusion(Ogura et al., 2008). Similarly, another study found that 60 minutes of intestinal ischemia-reperfusion in the rat significantly decreased mRNA expression of p-gp in the ileum, as well as decreased p-gp function as measured by Rhodamine123 (a p-gp substrate) efflux(Tomita et al., 2008). Most studies have examined intestinal ischemia-reperfusion in the short term, and the actual duration of effects is unknown, although it may last weeks to months. A study in rats found continued impairment in intestinal morphology and function even after 90 days in rats that had undergone more than one 45-minute episode of intestinal ischemia-reperfusion, and the number of macrophages in the lamina propria remained significantly higher than in control rats, even after only a single 45-minute ischemia-reperfusion episode(Morini et al., 2010).

1.5   ANIMAL AND HUMAN STUDIES OF SMALL BOWEL TRANSPLANTATION

1.5.1   Vitamin deficiency after small bowel transplantation

The majority of small bowel transplant recipients achieve nutritional autonomy after transplant, with weaning of TPN on average by post-transplant day 22 in one study(O'Keefe et al., 2007). After TPN weaning, serum vitamin levels are monitored regularly and supplementation administered in the case of deficiencies. The most striking vitamin deficiency noted in adult small bowel transplant recipients is that of pyridoxyl-5'-phosphate (the active form of vitamin B6). Although the exact mechanism of the deficiency is unknown, it has been observed in up to 96 percent of small bowel transplant recipients in the first 30 days post-transplant(Matarese et al., 2009)   Deficiencies in zinc and red blood cell folate levels have been observed in pediatric intestinal transplant recipients at one year post-transplant(Rovera et al., 1998) and vitamin E deficiency has been reported in pediatric intestinal transplant recipients, especially in the first 160 days post-transplant(Kaufman et al., 2000).

1.5.2   Effects of small bowel transplantation on intestinal smooth muscle contractility

Intestinal smooth muscle contractility is impaired in animal models of allogeneic small bowel transplantation, although this effect is partially ameliorated by immunosuppression. In a rat model, rats treated with intramuscular tacrolimus or intraperitoneal sirolimus showed a

significant reduction in the number of apoptotic cells and leukocyte infiltration into the graft muscularis compared with untreated control animals at 24 hours and seven days post-transplant. In addition, smooth muscle strips from the graft in tacrolimus- and sirolimus-treated animals showed significantly better bethanechol-induced contractility as compared with untreated transplanted rats at 24 hours and seven days after surgery. However, none of the groups of transplanted rats, including those under immunosuppression, had muscle contractility as high as untransplanted rats, indicating that some contractility impairment exists despite immunosuppressive treatment(Fujishiro et al., 2010). Reductions in contractility may persist into the later post-transplant period. In a dog model of small bowel allotransplantation, dose-response curves of bethanechol-stimulated circular muscle contractility obtained between post-transplant days 104 and 205 showed no difference in contractile function between autotransplanted dogs and controls, but a significant decrease in contractility was still observed in allotransplanted dogs as compared with control dogs(Iwanami et al., 2003).

1.5.3   Gastric emptying and intestinal transit times after small bowel transplantation

As mentioned in a previous section, denervation of the stomach leads to impaired phase III MMC contractions of that organ, and likely to impairment of gastric emptying. This has been confirmed in human small bowel transplant recipients. In a study of 16 transplant recipients (17 allografts due to one retransplant), eight of whom were isolated intestine, six liver-intestine, and three multivisceral, contrast studies were performed using either diatrizoate or barium sulfate at 1-2 weeks and 1-3, 6, 12, and 24 months after transplant. Delays in gastric emptying (as measured by amount of liquid or food remaining in the stomach after overnight fasting as well as how much contrast material remained in the stomach after one hour as compared with five

minutes after administration) were noted in 76% of the contrast studies done within two months post-transplant. At four months post-transplant, delayed emptying persisted in 33% of patients and by six months it was only present in 16% (Campbell et al., 1993). Abnormally rapid gastric emptying may also be present in multivisceral transplant recipients, possibly due to the effects of pyloroplasty of the transplanted stomach. A study of gastric emptying times of radiolabeled liquids and solids in small bowel transplant recipients found that three out of four multivisceral patients had rapid gastric emptying of solids and in one out of the four it was delayed. By contrast, rapid emptying of liquid was observed in one multivisceral subject but in the rest it was normal. By contrast, two out of the five isolated intestinal recipients had delayed gastric emptying of solids and one out of five had delayed emptying of liquids. The rest were normal(Furukawa et al., 1994).

As also mentioned previously, extrinsic denervation of the small intestine leads to a shortened intestinal transit time in dogs, but the contractile activity of intestinal smooth muscle is decreased secondary to infiltration of inflammatory mediators into the muscularis after small bowel transplantation. In addition, severe acute rejection may lead to neuronal cell loss(Watanabe et al., 2008). Therefore, the motility effects of denervation and inflammation on the transplanted small bowel combine to varying degrees depending on the patient and the time post transplant. In the aforementioned contrast study, small intestinal transit times were found to be variable and sometimes rapid: using barium they ranged from 0.2 to 17.8 hours (median of 2 hours) and using diatrizoate they ranged from 0.25 to 7.75 hours (median of 0.5 hours). However, in this study intestinal transit times were not related to time post transplant, unlike gastric emptying(Campbell et al., 1993).

1.5.3.1 The MMC and postprandial motility after small bowel transplantation

Phase III of the MMC in the transplanted intestine may be dissociated from the native intestine or absent in small bowel transplant recipients. A study measured intestinal motility patterns via 8-channel catheters placed inside the lumen of the transplanted intestine in eight pediatric small bowel transplant recipients, ages 2 to 22 years old (2 isolated intestine, 5 liver intestine, and 1 multivisceral). Time since transplant ranged from 3 to 23 months, with a mean of 12.5 months. Intestinal motility was measured for three hours during a period of fasting and one hour after a feeding. Normal MMC patterns were seen in the transplanted small intestine in 5 of the 8 patients, although the phase III contractions were dissociated from the native remainder of the GI tract. Two of the remaining patients only produced normal phase III patterns after injection of 1 μg/kg octreotide acetate, and the remaining patient, who had only recently recovered from severe exfoliative rejection, did not produce any phase III contractions. After feeding, normal post-prandial motility increases were observed only in the multivisceral transplant recipient(Mousa et al., 1998).

1.5.4   Immune activity after small bowel transplantation

The small intestine is a highly immunogenic organ, containing a large number of organized lymphoid tissues, including Peyer's patches, mesenteric lymph nodes, and isolated lymphoid follicles. Immediately after transplantation, recipient T and B cells migrate rapidly into these lymphoid tissues and begin to proliferate, composing 50% of lymphocytes inside graft lymphoid tissues within 24 hours(Wang et al., 2006). A study of lymphocyte populations in drainage fluid from six isolated intestinal transplant recipients showed that on post-transplant days 1 to 2, recipient cells already made up slightly greater than 50% of the CD8+ lymphocyte population

draining from the graft. By day 4, recipient cells accounted for 90%, and by day 11, 99%, of lymphocytes in the drainage(Meier et al., 2010). During this time, immune activity and the risk of acute rejection are especially high.

1.5.5   Inflammatory mediator expression after small bowel transplantation

The increased trafficking of lymphocytes from the small intestine into the bloodstream via lymphatic channels leads to increases in pro-inflammatory cytokine concentrations in the systemic circulation as well as graft tissue. Increases in serum concentrations of IL-2, IL-6, and TNF-α have been observed in the first 40 days after small bowel transplantation and during some episodes of acute rejection(Kita et al., 1996a; Noguchi et al., 1992). Samples of intestine taken before and at various time points during the reperfusion phase during human small bowel transplantation show a 90.3-fold upregulation of IL-6 mRNA in the graft muscularis during the ischemic phase that rises to 371.8-fold during the first few hours of reperfusion(Tuler et al., 2002). A rat model of allogeneic intestinal transplantation under tacrolimus immunosuppression has shown increases in IL-6, IL-2, and IFN-γ mRNA transcripts in the graft muscularis, leading to impaired smooth muscle contractile function, up to seven days post-transplant(Fujishiro et al., 2010). It should also be noted that enterocytes are able to produce proinflammatory cytokines directly, including IL-1, IL-6, and TNF-α, at the epithelial level of the intestinal mucosa(Ogle et al., 1994; Pratt et al., 2000), although this has not been studied within a transplant context.

1.5.6 Effects of small bowel transplantation on CYP3A and p-gp expression and function

Numerous studies have shown the suppression of CYP3A and transporter expression and function by pro-inflammatory cytokines. These effects are mediated through inhibition of PXR, constitutive androstane receptor (CAR), and RXRα gene transcription, DNA binding, and CYP3A and transporter gene transcription(Assenat et al., 2004; Beigneux et al., 2002; Yang et al., 2010). In particular, IL-1β(Aitken and Morgan, 2007; Assenat et al., 2004; Sukhai et al., 2001), IL-6(Aitken and Morgan, 2007; Sukhai et al., 2001; Yang et al., 2010), TNF-α(Aitken and Morgan, 2007; Belliard et al., 2004), and IFN-γ(Aitken and Morgan, 2007) have shown significant inhibition of CYP3A and p-gp expression and function *in vitro*.

Because of the inflammation and associated cytokine release caused by ischemia-reperfusion injury and early immune activation, impairments in CYP3A and p-gp are expected to be present after small bowel transplantation, especially in the early post-transplant period. This effect has been demonstrated in a dog model of intestinal autotransplantation. Intestinal microsomal CYP3A4 activity (as measured by 6β-hydroxytestosterone formation) was significantly diminished 60 minutes after reperfusion as compared with microsomes taken from non-transplanted dogs as well as microsomes from tissue taken after organ preservation but before reperfusion. P-glycoprotein expression levels 60 minutes after reperfusion (as measured by Western blot) were slightly decreased compared with expression levels before reperfusion(Ishikawa et al., 2003). In addition, the AUC and Cmax of tacrolimus were significantly increased compared with control dogs at post-operative days 1 and 7, whereas there was no significant difference at days 14 and 28(Ishikawa et al., 2003), implying impaired

intestinal CYP3A and p-gp-mediated first-pass metabolism leading to increased bioavailability that returned to normal within the first month after transplant.

Since these effects occurred in an autotransplant model, they reflect the effects of surgical manipulation and ischemia-reperfusion injury without the component of cell-mediated immunity. If different, the effects after allogeneic small bowel transplantation would be expected to be stronger and of longer duration post-transplant, but this not been studied. However, several studies have shown that the pharmacokinetic parameters of tacrolimus are not significantly different from those seen in other transplant populations in clinically stable small bowel transplant patients two to 24 months post-transplant taken as a whole, although the effect of time post-transplant on recovery of intestinal CYP-mediated metabolism was not examined(Jain et al., 1994; Jain et al., 1992; Schubert et al., 2004).

1.6   HYPOTHESIS AND SPECIFIC AIMS OF THIS RESEARCH

Small bowel transplant recipients take numerous medications, many of them by mouth, and a significant number of them CYP3A and/or transporter substrates. Ischemia-reperfusion injury and immune activation within the transplanted organ lead to inflammatory mediator release both within the graft and into the systemic circulation. *In vitro* studies have shown that pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ decrease CYP3A and transporter expression and function via suppression of gene transcription. Clinical studies in other inflammatory conditions such as cancer and sepsis have shown the clinical correlation between inflammatory mediator expression and suppression of drug metabolism. That these effects of cytokines on drug-metabolizing enzymes and transporters have clinical relevance in a transplant

setting is further evidenced by animal studies of small bowel transplantation that have shown not only impaired enzyme and transporter expression and function, but also impairments of intestinal first pass metabolism of the widely-used immunosuppressive drug tacrolimus, in the first two weeks after transplant, returning to normal approximately one month after surgery. Whether or not these changes are seen in intestinal transplant patients and when the functional recovery occurs are key questions. As mentioned earlier, the pharmacokinetic parameters of tacrolimus, a CYP3A and p-gp substrate, in clinically stable small bowel transplant recipients several months to years after transplant are similar to those seen in other transplant populations, implying eventual recovery of intestinal CYP3A and p-gp function.

Based on these observations, the central hypothesis of this study is that intestinal CYP3A and transporter expression and function in small bowel transplant recipients will be suppressed in the early post-transplant period (estimated at approximately the first 30 to 40 days after transplant), but will be similar to that seen in healthy control subjects in stable patients without evidence of rejection in the later post-transplant period (by four to six months post-transplant). In order to examine the effects of the transplanted intestine without the confounding variable of the transplanted liver, only isolated intestinal and modified multivisceral transplant recipients were included in the study.

The study therefore has the following specific aims:

1. To measure intestinal CYP3A expression and function (chapter 5) in stable small bowel transplant recipients in the early post-transplant period and late post-transplant period in comparison to CYP3A activity in healthy control subjects.

a. This will be accomplished by characterizing the pharmacokinetics of oral and intravenous midazolam, administered as a probe drug for assessment of intestinal and hepatic CYP3A, within 40 days post-transplant and approximately four to 12 months post-transplant in transplant recipients as well as in healthy control subjects. In addition, in transplant subjects, mRNA transcripts of *CYP3A4*, *CYP3A5*, and *NR1I2 (PXR)* will be measured in ileal mucosal biopsy samples taken within 48 hours of each midazolam study and compared between time periods.

b. It is predicted that the oral AUC and bioavailability of midazolam in the small bowel transplant recipients in the early post-transplant period will be significantly higher, and the AUC ratio of 1'hydroxymidazolam to midazolam significantly lower, than that seen in age- and gender-matched healthy control subjects and also significantly different than the same subjects in the later post-transplant period. In the later post-transplant period it is predicted that the pharmacokinetic parameters of oral midazolam will be no different than those seen in healthy controls. In addition, expression levels of *CYP3A4* and *CYP3A5* will be significantly lower in the early post-transplant period as compared with the later.

2. To measure intestinal transporter expression and function (chapter 6) in stable small bowel transplant recipients in the early post-transplant

period and later post-transplant period in comparison to transporter activity in normal healthy control subjects.

a. This will be accomplished by the characterization of the pharmacokinetics of oral fexofenadine as a probe drug for transporter function assessment, within 40 days post-transplant and approximately four to 12 months post-transplant. In addition, in transplant subjects, mRNA transcripts of *ABCB1* (p-gp), *SLCO1A2* (OATP1A2), and *SLCO2B1* (OATP2B1) will be measured in ileal mucosal biopsy samples taken within 48 hours of each fexofenadine study and compared between time periods.

b. It is predicted that the oral AUC of fexofenadine in small bowel transplant recipients in the early post-transplant period will be significantly higher than that seen in age- and gender-matched healthy control subjects and also significantly different than the same subjects in the later post-transplant period. In the later post-transplant period, it is predicted that the pharmacokinetic parameters of fexofenadine will be no different from those seen in healthy controls. In addition, expression levels of the above-mentioned transporters will be significantly lower in the early post-transplant period as compared with the later.

3. To characterize the pattern and magnitude of inflammatory mediator expression in plasma in the early and later post-transplant periods as compared to that seen in healthy control subjects (chapter 4).

a. This will be accomplished by the measurement of plasma IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ, and TNF-α at both the early post-transplant study session and the later. Plasma concentration of each cytokine will be compared between transplant subjects at each study session as well as with the group of healthy control subjects. In addition, in transplant subjects, mRNA transcripts of *IL-1β, IL-2, IL-6, TNF-α,* and *IFN-γ* genes will be measured in ileal biopsy samples taken within 48 hours of each study session and compared between time periods.

4. To assess the correlation between plasma concentrations of inflammatory mediators and the expression and function of CYP3A and transporters (chapters 4, 5, and 6).

   a. This will be accomplished by correlation analysis between pharmacokinetic parameters of midazolam and fexofenadine and plasma cytokine concentrations, between plasma cytokine concentrations and ileal *CYP3A* and transporter gene expression, between ileal *CYP3A* expression and pharmacokinetic parameters of midazolam, and between ileal *ABCB1*, *SLCO1A2*, and *SLCO2B1* gene expression and pharmacokinetic parameters of fexofenadine.

5. To characterize the pharmacokinetics of oral tacrolimus, a CYP3A and p-gp substrate, in small bowel transplant recipients in the first 40 days

post-transplant as compared with the later post-transplant period (four to 12 months post-transplant) (chapter 7)

a. This will be accomplished by measurement of whole blood tacrolimus concentrations in blood samples taken at the time of the early and late post-transplant study sessions mentioned previously. Tacrolimus dose-adjusted AUC will be compared between the early and later post-transplant periods.

b. It is predicted that the dose-adjusted AUC of tacrolimus will be significantly higher in small bowel transplant recipients at the early post-transplant period than during the later period.

2.0   MATERIALS AND METHODS

This chapter outlines the materials and methods used for the studies described in this document.

2.1   STUDY DESIGN

The study protocol was approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from all subjects before any study procedures were performed.

2.1.1   Subject recruitment – transplant subjects

Transplant recipients were recruited prior to transplant, either in the outpatient transplant clinic or as inpatients during hospital admissions not related to transplant. Inclusion criteria for transplant recipients were age 18 to 65, listed for isolated small bowel or modified multivisceral transplant, and weight within 30 percent of ideal body weight. Candidates were excluded if they were scheduled to get any other organ transplanted other than small intestine, stomach, or pancreas. In addition, transplant candidates were excluded before transplant if they had a history of previous organ transplant, a creatinine clearance less than 30 mL/minute, hemoglobin less than 8.5 gm/dL, were a smoker, or had any known allergy to midazolam or fexofenadine. Transplanted subjects were not studied after transplant if they required supplemental oxygen,

were receiving a non-standard immunosuppression protocol, had an ostomy output of greater than 2500 mL per day, were taking any major CYP3A or p-gp inhibitors or inducers or had clinical or histological evidence of acute or chronic rejection.

2.1.2 Subject recruitment – control subjects

Age (±5 years)- and gender-matched healthy subjects were recruited as a control group for comparison with the transplant patients. Inclusion criteria for control subjects were age between 18 and 65 years, weight within 30 percent of ideal body weight, and normal renal and hepatic function (as measured by total bilirubin, AST, ALT, BUN, and serum creatinine within normal limits). Exclusion criteria for control subjects were smoking, history of bariatric surgery or intestinal resection, intestinal disease (i.e. Crohn's disease or ulcerative colitis), any other condition, such as diabetes mellitus, which might affect gastrointestinal motility, use of major CYP3A or p-gp inhibitors or inducers (oral contraceptives were allowed), and for females, pregnancy or nursing. Control subjects underwent a physical examination and ECG as part of the screening process. All female subjects of childbearing age were given a urine pregnancy test during the screening and on the day of the study session before any study procedures were performed.

2.1.3 Study procedure

Transplant subjects underwent two separate pharmacokinetic study sessions, one in the early post-transplant period while patients were still in the hospital after surgery (PK session 1) and one four to 14 months after transplant, but prior to ostomy closure (PK session 2).   Healthy

control subjects underwent one pharmacokinetic study session after a screening session. Subjects were required to abstain from all fruits and fruit juices for at least 72 hours before the start of the study session due to the well-documented effects of certain juices on fexofenadine pharmacokinetics. (A study in healthy volunteers found that consumption of grapefruit juice with an oral dose of fexofenadine or 2 hours before the dose reduced fexofenadine AUC_{0-8} by 52%, possibly via inhibition of OATP1A2-mediated uptake(Glaeser et al., 2007). Another study observed a 30 to 40% decrease in AUC and Cmax of oral fexofenadine after consumption of grapefruit, orange, and apple juice(Dresser et al., 2002), and a third noted a large and significant decrease in mean AUC in subjects administered apple juice with fexofenadine compared to those given the drug with water alone (1342 vs. 284 ng*hr/mL)(Imanaga et al., 2011).) In addition, study subjects were asked to avoid caffeine for 48 hours before the session, and required to fast after midnight on the day of the study session (water was allowed).

On the morning of the study session, two blood samples were taken immediately prior to study drug administration: one whole blood sample for genotyping purposes and one sample for time zero drug concentration measurement. Five mg (2.5 mL) of oral midazolam syrup (Roxane Laboratories, Columbus, OH) was administered enterally via an oral syringe. To make sure that no drug was sticking to the syringe, the syringe was refilled with water twice, which was given to the subject. The subject then drank approximately four ounces of water. Sixty minutes after administration of oral midazolam, a 60 mg tablet of fexofenadine (Teva Pharmaceuticals, North Wales, PA) was given to the subject with four ounces of water. Subjects were required to remain in bed with the head of the bed set to a 60-degree angle for six hours after oral midazolam administration. At the 7-hour timepoint, 2 mg of intravenous midazolam (Bedford Laboratories, Bedford, OH) in 120 mL of normal saline was given as an infusion over 30 minutes. Subjects

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were given a meal two hours after administration of oral midazolam, but were required to fast for 30 minutes before and one hour after administration of IV midazolam. Blood samples (3 mL) were drawn into EDTA tubes at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, and 7, 7.25, 7.5, 8, 10, 12, and 20 hours after oral administration of midazolam. Blood samples were spun down and plasma stored at -80 degrees Celsius until analysis.

Subjects were monitored by the principal investigator and the nursing staff during the study sessions. Subjects remained in bed for six hours after each administration of midazolam, with bathroom privileges with assistance after two hours. Blood pressure, pulse, and oxygen saturation were measured every 15 minutes for the first two hours after each administration of midazolam and additionally as deemed necessary. Supplemental oxygen was administered for an oxygen saturation less than 94%. A study physician was required to be notified if supplemental oxygen failed to improve saturation, if the oxygen saturation was below 90% at any time, in the event of any changes in blood pressure greater than 20 mm Hg from baseline, or they exhibited any other signs or symptoms of adverse effects of any kind.

2.1.4 Ileal biopsy sampling in transplant subjects

Small bowel transplant recipients undergo routine biopsies of intestinal mucosa. For this study, two extra tissue samples (in addition to the two or three taken for clinical purposes) were taken during the routine biopsy session closest to the study date. This was often done on the same day as the PK study session, but could be ± 2 days apart from the session. All biopsy samples were taken from 4 to 13 cm inside the proximal ileum. Samples were placed on dry ice immediately after removal from the subject, and stored at -80 degrees Celsius until analysis of mRNA expression of enzymes, transporters, and cytokines by RT-PCR.

2.2 MEASUREMENT OF MIDAZOLAM, 1'HYDROXYMIDAZOLAM, AND FEXOFENADINE IN PLASMA BY LC-MS

2.2.1 Materials

Midazolam, 1'hydroxymidazolam, midazolam-D5 (internal standard), and fexofenadine-D6 (internal standard) were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Fexofenadine was purchased from the United States Pharmacopeia (Rockville, MD). HPLC-grade methanol and water were purchased from Fisher Scientific (Pittsburgh, PA).

2.2.2 Sample preparation

Frozen plasma samples were thawed at room temperature and vortexed for 30 seconds. Each aliquot of 0.4 mL of plasma was mixed with 0.6 mL of HPLC-grade water (Fisher Scientific, Pittsburgh, PA), 50 μL of five percent ammonium hydroxide, and 25 μL of midazolam-d5 (2 μg/mL, internal standard). After vortexing for 30 seconds, solid phase extraction (SPE) was performed using Oasis® HLB extraction cartridges (Waters Corporation, Milford, MA) that had been conditioned with one mL of HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA) followed by one mL of HPLC-grade water. Samples were loaded onto cartridges and the plasma mixture allowed to filter through. After filtration, each cartridge was washed with one mL of

water. Samples were eluted with one mL of methanol and dried under air. The resulting residue was reconstituted with 150 μL of mobile phase mixture (described below).

2.2.3   Sample analysis

Plasma samples were analyzed by LC-MS, using a system consisting of a Waters model 2795 separations module (Waters Corporation, Milford, MA), a Waters Micromass Quattro Micro triple quadrupole mass spectrometer (Waters Corporation, Milford, MA), and MassLynx 4.1 software (Waters Corporation, Milford, MA). HPLC conditions included a Symmetry C18 (3.5 μm 2.1 x 50 mm) column with guard (Waters Corporation, Milford, MA) and mobile phase components consisting of mixture A: 2 mM ammonium acetate, 0.1 percent formic acid, and 5 percent methanol in water and B: 2 mM ammonium acetate and 0.1 percent formic acid in 100 percent methanol running at a flow rate of 0.3 mL/minute. A gradient procedure was used for the mobile phase mixture proportions throughout the ten-minute run time. The ratios of A:B were as follows: 0-1.5 minutes, 70:30; 1.5-1.55 minutes, 50:50, 1.55-3.1minutes, 10:90; 3.1-10 minutes, 70:30.

Mass spectrometry parameters for the assay were as follows: Capillary voltage, 0.8 kV; source temperature, 100°C; desolvation temperature, 500°C; desolvation gas flow, 550 L/hr; cone gas flow, 50 L/hr; argon pressure, 20 ± 10 psig; nitrogen pressure, 100 ± psig. Multiple reaction monitoring parameters and conditions for midazolam were m/z of 326>295.97, for 1'hydroxymidazolam 342>323.9, and for midazolam-d5 331.09>295.97. Multiple reaction monitoring parameters and conditions for fexofenadine were m/z of 502.25>466.12 and for fexofenadine-d6 508.17>472.23.

Standard curves were generated, using the ratio of drug peak height to that of internal standard, with every batch analyzed using seven concentrations of each analyte and were accepted if the corresponding r^2 value was 0.990 or greater. The standard curve was linear between 0.5 and 75 ng/mL for midazolam, 0.25 to 50 ng/mL for 1'hydroxymidazolam, and 1 and 225 ng/mL for fexofenadine. Three quality control (QC) samples were also run with each batch. %CVs were less than 20% for the low range, and less than 15% for the medium and high ranges.

2.3   MEASUREMENT OF TACROLIMUS IN WHOLE BLOOD BY LC-MS

2.3.1   Sample preparation

Whole blood samples were gently mixed by placing on a Thermolyne speci-mix rocker (Sybron, Milwaukee, WI). After mixing, 50 μL of each sample was placed into a microcentrifuge tube with 0.2 mL of 0.1M zinc sulfate heptahydrate solution (Fisher Scientific, Pittsburgh, PA) and 0.5 mL of 1.75 ng/mL ascomycin internal standard (Sigma-Aldrich, St. Louis, MO). Tubes were vortexed on speed 10 for 60 seconds using a multi-tube vortexer (VWR, Radnor, PA), then centrifuged. The resulting supernatant was placed into a vial and loaded into the mass spectrometer unit.

2.3.2   Sample analysis

Tacrolimus concentrations were measured in whole blood by LC-MS, using a system consisting of a model 2795 separations module (Waters Corporation, Milford, MA), a Micromass Quattro

Micro API mass spectrometer (Waters Corporation), and MassLynx 4.1 software (Waters Corporation). HPLC conditions included a Nova-Pak® C18 2.1 x 10 mm column (Waters Corporation) and mobile phase components consisting of equal amounts of mixture A: 1L HPLC-grade water to which 2 mL of 1M ammonium acid (Fisher Scientific, Pittsburgh, PA) and 1 mL of formic acid (Fisher Scientific) were added and mixture B: 1L HPLC-grade methanol to which 2 mL of 1M ammonium acid (Fisher Scientific, Pittsburgh, PA) and 1 mL of formic acid (Fisher Scientific) were added, running at a flow rate of 0.6 mL/minute.

Mass spectrometry parameters for the assay were as follows: Capillary voltage, 1.00 kV; cone voltage, 30.00 V; source temperature, 140°C; desolvation temperature, 350°C; desolvation gas flow, 600 L/hr. Mass to charge ratio of tacrolimus was 821.4→768.3 and of ascomycin 809.5→756.3. Standard curves consisting of six standard concentrations were calculated using the ratio of drug peak height to that of internal standard. Curves were accepted if the r^2 was 0.990 or greater. The standard curve was linear within the range of 2.0 to 40.0 ng/mL. Samples with concentrations above 40.0 ng/mL were diluted and re-analyzed. Three whole blood quality control (QC) samples (Tri-level immunosuppressants, UTAK Laboratories, Valencia, CA) were run with each batch of patient samples. Low QC was 4 ng/mL, medium QC was 15 ng/mL, and high QC was 25 ng/mL. QC readings had to be within ±15% of the verified value given by the manufacturer for each batch in order for the results to be accepted. %CVs were 9.3% in the low range, 9.9% in the medium range, and 7.5% in the high range.

2.4   ANALYSIS OF CYP3A4/5 AND TRANSPORTER EXPRESSION IN ILEAL BIOPSY SAMPLES

2.4.1   Materials

DEPC water, chloroform, isopropyl alcohol, and 75% ethanol were purchased from Sigma-Aldrich (St. Louis, MO). Trizol® reagent was purchased from Invitrogen (Carlsbad, CA). RQ1 DNAse, RQ1 DNase buffer, RQ1 DNase stop solution, random primers, M-MLV reverse transcriptase, M-MLV RT buffer, dNTPs, and RNase inhibitor (rRNasin®) were all purchased from Promega (Madison, WI).   TaqMan® gene expression assays for *CYP3A4* (Hs00604506_m1), *CYP3A5* (Hs00241417_m1), *ABCB1* (Hs00184500_m1), *MRP2* (Hs00166123_m1), *BCRP* (ABCG2) (Hs00184979_m1), *PXR (NR1I2) (*(Hs00243666_m1), *SLCO1A2* (Hs00245360_m1), *SLCO2A1* (Hs00194554_m1), *SLCO2B1* (Hs00200670_m1), *IL-1β* (Hs00174097_m1), *IL-2* (Hs99999150_m1), *IL-6* (Hs00985639_m1), *TNF-α* (Hs00174128_m1), and *IFN-γ* (Hs00989291_m1) were purchased from Applied Biosystems (Foster City, CA). TaqMan® Universal PCR MasterMix was also purchased from Applied Biosystems.

2.4.2   RNA extraction

Frozen biopsy samples were thawed and placed in polypropylene tubes. To each sample (weighing 25-50 mg), 0.5 mL of Trizol® reagent was added and the tissue crushed and stirred with a pestle. Samples were incubated for five minutes at room temperature, then 0.1 mL chloroform was added. Sample tubes were then shaken vigorously for 15 seconds, incubated at

room temperature for two to three minutes, and centrifuged at 12,000 x g for 15 minutes at 4 degrees Celsius. The resulting top layer (aqueous phase) in each tube was transferred to a fresh tube and 0.25 mL of isopropyl alcohol added. Tubes were then incubated at room temperature for ten minutes, and centrifuged at 12,000 x g for ten minutes at 4 degrees Celsius. At this point, a small white pellet of RNA could be seen at the bottom of each tube. Supernatant was removed, and pellets were washed with approximately 0.5 mL of 75 percent ethanol. After removal of the ethanol, pellets were air dried, then reconstituted with 20 μL of DEPC water.

2.4.3   cDNA synthesis

After reconstitution to 5 μL with DEPC water according to concentrations obtained from OD measurement, each RNA sample was used to create a cDNA sample according to the following procedure. A 5 μL mixture of 1μL DNase buffer, 2 μL DNase I and 2 μL DEPC water was added to each 5 μL RNA sample. Samples were then incubated at 37° C for 30 minutes in a thermal cycler (PCR Express, ThermoHybaid, Pittsburgh, PA). Samples were then placed on ice and 1 μL stop solution was added to each and samples reincubated at 65°C for 10 minutes. Afterwards, samples were removed and placed on ice, and 1 μL of random primers was added to each and samples placed in the thermal cycler at 70°C for 10 minutes. Samples were then removed and 13 μL of a mixture containing 5 μL reverse transcriptase buffer, 1 μL reverse transcriptase, 1.25 μL dNTPs, 0.65 μL RNase inhibitor, and 5.1 μL DEPC water was added to each. Samples were then incubated at 37°C for 60 minutes followed immediately by 70°C for 10 minutes. After removal from the thermal cycler, 200 μL of DEPC water was added to each cDNA sample and samples were frozen at -20°C until use.

2.4.4   RT-PCR procedure

In each well of a 96-well plate, 5 µL of cDNA solution was combined with 12.5 µL of master mix, 6.25 µL DEPC water, and 1.25 µL of the relevant gene expression assay. All samples were assayed in triplicate. After preparation, plates were covered and centrifuged at 600 rpm for 10 seconds to remove droplets from the sides of wells and any air bubbles. Cyclophilin A, a housekeeping gene, was used as the reference gene.

Plates were placed in an ABI PRISM® 7000 sequence detection system (Applied Biosystems). Corresponding 7000 system sequence detection software version 1.2.3 (Applied Biosystems) was used for sample analysis. Thermal cycler settings consisted of three stages as follows: Stage 1, 50°C for 2 minutes (one rep); Stage 2, 95°C for 10 minutes (one rep); Stage 3, 95°C for 15 seconds followed by 60°C for 60 seconds (45 reps). Gene expression levels are given relative to cyclophilin. Integrity of PCR products was verified by gel electrophoresis.

2.5   ANALYSIS OF PLASMA CYTOKINE CONCENTRATIONS

2.5.1   Materials

Interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were measured in plasma using a MILLIPLEX® MAP High Sensitivity Human Cytokine Multiplex Panel kit (#HSCYTO-60SK, Millipore, Billerica, MA). Reagents included in the kit were a high sensitivity human cytokine standard, high sensitivity

human cytokine quality controls 1 and 2, serum matrix containing 0.08% sodium azide, 96-well filter plate with sealers, 10X wash buffer containing 0.05% Proclin, high sensitivity human cytokine detection antibodies, and streptavidin-phycoerythrin (reporter molecule) solution.

2.5.2   Assay method

Frozen plasma samples were thawed, mixed, and centrifuged to remove particulate matter. Antibody beads were mixed and quality controls and serum matrix were reconstituted according to manufacturers directions.   The manufacturer-supplied standard was reconstituted to a concentration of 2,000 pg/mL, then serially diluted to make standards of 400, 80, 16, 3.2, 0.64, and 0.13 pg/mL. The filter plate was prewetted by the addition of 200 μL of 1X wash buffer to each well. After the plate was shaken for ten minutes, the wash buffer was removed from the wells. After sonicating the bead bottle for 30 seconds and vortexing for one minute, 25 μL of premixed beads were added to each well. After removal of excess liquid from wells, 50 μL of each of the standards and quality controls 1 and 2 were added to the designated wells along with 50 μL of serum matrix. Subsequently, 50 μL of assay buffer was added to each of the sample wells, followed by 50 μL of sample. Filled plates were sealed and incubated overnight on a plate shaker at 4°C.

The next day, fluid was removed from each well by vacuum and wells were each washed twice with 200 μL of wash buffer. Fifty μL of detection antibodies were added to each well and the plates resealed and incubated at room temperature for one hour on a plate shaker. After incubation, 50 μL of streptavidin-phycoerythrin (reporter molecule) was added to each well and the plates sealed and reincubated on a plate shaker for 30 minutes at room temperature. After

removal of liquid by vacuum, each well was washed twice with 200 µL of wash buffer. One hundred µL of sheath fluid was added to each well in which the beads were resuspended on a plate shaker for five minutes.

The plates were read on a Luminex® 100™ analyzer (Luminex Corporation, Austin, TX). Fluorescent intensity (FI) output was analyzed using Bio-Plex Manager™ software (Bio-Rad Laboratories, Hercules, CA). A minimum of five points were used to generate each standard curve. Points were excluded from the curve if the (observed concentration/expected concentration)*100 was less than 70 or greater than 130. Two plates were run, with the within-range standard curve ranges for each plate as follows:

IL-1β: Plate 1: 0.13 - 359.86 pg/mL Plate 2: 0.79 - 334.65 pg/mL

IL-2: Plate 1: 0.13 – 2122.24 pg/mL Plate 2: 0.77 – 2395.94 pg/mL

IL-4: Plate 1: 3.72 – 2002.91 pg/mL Plate 2: 3.02 – 2000.03 pg/mL

IL-6: Plate 1: 0.14 – 2156.73 pg/mL Plate 2: 0.79 – 343.75 pg/mL

IL-8: Plate 1: 0.13 – 1721.55 pg/mL Plate 2: 0.13 – 2308.77 pg/mL

IL-10: Plate 1: 3.25 – 2019.26 pg/mL Plate 2: 3.08 – 2018.59 pg/mL

IL-12: Plate 1: 0.72 – 2034.93 pg/mL Plate 2: 0.83 – 2050.18 pg/mL

TNF-α: Plate 1: 0.13 – 2286. 95 pg/mL Plate 2: 0.14 – 366.14 pg/mL

IFN-γ: Plate 1: 0.16 – 2010.6 pg/mL Plate 2: 4.15 – 2027.17 pg/mL

2.6   PHARMACOKINETIC DATA ANALYSIS

Pharmacokinetic parameters of midazolam and fexofenadine were calculated by noncompartmental analysis using WinNonLin software, version 6.1 (Pharsight Corporation, St.

Louis, MO). At least three points were used to calculate the terminal disposition rate constant (λ_z) by log regression using uniform weighting. The bioavailability (F) of midazolam was calculated using the formula F = (AUC$_{po}$ × DOSE$_{IV}$)/(AUC$_{IV}$ × DOSE$_{po}$). Areas under the curve (AUCs) were calculated using the linear trapezoidal method, and Tmax and Cmax were obtained from visual inspection of the data. AUC of midazolam after IV administration (AUC$_{IV}$) was calculated by calculating the AUC from the start of the intravenous infusion to infinity, then subtracting the remaining AUC from the oral dose (the last concentration before administration of the IV divided by the slope of the intravenous elimination curve (C7/λ_z)). Clearance (CL), apparent oral clearance (CL/F), volume of distribution (Vz), and apparent volume of distribution (Vz/F) were normalized to subject weight in kg.

2.7 STATISTICAL ANALYSIS

2.7.1 Sample size calculation

The required sample size for the study was calculated based on a main outcome measure, the oral AUC of midazolam (a measure of systemic exposure to the drug and hence CYP3A activity). Calculation of the necessary sample size to have a power of 0.80 of observing a 50% difference in the oral AUC of midazolam between transplant subjects and controls using a two-sided test with a significance level of α=0.05 was performed using the following equation(Rosner, 2006):

$$n = \frac{\left(\sigma_1^2 + \sigma_2^2\right)\left(z_{1-\alpha/2} + z_{1-\beta}\right)}{\Delta^2}$$

Estimates of the likely mean of the AUC of 5 mg oral midazolam in healthy volunteers was taken from Lee et al. (Lee et al., 2002) and was 4124 ng*min/mL with a standard deviation of 2018 ng*min/mL. No midazolam data from small bowel transplant populations is available in the literature, so mean oral AUC in transplant subjects was predicted to be 50% higher (6186 ng*min/mL). The calculated sample size needed to fulfill the above requirements was 15 subjects in each group.

2.7.2 Comparison of pharmacokinetic parameters and plasma cytokine concentrations between subject groups

Since the pharmacokinetic parameters of midazolam, fexofenadine, and tacrolimus as well as plasma cytokine concentrations were not normally distributed, they were compared between transplant groups using the Wilcoxon matched-pairs signed rank test. Transplant session one parameters were compared with controls and transplant session two with controls using the Mann-Whitney U test, as were pharmacokinetic parameters between enteral routes of administration and transplant subtype. A two-tailed p value of less than 0.05 was considered statistically significant for these comparisons.

Plasma concentrations of several cytokines were below the lower limit of detection of the assay. These included:

- 4 Tx1 samples below the standard curve range for IL-1β
- 3 Tx1 samples below the standard curve range for IL-2
- 5 Tx1 samples below the standard curve range for IL-4
- 5 Tx1 samples below the standard curve range for IL-12
- 2 Tx1 samples below the standard curve range for IFN-γ

- 2 Tx2 samples below the standard curve range for IL-4

- 1 Tx2 sample below the standard curve range for IL-12

- 3 control samples below the standard curve range for IL-12

These samples were assigned a concentration of 0.13 pg/mL, the concentration of the lowest point on the standard curve of each cytokine's assay. Because this point fell out of range ((observed concentration/expected concentration)*100 was less than 70 or greater than 130) in 8 out of the 18 standard curves generated, 0.13 pg/mL was chosen rather than the LLOD/2, since the actual concentration could be above or below 0.13 pg/mL depending on the direction of the %CV.

2.7.3 Correlation analysis

Individual correlations between pharmacokinetic parameters of study drugs, *CYP3A* and transporter expression, and plasma cytokine concentrations were analyzed by Spearman rank correlations. A two-tailed p-value less than 0.10 was considered statistically significant. Separate correlations were performed for study sessions 1 and 2. In addition, within-subject correlation was calculated for the 10 subjects who completed both study sessions, based on a method proposed by Bland and Altman (Bland and Altman, 1995). Since there were two observations per subject, correlation analysis was performed on the difference in values between study sessions 1 and 2.

3.0   SUBJECT DEMOGRAPHICS

Sixteen small bowel transplant recipients (12 isolated small bowel, 4 modified multivisceral) underwent study session 1 between post-transplant day 10 and 40 (median day 19) and ten were studied a second time between post-transplant day 125 and 428 (median day 239). Out of the sixteen transplant subjects who underwent study session 1, six were men and ten were women. Of these, five men and five women returned for the second study session.   Sixteen healthy age- and gender-matched control subjects (six men and ten women) were also studied.   There was no significant difference in ages or weights between the transplant subjects at session one and the control subjects, nor any significant difference in weight between transplant subjects at session two and control subjects (see Table 2). However, the ten transplant subjects who underwent the second study session had significantly higher body weight than at the first study session (median weight 62.8 vs. 72.8 kg, p=0.0059). This was due to the fact that most of the transplant subjects were somewhat underweight prior to transplant secondary to intestinal failure and had significant improvements in nutritional autonomy afterwards, resulting in weight gain.   Total bilirubin, ALT, and AST were within normal limits in all subjects on the days of study sessions, although all transplant subjects showed some degree of hepatic disease on pre-transplant liver biopsy. Estimates of steatosis ranged from less than 5% to 60% of the lobule biopsied, which may negatively affect hepatic CYP3A protein expression and function(Fisher et al., 2009).   All subjects had some degree of fibrosis, ranging from "minimal" or "early" to severe (3-4/4) with

bridging. Three out of the sixteen subjects showed significant cholestasis. Median total bilirubin at the time of transplant was 2.3 mg/dL (25th percentile: 1.55 mg/dL, 75th percentile: 3.35 mg/dL) but typically decreased to within normal limits (less than 1.5 mg/dL) in the first week after transplant.   Bilirubin levels were within normal limits in all subjects on the day of pharmacokinetic study.

Creatinine clearance (CrCL) was calculated for all subjects using the Modification of Diet in Renal Disease (MDRD) formula of CrCL (in mL/min/1.73m^2) = 186.3 x SCr$^{-1.154}$ x (Age in years)$^{-0.203}$ x 1.212 (if African-American) x 0.742 (if female). There was no difference in CrCL between transplant session 1 and control subjects (91.5 vs. 99.0 mL/min/1.73m^2, p=0.98). However, CrCL was significantly lower at the second study session as compared with the first (55.5 vs. 94.0 mL/min/1.73m^2, p=0.0098) and with controls (55.5 vs. 99.0 mL/min/1.73m^2, p=0.0054). This may have been due to the nephrotoxic effects in some subjects of several months to a year of tacrolimus therapy, and the nephrotoxic potential of certain other drugs that may have been used such as aminoglycosides and vancomycin.

All of the transplant recipients were Caucasian, as were 12 out of the 16 donors. Of the remaining donors, three were African-American and one was categorized as Caucasian/Asian. Of the sixteen control subjects, fourteen were Caucasian and two were African-American. Four of the transplant subjects were receiving medications via jejunostomy tube (J-tube) at the time of the first study session; In those subjects, the midazolam syrup was administered directly into the J-tube followed by two oral syringe rinses of 2.5 mL and one chaser of four ounces of water. The fexofenadine tablet was crushed to a powder and mixed with four ounces of water before administration via the tube. After drug administration the tube was flushed twice with four ounces of water.

Table 2. Subject demographics

	Transplant Subjects Session 1 (n=16)	Transplant Subjects Session 2 (n=10)	Control Subjects (n=16)	P value* Tx1 vs. Tx2	P value§ Tx1 vs. C	P value§ Tx2 vs. C
Males/Females	6/10	5/5	6/10	n/a	n/a	n/a
Age (yr)	45.5 (27.3,55.5)	48.0 (27.3,55.5)	40.5 (22.5,53.5)	n/a	0.36	0.33
Body weight (kg)	63.0 (52.6,81.0)	72.8 (66.3,94.5)	68.0 (53.8,80.2)	**0.0059**	0.69	0.18
Post-tx day studied	19.0 (13,23)	213 (178,256)	n/a	n/a	n/a	n/a
Race (African-American/Caucasian)	Recipients: 0/16 Donors: 3/12[a]		2/14	n/a	n/a	n/a
Creatinine clearance (mL/min/1.73m2)[b]	91.5 (79.5, 136.5)	55.5 (46.8, 62.5)	99.0 (79.5, 121)	**0.0098**	0.98	**0.0054**

Age, body weight, post-tx day, and creatinine clearance are expressed as median (25th, 75th percentile)

*Comparisons between transplant sessions 1 and 2 made using the Wilcoxon matched-pairs signed rank test on ten matched pairs. §Comparisons between transplant session 1 and controls and transplant session 2 and controls made using the Mann-Whitney U test. Two-tailed p values reported. [a]One donor was categorized as Caucasian/Asian. [b]Creatinine clearance was calculated using the MDRD formula: $186.3 \times SCr^{-1.154} \times (\text{Age in years})^{-0.203} \times 1.212$ (if African-American) $\times 0.742$ (if female).

Median cold ischemia time of the transplanted organs was 6.5 hours (6.1, 7.5). Primary reasons for intestinal failure in the transplant subjects included SMA and/or SMV thrombosis (n=4), desmoid tumors (n=3), Crohn's disease (n=3), complications of gastric bypass (n=2), trauma due to motor vehicle accident (n=1), radiation enteritis (n=2), and intestinal pseudo-obstruction (n=1). Fifteen out of the 16 transplant subjects had graft drainage via the superior mesenteric vein connected to the native hepatic portal venous system. One of the isolated intestinal transplant patients had the superior mesenteric vein of the graft connected to the inferior vena cava, bypassing the hepatic portal system. All of the four modified multivisceral transplant recipients had undergone pyloroplasty (surgical widening of the pylorus of the

transplanted stomach) during the transplant surgery to facilitate gastric emptying. One modified multivisceral transplant recipient had preservation of the native spleen, pancreas, and a small portion of duodenum with a side-to-side anastomosis to the transplanted duodenum. Two of the remaining three had preservation of the native spleen without the pancreaticoduodenal complex, and one had no preservation of spleen, pancreas, or native duodenum. These three subjects had T-tubes placed during transplant surgery to connect the common bile duct.

All of the transplant subjects had ileostomies at the time of both study sessions. Five out of the sixteen transplant subjects had a permanent end ileostomy after transplant, with the result that small intestinal contents could only exit the body via the ostomy bag. The remaining 11 had chimney (n=8) or loop (n=3) ileostomies placed with an additional anastomosis with remaining colon and/or rectum. Of these, five subjects had ileotransverse anastomoses, two had ileosigmoid anastomoses, three had ileorectal anastomoses, and one had a colocolonic anastomosis. In the three subjects with ileorectal anastomoses, intestinal contents could exit the body either via the ileostomy or via the rectum with no colon involved. However, in the remaining eight subjects with the intestinal graft connected to some portion of colon, intestinal contents could exit via the ileostomy or travel through the remaining colon.

There were no unanticipated adverse events during the study. One transplant subject required temporary supplemental oxygen for an oxygen saturation below 94%, and in two cases (1 transplant subject and 1 control subject) administration of IV midazolam was delayed by approximately one hour due to temporary decreases in blood pressure requiring consultation with study physicians. In one transplant subject, the dose of IV midazolam was reduced to 1 mg after physician consult due to concerns about sustained blood pressure decreases since the patient had

just taken a dose of metoprolol. In one control subject, the dose of IV midazolam was not given because of sustained decreases in blood pressure after oral midazolam.

3.1.1   Other medications taken by transplant subjects

None of the study subjects, controls or transplant, were taking moderate or strong CYP3A or p-gp inhibitors or inducers at the time of study sessions. However, some of the transplant subjects required concurrent therapy with medications that may have weak CYP3A inhibitory or induction effects. Small bowel transplant recipients require acid suppression therapy after transplant to prevent ulcers and bleeds in the delicate newly transplanted graft. All transplant subjects except one were taking proton pump inhibitors (PPIs) during the study sessions. Lansoprazole, pantoprazole, or omeprazole were given, typically once per day. All of the PPIs are known to be weak inhibitors of CYP3A4 *in vitro* to varying degrees. Ki values of 42 μM for omeprazole and 22 μM for pantoprazole with regards to 1'hydroxymidazolam formation have been seen in *in vitro*. Lansoprazole has been shown to have extremely weak (practically negligible) CYP3A4 inhibitory activity *in vitro* (IC_{50} of >200 μM)(Li et al., 2004). These Ki values for the PPIs are significantly higher than, for example, the Ki of 0.0052 μM for the prototypical CYP3A4 inhibitor ketoconazole.   Considering that typical Cmax values of 20 mg omeprazole at steady state are 1.6 μM, for 40 mg pantoprazole 5.4 μM, and 30 mg lansoprazole 2.2 μM(Li et al., 2004), the risk of interactions *in vivo* between these PPIs and the CYP3A substrates midazolam and tacrolimus is low.

In particular, the risk of interaction with omeprazole appears low. Another *in vitro* study calculated a Ki of 367.5 μM for omeprazole inhibition of nifedipine oxidation, another marker of CYP3A4 function(Furuta et al., 2001). In addition, Katsakiori et al. found no difference in

tacrolimus pharmacokinetics in renal transplant patients during omeprazole treatment versus without omeprazole(Katsakiori et al., 2010). It has been suggested that any interaction between tacrolimus and lansoprazole is mediated via p-glycoprotein rather than CYP3A4, since lansoprazole is a p-gp substrate(Miura et al., 2007a). Therefore, given the low risk of interactions between PPIs and study drugs, the clinical need for PPI therapy in the transplant recipients, and the risk of gastric acid rebound if the drugs were discontinued, transplant subjects remained on PPI therapy during the study period.

All of the 16 transplant subjects required corticosteroid treatment at some point either during or after transplant, either for adrenal insufficiency or rejection treatment. Most of the corticosteroids now on the market are inducers of CYP3A to some degree, but level of induction depends on the steroid, with the most potent inducer being dexamethasone, which was not used in any of the subjects(El-Sankary et al., 2000; Pascussi et al., 2000). Methylprednisolone has been shown to have no significant effect on CYP3A *in vitro* or *in vivo*(El-Sankary et al., 2000; Pichard et al., 1992; Villikka et al., 2001) but may be an inducer of p-glycoprotein at very high doses(Konishi et al., 2004). Prednisolone is also been shown not to significantly induce CYP3A(Pichard et al., 1992), but hydrocortisone and prednisone have induced CYP3A in *in vitro* systems via regulation of PXR, CAR, and the glucocorticoid receptor(El-Sankary et al., 2000; Pascussi et al., 2000; Pichard et al., 1992). A clinical study in kidney transplant recipients found that daily tacrolimus dose requirements were significantly higher, and concentration/dose ratios significantly lower, in patients taking 0.25 mg/kg/day or greater of oral prednisone as compared with those taking less than 0.15 mg/kg/day(Anglicheau et al., 2003).

Steroid doses could not be withheld in the transplant subjects without significant patient discomfort and risk, and drug choice and doses were individualized based on steroid use prior to

transplant, degree of adrenal insufficiency, and risk of rejection. All subjects received a bolus of 1 to 3 mg of methylprednisolone intravenously for induction immediately before or during surgery, then an IV methylprednisolone taper after surgery in which the drug was tapered from 60 mg IV four times a day to 20 mg IV every 12 hours. At the time of study session 1, transplant subjects were receiving methylprednisolone IV, hydrocortisone IV, hydrocortisone by mouth, or prednisone by mouth. In addition, single bolus doses of 1000 or 2000 mg of methylprednisolone were administered to some transplant subjects during episodes of suspected immune activation or risk of rejection. All of these bolus doses occurred at least three days prior to the study session (2 subjects) and most were many days before (see table 4). At the time of study session 2, all transplant subjects were receiving either oral hydrocortisone or prednisone. Steroid and PPI doses in transplant subjects on study session days are listed in table 3.

Table 3. Proton pump inhibitor and corticosteroid doses in transplant subjects at both study sessions

Transplant Subject	PPI dose		Steroid dose	
	Session 1	Session 2	Session 1	Session 2
1	Lans 30 mg JT qd	n/a	HC 50 mg IV q8h	n/a
2	Lans 30 mg JT qd	Pant 40 mg po qd	MP 20 mg IV q12h	HC 15 mg po q8h
3	Lans 30 mg JT qd	n/a	MP 20 mg IV q12h	n/a
4	Lans 30 mg JT qd	Pant 40 mg po qd	HC 50 mg IV q8h	HC 20 mg po qAM/ 10 mg po qPM
5	none	none	PD 20 mg po BID	PD 5 mg po BID
6	Pant 40 mg IV qd	Pant 40 mg po BID	MP 20 mg IV q12h	HC 25 mg po q8h
7	Pant 40 mg po qd	Pant 40 mg po qd	HC 40 mg IV q8h	HC 20 mg po qAM/ 10 mg po qPM
8	none	Omep 20 mg po qd	MP 20 mg IV q8h	HC 15 mg po q8h
9	Omep 20 mg po qd	none	HC 50 mg IV q8h	HC 15 mg po q8h
10	Lans 30 mg po qd	n/a	MP 20 mg IV q8h	n/a
11	Lans 30 mg po qd	n/a	HC 50 mg IV q8h	n/a
12	none	Pant 40 mg po BID	MP 20 mg IV q12h	PD 5 mg po BID
13	Omep 20 mg po qd	Omep 20 mg po qd	HC 30 mg IV q8h	HC 15 mg po q8h
14	Lans 30 mg po qd	n/a	HC 50 mg IV q8h	n/a
15	Omep 20 mg po qd	Omep 40 mg po qd	HC 50 mg IV q8h	HC 30 mg po q8h
16	Omep 20 mg po qd	n/a	HC 50 mg po q8h	n/a

Lans=lansoprazole, Pant=pantoprazole, Omep=omeprazole

MP=methylprednisolone sodium succinate (Solu-Medrol®)

HC=hydrocortisone sodium succinate (Solu-Cortef® IV) or hydrocortisone tablets (po)

PD=prednisone tablets

Table 4. High-dose corticosteroid boluses in transplant subjects prior to study session 1

Transplant Subject	Steroid Bolus	Days before study session 1
1	none	n/a
2	MP 1 gm IV x 1	10
3	MP 1 gm IV x 1	11
4	MP 1 gm IV x 1	10
5	MP 1 gm IV x 1	18
	MP 2 gm IV x 1	12
6	MP 1 gm IV x 1	12
7	MP 1 gm IV x 1	34
	MP 2 gm IV x 1	23
	MP 2 gm IV x 1	19
8	MP 1 gm IV x 1	20
	MP 2 gm IV x 1	6
	MP 1 gm IV x 1	3
9	MP 2 gm IV x 1	10
10	MP 1 gm IV x 1	16
	MP 0.5 gm IV x 1	15
11	MP 2 gm IV x 1	12
12	MP 1 gm IV x 1	18
	MP 2 gm IV x 1	13
	MP 1 gm IV x 1	6
13	MP 1 gm IV x 1	26
14	MP 1 gm IV x 1	19
15	MP 1 gm IV x 1	18
	MP 2 gm IV x 1	3
16	MP 1 gm IV x 1	21

MP=methylprednisolone sodium succinate (Solu-Medrol®)

Transplant subjects also received 300 mg oral ursodiol (ursodeoxycholic acid) three times a day at session 1 to encourage bile flow. Ursodiol has been shown to weakly induce CYP3A *in vitro* by induction of PXR(Becquemont et al., 2006). A study in rats found that although the AUC and Cmax of midazolam were significantly increased by a single dose of 300 mg/kg ursodiol, repeated dosing of 300 mg/kg/day had no discernable effect on the pharmacokinetics of either oral or IV midazolam. In addition, the repeated dosing had no effect on CYP3A1 or 3A2 mRNA expression in rat liver(Kurosawa et al., 2009). In addition, a study in eight healthy human volunteers given oral midazolam before and after a 14 day course of 13 mg/kg/day oral ursodiol in divided doses found no difference in midazolam disposition before and after the therapy. Gene expression levels of *CYP3A4* and *ABCB1* in duodenal biopsy samples from the subjects also showed no difference between before and after ursodiol treatment. There was, however, a small but statistically significant decrease in the bioavailability of oral digoxin seen after ursodiol treatment. It may be that ursodiol is a weak inducer of p-glycoprotein, but the risk of interaction was considered low(Becquemont et al., 2006).

Subjects used nystatin swish and swallow for candida esophagitis prevention. Nystatin does not affect CYP3A4 or transporters. Clotrimazole troches were not allowed because clotrimazole, although poorly absorbed when taken orally, is a CYP3A4 inhibitor and has been shown to significantly decrease the oral clearance of midazolam in healthy volunteers(Shord et al., 2010). All subjects received sulfamethoxazole/trimethoprim 400/80 mg tablet by mouth three times a week for prevention of *Pneumocystis carinii* (PCP) pneumonia. At the first study session, 12 subjects were still receiving ganciclovir IV and four had been switched to oral ganciclovir for cytomegalovirus (CMV) prophylaxis.

Some transplant subjects were receiving medications that could affect gastrointestinal motility. Before and after the first study session, seven out of 16 subjects were receiving metoclopramide (six IV and one by mouth) three times a day to encourage more rapid gastric emptying and to prevent nausea. Because of the prokinetic effects of metoclopramide and the fact that not all subjects required it, the drug was held for 24 hours during the study sessions. In addition, at the first study session two subjects were receiving low doses of hydromorphone by mouth (2 mg) and three received one or two doses of hydromorphone 0.5 mg IV for pain during the study session. Two subjects were wearing fentanyl transdermal patches for pain control at the first study session, and four at the second. Transdermal fentanyl doses ranged from 25 mcg/hr to 75 mcg/hr.

Transplant subjects received other medications, including antihypertensives, antidepressants, warfarin, vitamin supplements, and others. These are listed in table 5. Median number of oral medications taken by transplant subjects at session 1 was seven (range 4 to 11) and at session 2 was 14 (10 to 22).

Table 5. Other miscellaneous oral medications taken by small bowel transplant recipients

Drug	Number of transplant subjects taking drug	
	Session 1	Session 2
Metoprolol	2	3
Amlodipine	0	1
Warfarin	0	6
Sertraline	3	2
Trazodone	1	0
Mirtazepine	3	0
Bupropion	0	1
Hydromorphone	3	0
Loperamide	0	4
Diphenoxylate/atropine	0	4
Ganciclovir	4	2
Acyclovir	0	4
Valganciclovir	1	4
Clonazepam	5	6
Zolpidem	0	1
Sodium bicarbonate	1	7
Magnesium gluconate	11	8
Pyridoxine	1	7
Cholecalciferol	0	3
Calcium citrate + Vit. D	0	4
Folic acid	0	1
Ferrous gluconate	0	2
Zinc sulfate	0	4
Riboflavin	0	1
Methylphenidate	0	1
Leviracetam	0	1
Pravastatin	1	0
Median number of daily oral medications not including tacrolimus (range)	**7 (4 – 11)**	**14 (10 – 22)**

3.1.2 Crossmatch and donor-specific antibodies prior to transplant and induction therapy at time of transplant

All transplant subjects except one received an intravenous methylprednisolone bolus as part of induction therapy before and during transplant surgery. Twelve out of the 16 subjects also received alemtuzumab (Campath®, Genzyme, Cambridge, MA) 30 mg IV immediately before transplant surgery as induction therapy. Three out of the 16 transplant subjects showed a strong positive crossmatch upon pre-transplant testing and received 3 gm of methylprednisolone IV and 2 gm/kg intravenous immunoglobulin in addition to alemtuzumab. Four of the 16 transplant subjects exhibited donor-specific antibodies upon pre-transplant testing: two had class I only, one had class II only and one had both class I and class II. One of these subjects received bortezomib (Velcade®, Millenium Pharmaceuticals, Cambridge, MA) intravenously during the transplant surgery and several times in the early post-transplant period in response to donor-specific antibody formation.

3.1.3 Intestinal morphology and biopsy results at time of pharmacokinetic study sessions

Intestinal biopsy pathology reports at the times of each study session are summarized in tables 6 and 7.

Table 6. Intestinal biopsy results at time of study session 1

Subject	Mucosal Architecture	Apoptotic bodies/10 crypts	Other comments by pathologist
1	intact	0-1	no ACR
2	intact	1	no ACR
3	focal crypt loss	6-8	mild ACR
4	intact	2-3	no ACR, mild LP inflammation
5		1-2	mild nonspecific infiltrate
6	intact	1-2	focal, mild LP inflammation
7	intact	1	
8	intact, some focal crypt loss	1-2	mild nonspecific LP inflammation
9	intact	1-2	no ACR
10		1-2	mild infiltrate
11	mostly intact, mild crypt loss	2	mild regenerative changes
12	intact	1	
13	intact	2	
14	intact	1-2	
15	intact	1-2	
16	intact	0-1	

Table 7. Intestinal biopsy results at time of study session 2

Subject	Mucosal Architecture	Apoptotic bodies/10 crypts	Other comments by pathologist
2	intact	1	
4	intact	2	mild chronic nonspecific inflammation
5		1-3	mild lymphoplasmacytic inflammation
6	intact	0-1	
7		2	mild lymphoplasmacytic infiltrate
8	intact	2-3	
9	intact	1-2	no ACR
12	intact	2	mild LP congestion
13	intact	2	mild LP inflammation
15	intact	2	

4.0   EXPRESSION OF INFLAMMATORY MEDIATORS AFTER SMALL BOWEL AND MODIFIED MULTIVISCERAL TRANSPLANTATION

4.1   OBJECTIVES

Since the suppressive effects of pro-inflammatory cytokines on CYP3A and p-gp expression and function have been well characterized, the objective of this component of the study is to assess the plasma concentrations of inflammatory mediators in small bowel and modified multivisceral transplant recipients in the early post-transplant period as compared with the concentrations observed in the later post-transplant period and in healthy controls.   Two secondary objectives are to examine the correlation between ileal mRNA expression of *CYP3A* and *ABCB1* and plasma cytokine concentrations and to measure cytokine mRNA transcripts in the ileal mucosa of small bowel and modified multivisceral transplant recipients.

4.2   METHODS AND DEMOGRAPHICS

The study design, methodology and demographics of the recruited subjects have been described in detail in chapters 2 and 3 of this dissertation.   Briefly, this study was approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from all subjects before any study procedures were performed.   Sixteen small bowel transplant recipients

(12 isolated intestine and 4 modified multivisceral) and 16 age-and-gender-matched healthy control subjects were recruited and studied. Transplant subjects were studied twice: once within the first 40 days post-transplant (median day–19, range–10 to 40 days); and again four to fourteen months post-transplant (median day–239, range–125 to 428 days). Sixteen transplant subjects (ten women and six men) underwent the first study session and ten (five women and five men) returned for the second. Control subjects underwent one study session. A time zero plasma sample from the associated pharmacokinetic analysis was used for measurement of cytokine concentrations.

Interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were measured in plasma using a MILLIPLEX® MAP High Sensitivity Human Cytokine Multiplex Panel kit (#HSCYTO-60SK, Millipore, Billerica, MA). The concentrations of each cytokine were compared between transplant subjects at the two study sessions using the Wilcoxon matched pairs signed-rank test, and between each transplant group and controls using the Mann-Whitney U test. In addition, mucosal biopsy samples from the transplanted ileum of the transplant subjects were obtained during routine intestinal biopsies done to monitor the graft for rejection. They were typically taken on the same day as the pharmacokinetic study session, although they were allowed to be within ±48 hours of the study session. These mucosal biopsy samples were subsequently analyzed for *IL-1β, IL-2, IL-6, IFN-γ,* and *TNF-α* gene expression by RT-PCR.

Plasma concentrations of several cytokines were below the lower limit of detection of the assay. These included:

- 4 Tx1 samples below the standard curve range for IL-1β

- 3 Tx1 samples below the standard curve range for IL-2

- 5 Tx1 samples below the standard curve range for IL-4

- 5 Tx1 samples below the standard curve range for IL-12

- 2 Tx1 samples below the standard curve range for IFN-γ

- 2 Tx2 samples below the standard curve range for IL-4

- 1 Tx2 sample below the standard curve range for IL-12

- 3 control samples below the standard curve range for IL-12

These samples were assigned a concentration of 0.13 pg/mL, the concentration of the lowest point on the standard curve of each cytokine's assay. Because this point fell out of range ((observed concentration/expected concentration)*100 was less than 70 or greater than 130) in 8 out of the 18 standard curves generated, 0.13 pg/mL was chosen rather than the LLOD/2, since the actual concentration could be above or below 0.13 pg/mL depending on the direction of the %CV.

4.3   INDIVIDUAL CYTOKINES AND THEIR CONCENTRATION IN PLASMA

Cytokines are proteins having autocrine, paracrine, and endocrine functions, and are produced by a number of different cell types. They are secreted by immune cells such as macrophages and lymphocytes, as well as by non-immune cells such as epithelial cells, fibroblasts, endothelial cells, and smooth muscle cells. They play a role in various types of immune activities, including the acute phase response, antibody synthesis, and cell-mediated immunity, among others(Blumberg and Stenson, 2003).

The amount of leukocyte traffic through the small intestine exceeds that of all other organs, even in healthy persons. As mentioned in chapter 1, recipient lymphocytes rapidly repopulate the lymphoid tissue of the transplanted small intestine where they undergo activation. Activated T and B lymphocytes migrate out of the intestine lymphoid follicles into afferent lymphatic channels and thence into mesenteric lymph nodes. These lymphocytes then pass through the thoracic duct into the bloodstream, returning to the lamina propria via endothelial cells of small intestinal venules(Blumberg and Stenson, 2003).

4.3.1   Interleukin- 1β

IL-1β is secreted by macrophages and acts on receptors as a costimulator of T cells and B cells, as well as on endothelial cells and hepatocytes, as a mediator of the acute phase response(Blumberg and Stenson, 2003).   IL-1β has been shown to down-regulate mRNA expression of *CYP3A4*(Aitken and Morgan, 2007; Assenat et al., 2004), *SLCO2B1* (OATP2B1), *ABCG2* (BCRP), *MRP2*, *MRP3*, and *MRP4* (Le Vee et al., 2008) as well as CYP3A4 protein expression(Aitken and Morgan, 2007) in human hepatocytes and p-glycoprotein expression and function in rat hepatocytes(Sukhai et al., 2001).   In the absence of inflammation, IL-1β concentrations in plasma are low, with a mean concentration of 0.96 ±0.12 pg/mL reported in a group of healthy female control subjects(Scully et al., 2010).

4.3.2   Interleukin-2

IL-2 is secreted by Th1 cells and acts on T and B cells to support the events leading to IgG1 production and cell-mediated immunity(Blumberg and Stenson, 2003). IL-2 has been shown to

suppress p-gp transport of rhodamine 123 and *ABCB1* mRNA production in Caco-2 cells(Belliard et al., 2002).   IL-2 concentrations in plasma were significantly elevated in the first 1 to 2 days post transplant but decreased sharply by days 4 to 6 in a study of 52 cadaveric renal transplant recipients over the first 10 days post-transplant(Sadeghi et al., 2003a).   IL-2 levels are typically low in healthy persons, with one study finding a mean plasma IL-2 concentration of 0.3 ±1.4 pg/mL (range 0-7) in a group of 37 healthy control subjects(Daniel et al., 2005).

4.3.3   Interleukin-4

IL-4 is produced by CD4 T cells and stimulates undifferentiated Th cells to become Th2 by activation of the transcription factor GATA-3.   Th2 cells then proceed to secrete more IL-4 in addition to other cytokines(Blumberg and Stenson, 2003).   A study of 52 cadaveric renal transplant recipients over the first 10 days post-transplant observed stability in mean IL-4 concentrations of 6 ±2 pg/mL on both days 1-2 and days 4-6, with a mean concentration of 6±3 pg/mL on days 8-10(Sadeghi et al., 2003a).   IL-4 concentrations in plasma are variable, even in healthy persons.   One study found a mean IL-4 concentration of 6 ±17 pg/mL in a group of 40 healthy control subjects(Sadeghi et al., 2003b), while another reported a mean plasma IL-4 concentration of 1.4 ±5.4 pg/mL (range 0-27) in a group of 37 healthy control subjects(Daniel et al., 2005).

4.3.4   Interleukin-6

IL-6 is produced by macrophages, endothelial cells, and Th2 cells, and plays a role in the acute phase response as well as in T and B cell growth(Blumberg and Stenson, 2003).   IL-6 has been

shown to suppress CYP3A4 mRNA and protein expression and activity in human hepatocytes(Aitken and Morgan, 2007; Yang et al., 2010). In Caco-2 cells, incubation with IL-6 has been shown to disrupt cell integrity, but have no effect on PEPT1-mediated uptake(Foster et al., 2009). IL-6 has been shown to decrease p-glycoprotein expression and function in rat hepatocytes(Sukhai et al., 2001).

Plasma concentrations of IL-6 have been positively correlated with lactulose-mannitol absorption (a measure of gut barrier permeability) after severe trauma in patients with and without multiple organ failure ($r=0.43$, $p<0.03$)(Spindler-Vesel et al., 2006) and negatively correlated with caffeine (a CYP1A2 probe) metabolism ($r_s=-0.56$, $p=0.0235$) and CYP2C19 activity (as measured by urinary recovery of 4'OH-mephenytoin after mephenytoin administration)($r_s=-0.63$, $p=0.0094$) in a group of congestive heart failure patients(Frye et al., 2002). IL-6 in plasma is also highly correlated with C-reactive protein, a marker of systemic inflammation, which has in turn been associated with suppression of CYP3A4-mediated metabolism in cancer patients(Rivory et al., 2002). Several studies in liver, liver-intestine, kidney, and isolated intestinal transplant patients have shown spikes in serum IL-6 concentrations immediately before or during episodes of rejection in some patients(Kita et al., 1994; Kita et al., 1996a; Kita et al., 1996b). A study in 81 stable cadaveric renal transplant recipients without infection or rejection reported that the median plasma IL-6 concentrations of patients taking tacrolimus were 3.9 pg/mL (range 3.9 to 6.1 pg/mL) at three months post-transplant and 3.2 pg/mL (2.5 to 3.7 pg/mL) at 12 months post-transplant(Lauzurica et al., 2007).

Plasma IL-6 concentrations are typically low but variable in healthy subjects. One study found a mean plasma IL-6 concentration of 2 ±12 pg/mL in a group of 40 healthy control subjects(Sadeghi et al., 2003b), while another study reported a mean plasma IL-6 concentration

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of 1.2 ±2.4 pg/mL (range 0-9) in a group of 37 healthy control subjects(Daniel et al., 2005). A third study found a mean plasma IL-6 concentration of 1.90 ±0.28 pg/mL in a group of healthy females(Scully et al., 2010).

4.3.5 Interleukin-8

IL-8 is a chemokine produced by macrophages and epithelial cells. It activates neutrophils and encourages their chemotaxis to sites of inflammation or infection(Blumberg and Stenson, 2003; Tixier et al., 2005). IL-8 concentrations increase rapidly during the reperfusion phase during lung(Mathur et al., 2006) and liver transplantation(Wen et al., 2004), and remained elevated but stable over the first seven days in a liver transplant population(Kubala et al., 2001). Mean plasma IL-8 concentrations of 9.76 ±1.31 pg/mL were seen in a group of healthy female controls(Scully et al., 2010).

4.3.6 Interleukin-10

IL-10 is produced by CD4 T cells and acts on other T cells to suppress cytokine production(Blumberg and Stenson, 2003). IL-10 has been shown to improve Caco-2 cell monolayer integrity and ameliorate the destructive effects of IL-6 and TNF-α on integrity(Foster et al., 2009). Plasma IL-10 levels have been shown to peak sharply at 2500% of baseline immediately after reperfusion during liver transplant surgery, and remain elevated but stable through post-transplant day 7(Kubala et al., 2001). A study of 52 cadaveric renal transplant recipients over the first 10 days post-transplant observed mean IL-10 concentrations of 38 ±13

pg/mL on days 1-2, 19±9 pg/mL on days 4-6, and 14±3 pg/mL on days 8-10(Sadeghi et al., 2003a). At 30 days post-transplant, a mean plasma IL-10 concentration of 3.47 pg/mL was observed in a group of 77 liver, heart, liver-kidney, and heart-kidney transplant recipients(Cervera et al., 2007). Plasma IL-10 concentrations in healthy subjects appear variable, with one study finding a mean plasma IL-10 concentration of 6.4 ±15.0 pg/mL (range 0-76) in a group of 37 healthy control subjects(Daniel et al., 2005).

4.3.7   Interleukin-12

IL-12 is produced by macrophages and stimulates the T-bet transcription factor to drive undifferentiated Th cells to become Th1, leading to further T and B cell development and cell-mediated immunity(Blumberg and Stenson, 2003). IL-12 concentrations in healthy persons are typically low—one study found a mean plasma IL-12 concentration of less than 10 pg/mL in a group of healthy female controls(Scully et al., 2010).

4.3.8   Tumor necrosis factor-α

TNF-α is secreted by macrophages, T cells, natural killer cells, and mast cells and acts on neutrophils and endothelial cells(Blumberg and Stenson, 2003). TNF-α has been shown to decrease *CYP3A4* mRNA and protein expression(Aitken and Morgan, 2007) in human hepatocytes as well as decrease *ABCB1* mRNA expression and p-glycoprotein transport(Belliard et al., 2004) in Caco-2 cells. A significant negative correlation between CYP2C19 activity (as measured by urinary recovery of 4'OH-mephenytoin after mephenytoin administration) and

plasma TNF-α (r_s=-0.61, p=0.0118) was observed in a group of 16 congestive heart failure patients(Frye et al., 2002).

A study of 52 cadaveric renal transplant recipients over the first 10 days post-transplant found that plasma TNF-α concentrations peaked between day 8 and day 10, with a mean concentration of 57 ±17 pg/mL on days 1-2, 115±56 pg/mL on days 4-6, and 155±46 pg/mL on days 8-10(Sadeghi et al., 2003a). At 30 days post-transplant, a mean plasma TNF-α concentration of 12.71 pg/mL was observed in a group of 77 liver, heart, liver-kidney, and heart-kidney transplant recipients(Cervera et al., 2007). A study in 81 stable cadaveric renal transplant recipients without infection or rejection reported median TNF-α concentrations of 10.2 pg/mL (range 7.8 to 13 pg/mL) at three months and 10.6 pg/mL (range 6.6 to 13.2 pg/mL) at 12 months(Lauzurica et al., 2007). In healthy subjects, a mean plasma TNF-α concentration of 1.3 ±2.6 pg/mL (range 0-14) has been reported(Daniel et al., 2005).

4.3.9   Interferon-γ

IFN-γ is produced by T cells and natural killer cells, and acts on macrophages, endothelial cells, natural killer cells, and epithelial cells(Blumberg and Stenson, 2003), and has been shown to significantly suppress CYP3A mRNA expression in human hepatocytes(Aitken and Morgan, 2007). IFN-γ concentrations in plasma have been shown to spike to high concentrations three to six days before and during episodes of rejection in the first month after transplant in kidney transplant patients(Sadeghi et al., 2003b). Plasma concentrations of IFN-γ are typically low in healthy persons. One study found a mean plasma IFN-γ concentration of less than 5 pg/mL in a group of healthy female controls(Scully et al., 2010).

4.4 COMPARISON OF PLASMA CYTOKINE CONCENTRATIONS ON THE DAY OF STUDY SESSION IN THE THREE STUDY GROUPS

Plasma samples were analyzed for cytokine concentrations from each study session in all subjects. Median cytokine concentrations (in pg/mL) are presented in Table 8. Only those samples with a concentration falling within the plate-specific standard curve were included in the analysis.

IL-6 and TNF-α concentrations were elevated during the early post-transplant period (Tx1), and remained elevated in the later period (Tx2) compared with controls. Concentrations of IL-6 in transplant patients were significantly higher at study session 1 than at study session 2 (13.5 pg/mL vs. 3.90 pg/mL, p=0.049), and were also higher in Tx1 than in controls (13.8 pg/mL vs. 1.21 pg/mL, p<0.0001) and in Tx2 than in controls (3.90 pg/mL vs. 1.21 pg/mL, p=0.0014). Similarly, TNF-α concentrations were significantly higher in Tx1 than in Tx2 (4.00 pg/mL vs. 3.13 pg/mL, p=0.020), higher in Tx1 than in controls (4.10 pg/mL vs. 1.37 pg/mL, p<0.0001), and higher in Tx2 than in controls (3.13 pg/mL vs. 1.37 pg/mL, p=0.0004).

Concentrations of the anti-inflammatory cytokine IL-10 were elevated during the early post-transplant period, but were no different from controls in the later period. Concentrations were significantly higher in Tx1 than in Tx2 (47.4 pg/mL vs. 10.8 pg/mL, p=0.0059) and higher in Tx1 than in controls (45.9 pg/mL vs. 5.98 pg/mL, p<0.0001). However, there was no significant difference in IL-10 between Tx2 and controls (10.8 pg/mL vs. 5.98 pg/mL, p=0.078). IL-8 concentrations did not differ between the early and later post-transplant periods (8.38 pg/mL vs. 3.40 pg/mL, p=0.11), but were significantly higher in the early period than in controls (12.7 pg/mL vs. 1.51 pg/mL, p<0.0001) and in the later period than in controls (3.40 pg/mL vs. 1.51 pg/mL, p=0.0048). No significant differences in median plasma concentrations of IL-1β,

IL-2, IL-4, IL-12, and IFN-γ were observed between any of the three groups, although a fairly

wide variability in IL-12 and IFN-γ was noted in the transplant subjects at session 1.

Table 8. Plasma cytokine concentrations on study session days

Plasma cytokine (pg/mL)	Transplant Session 1 (n=16)*	Transplant Session 2 (n=10)	Control Subjects (n=16)	P value* Tx1 vs. Tx2	P value§ Tx1 vs. C	P value§ Tx2 vs. C
IL-1β	0.490 (0.198,1.54)	0.550 (0.458,1.26)	0.640 (0.428,1.60)	0.92	0.31	0.58
IL-2	0.705 (0.335,1.08)	0.720 (0.608,1.16)	0.635 (0.470,1.38)	0.63	0.63	0.60
IL-4	5.57 (0.130,10.5)	6.23 (4.31,10.3)	5.05 (1.81,10.4)	0.91	0.46	0.98
IL-6	13.8 (5.22,29.0)	3.90 (1.98,5.70)	1.21 (0.763,1.61)	**0.049**	**<0.0001**	**0.0014**
IL-8	12.7 (5.18,17.1)	3.40 (2.36,5.62)	1.51 (1.33,1.97)	0.11	**<0.0001**	**0.0048**
IL-10	45.9 (25.5,52.9)	10.8 (5.54,14.5)	5.98 (2.70,7.85)	**0.0059**	**<0.0001**	0.078
IL-12	0.790 (0.130,2.17)	1.18 (0.703,2.76)	0.880 (0.225,2.22)	0.49	0.88	0.56
IFN-γ	4.30 (0.633,9.13)	3.30 (1.58,5.76)	2.84 (1.47,4.51)	1.0	0.84	0.92
TNF-α	4.10 (2.67,5.18)	3.13 (2.85,3.46)	1.37 (1.01,1.69)	**0.020**	**<0.0001**	**0.0004**

Concentrations are median (25th, 75th percentile)

*Comparisons between transplant sessions 1 and 2 made using the Wilcoxon matched-pairs signed rank test on matched pairs. Numbers in first column are median (25th, 75th percentile) for all 16 transplant session 1 subjects. Median concentrations in the Tx1 subjects used in the paired analysis only are reported in the text as appropriate. §Comparisons between transplant session 1 and controls and transplant session 2 and controls made using the Mann-Whitney U test. Two-tailed p values reported.

4.5 CORRELATION BETWEEN PLASMA CYTOKINE CONCENTRATIONS AND ILEAL CYP3A AND TRANSPORTER GENE EXPRESSION

4.5.1 Relationship between plasma cytokines and ileal *CYP3A* expression in transplant subjects at study session 1

Significant correlations between *CYP3A* expression and plasma cytokine concentrations are shown in figures 8 through 12. There was a strong negative correlation between relative *CYP3A* mRNA expression in ileal biopsy samples and plasma IL-2 (r_s=-0.58, p=0.036), IL-4 (r_s=-0.53, p=0.062), IL-12 (r_s=-0.51, p=0.077), and IFN-γ (r_s=-0.53, p=0.062) concentrations at study session 1. There was a significant positive correlation between *CYP3A* mRNA and plasma IL-8 concentrations (r_s=0.62, p=0.025).

Figure 8. Correlation between ileal *CYP3A* expression and plasma IL-2 in transplant subjects at study session 1.
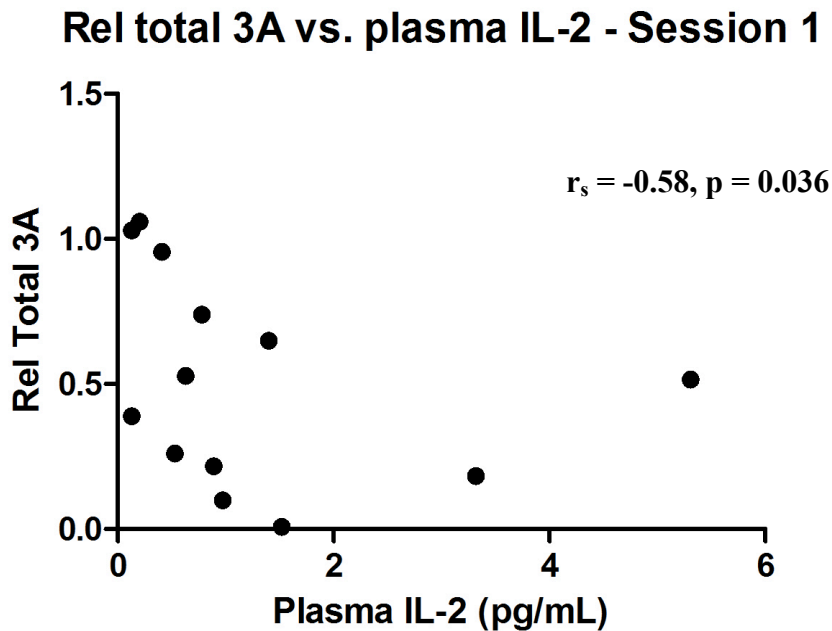
Figure 9. Correlation between ileal *CYP3A* expression and plasma IL-4 in transplant subjects at study session 1.

Rel total 3A vs. plasma IL-4 - Session 1

$r_s = -0.53$, $p = 0.062$
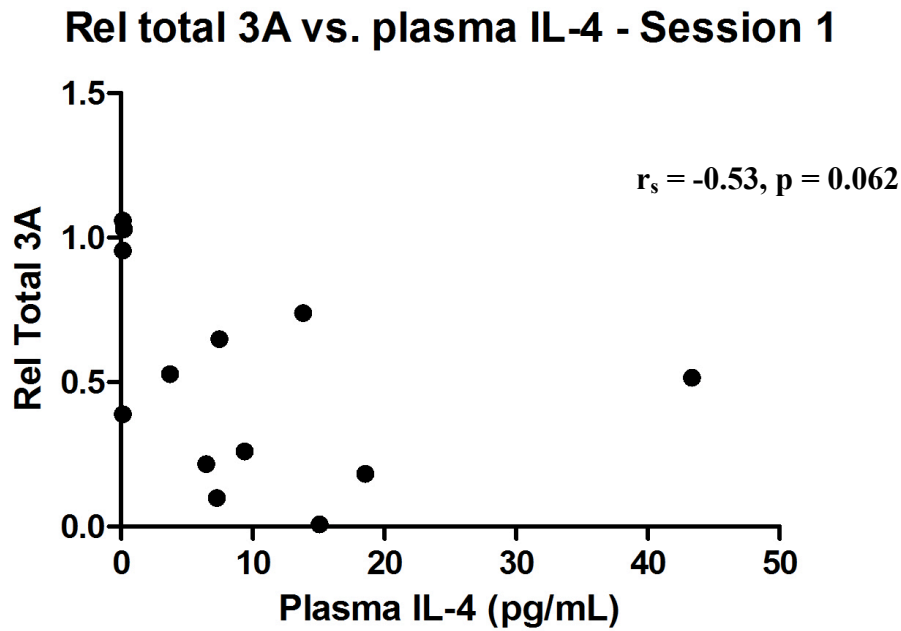
Figure 10. Correlation between ileal *CYP3A* expression and plasma IL-8 in transplant subjects at study session 1.

Rel Total 3A vs. Plasma IL-8 -- Session 1

$r_s = 0.62$, $p = 0.025$
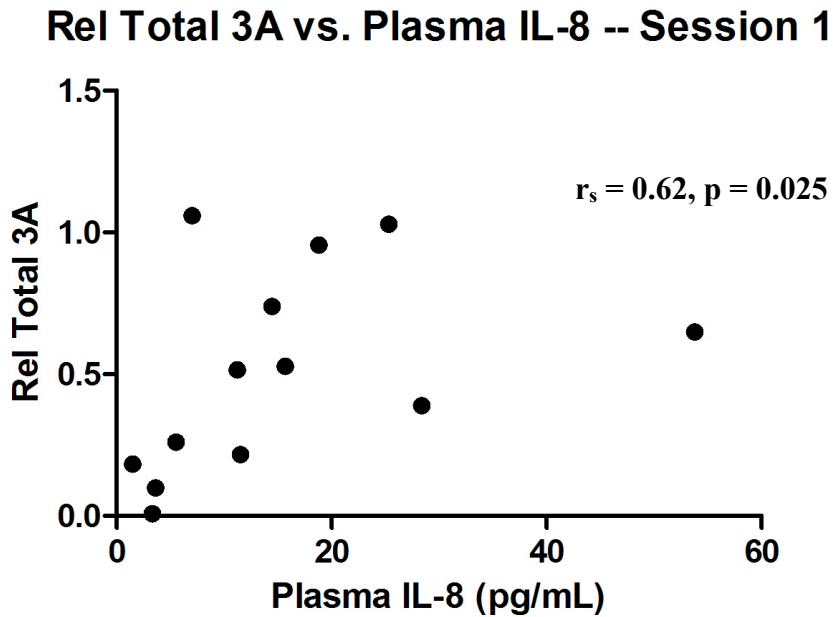
Figure 11. Correlation between ileal *CYP3A* expression and plasma IL-12 in transplant subjects at study session 1.

Rel Total 3A vs. Plasma IL-12 - Session 1

$r_s = -0.51, p = 0.077$
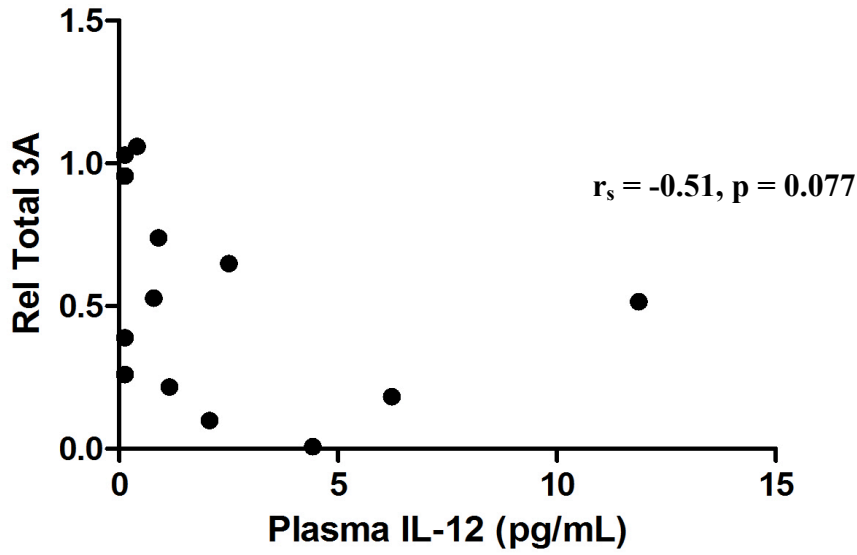
Figure 12. Correlation between ileal *CYP3A* expression and plasma IFN-γ in transplant subjects at study session 1.

Rel total 3A vs. IFNy - Session 1

$r_s = -0.53, p = 0.062$
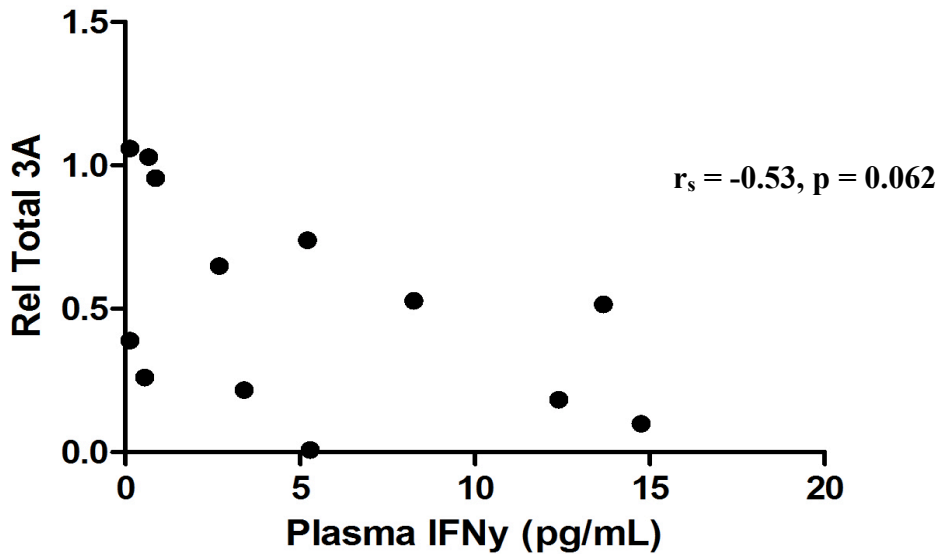
In addition, at study session 1 there was a negative correlation between total *CYP3A* expression and plasma IL-1β (r_s=-0.33, p=0.27) and TNF-α (r_s=-0.19, p=0.53). All correlations between ileal *CYP3A4* and total *CYP3A* and plasma cytokine concentrations are listed in Table 34 of Appendix A.

4.5.2   Relationship between plasma cytokines and *CYP3A* in transplant subjects at study session 2

A strong negative correlation was observed between ileal *CYP3A4* expression and plasma IL-10 concentrations in transplant subjects at study session 2 (r_x=-0.65, p=0.049). This relationship is shown in figure 13. Similarly, a negative correlation was seen between ileal *CYP3A* expression and plasma IL-12 concentrations (r_x=-0.62, p=0.060), shown in figure 14.

Figure 13.   Correlation between ileal *CYP3A4* expression and plasma IL-10 in transplant subjects at study session 2
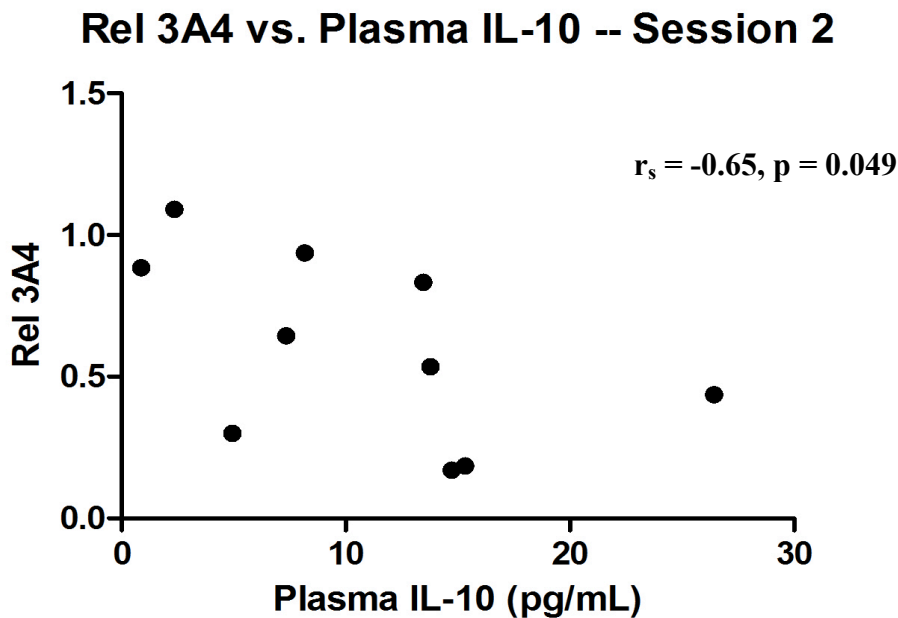
Figure 14. Correlation between ileal CYP3A expression and plasma IL-12 in transplant subjects at study session 2

Rel Total 3A vs. Plasma IL-12 - Session 2

$r_s = -0.62, p = 0.060$
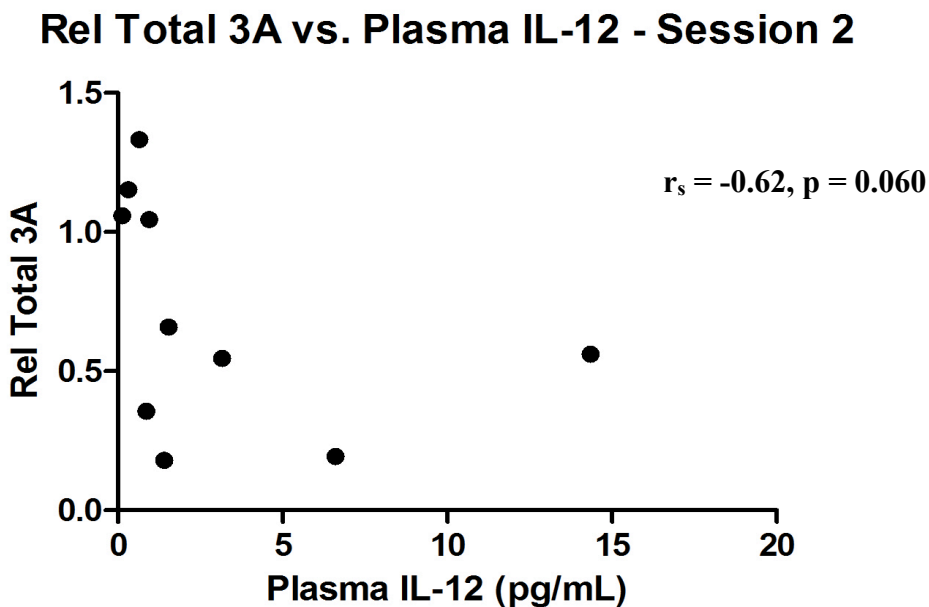
In addition, at study session 2 negative correlations between total *CYP3A* expression and IL-2 (r_s=-0.49, p=0.15), IL-4 (r_s=-0.34, p=0.33), IFN-γ (r_s=-0.54, p=0.11), and TNF-α (r_s=-0.30, p=0.41) were observed.

4.6   ASSOCIATION OF PLASMA CYTOKINE CONCENTRATIONS WITH EPISODES OF ACUTE REJECTION EARLY POST-TRANSPLANT

Figure 15. Plasma cytokine profile in subject with acute rejection on biopsy
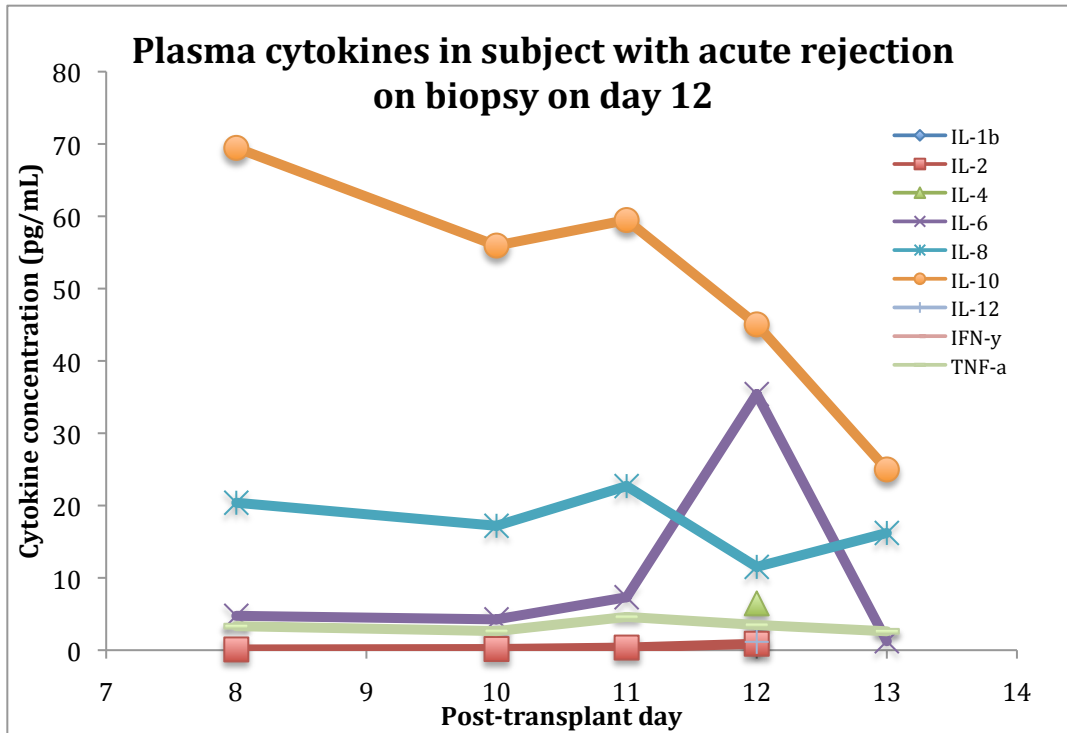
Biopsy taken on day 12 showed acute rejection with 6-7 apoptotic bodies per 10 crypts. 2 methylprednisolone IV x

1 was given on day 12 and OKT3 was started on day 13.

Figure 16. Plasma cytokine profiles in two subjects with clinical signs of rejection

Plasma cytokines in subject with clinical signs of rejection on day 15

Plasma cytokines in subject with signs of rejection on day 15
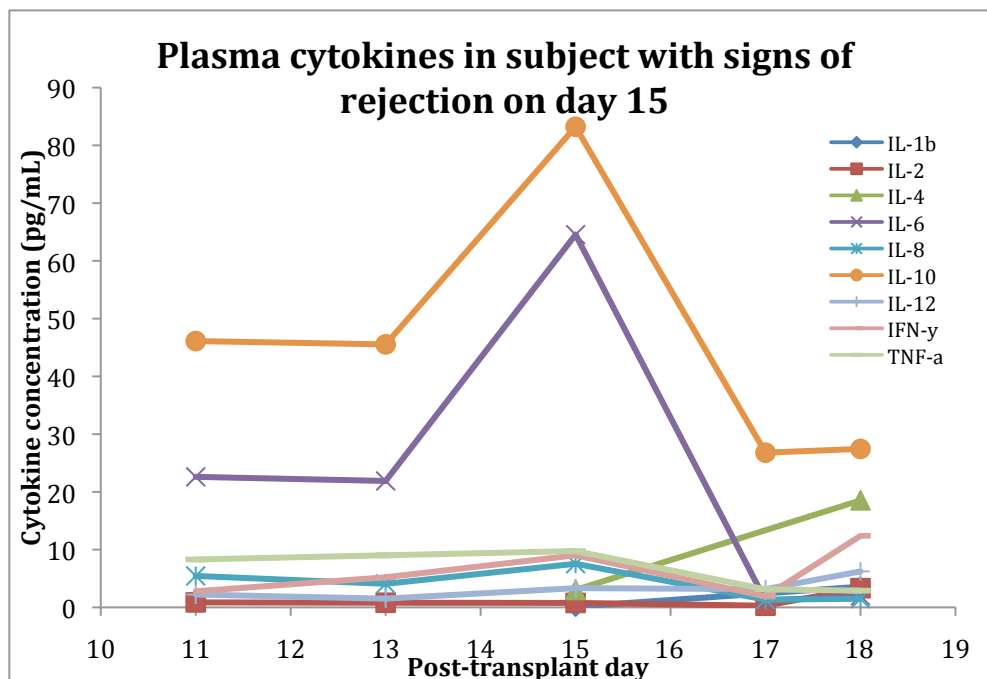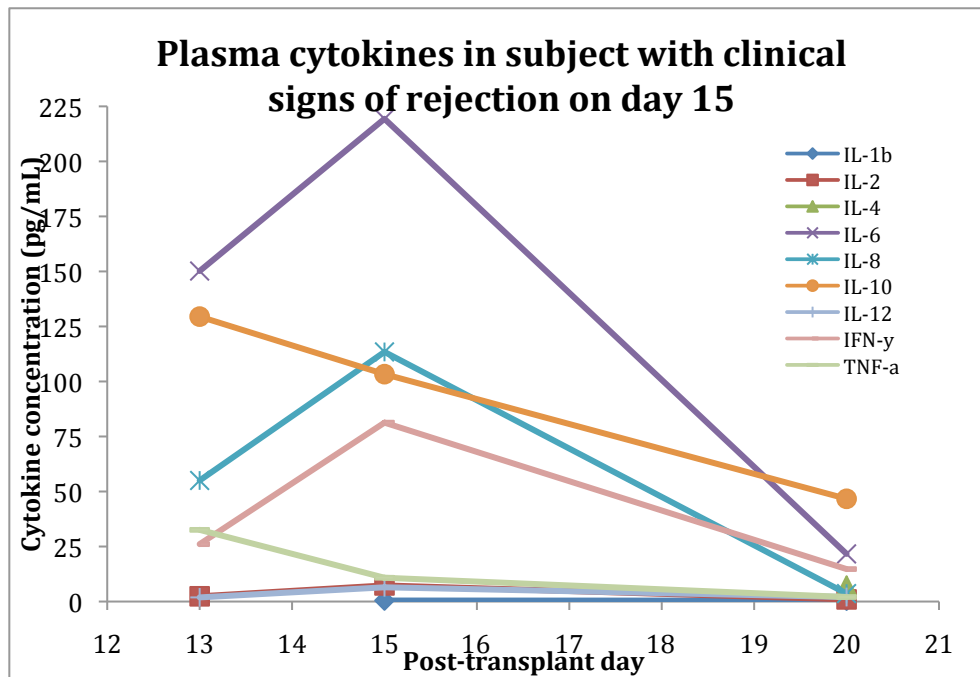
One subject received 1 gm IV methylprednisolone x 1 and one received 2 gm IV methylprednisolone x 1 on day 15 for clinical symptoms. Biopsies turned out to be normal. Areas of rejection may be focal/patchy.

4.7 RELATIONSHIP BETWEEN PLASMA IL-6 CONCENTRATIONS AND PRE-TRANSPLANT DONOR-SPECIFIC ANTIBODIES AND CROSSMATCH

There was no observed association between plasma cytokine concentrations on the day of study and the presence of strong positive crossmatch, donor-specific antibodies, or use of alemtuzumab as induction therapy in the transplant subjects.

Figure 17. Plasma IL-6 concentrations in transplant subjects at study session 1 by presence of crossmatch
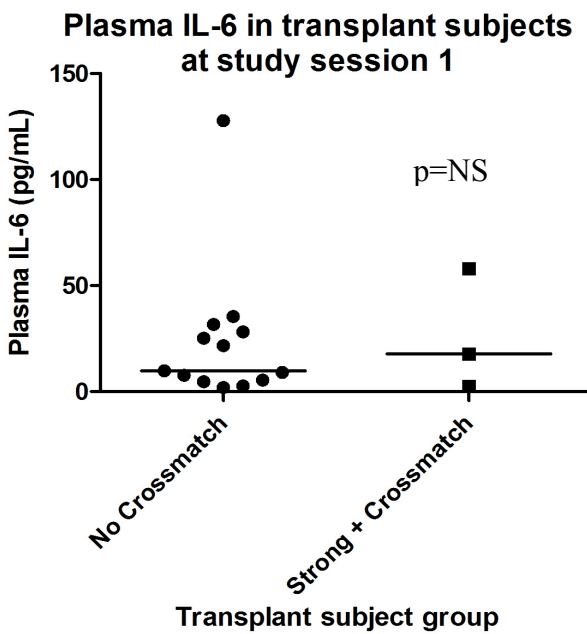
Figure 18. Plasma IL-6 concentrations in transplant subjects at study session 1 by presence of donor-specific antibodies

Plasma IL-6 in transplant subjects at study session 1
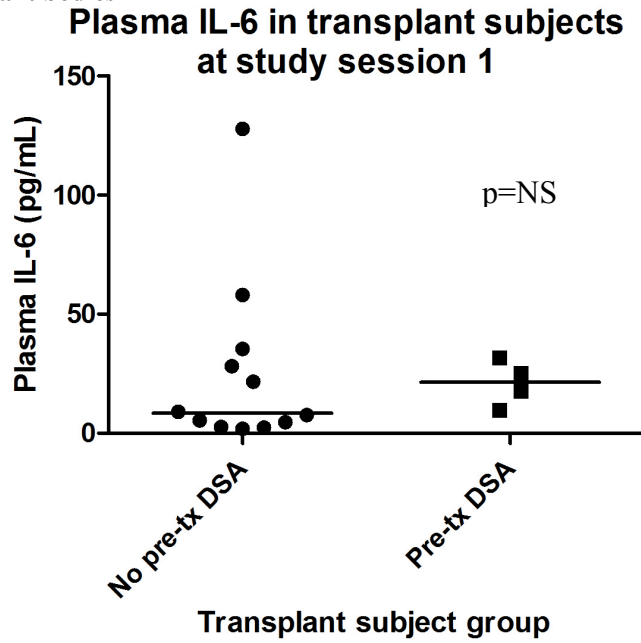
Figure 19. Plasma IL-6 concentrations in transplant subjects at study session 1 by crossmatch and/or donor-specific antibodies

Plasma IL-6 in transplant subjects at study session 1
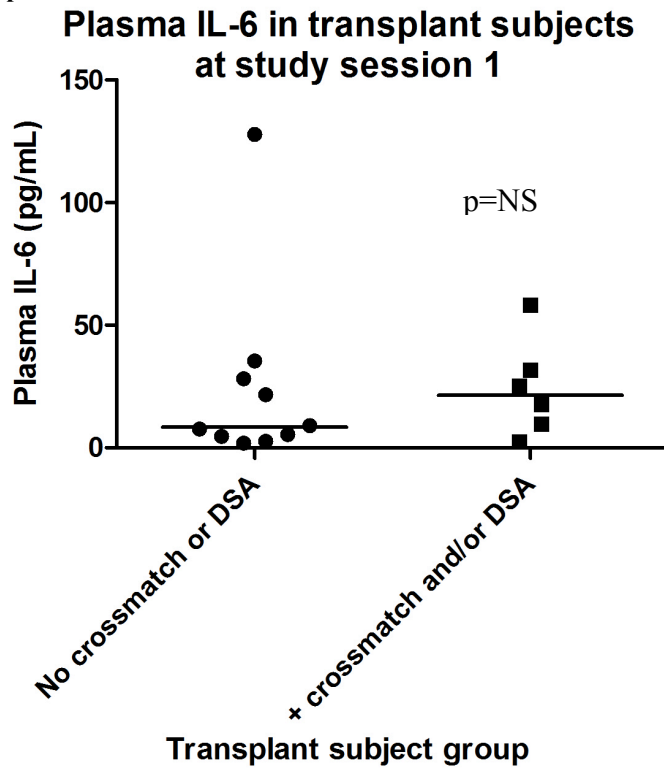
Figure 20. Plasma IL-6 concentrations in transplant subjects at study session 1 by alemtuzumab induction
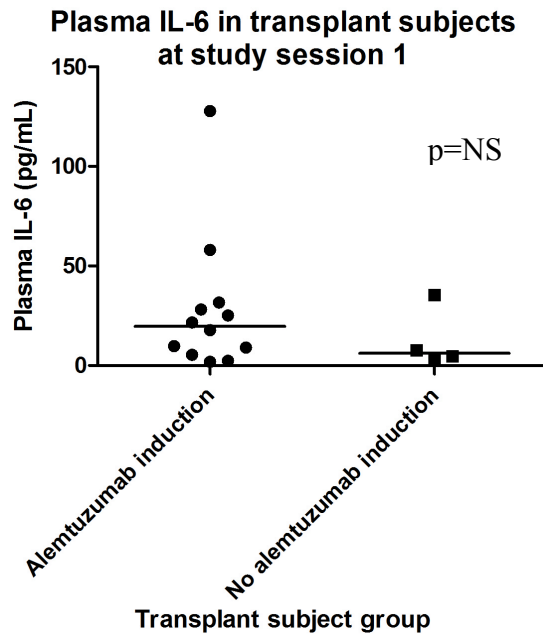
4.8   CYTOKINE GENE TRANSCRIPTS IN ILEAL BIOPSY SAMPLES

Low amounts of cytokine mRNA transcripts in ileal biopsy tissue were detected in all but one of the transplant subjects at session 1, and all but two at session 2. Transcript amounts relative to cyclophilin are shown in Tables 7 and 8. IL-1β was the most widely expressed cytokine in the transplanted mucosa, detected in 12 out of 13 biopsies at session 1 and six out of ten at session 2. TNF-α was the second most ubiquitous, expressed in 11 out of 13 subjects at session 1, and seven out of ten at session 2.   IFN-γ was expressed in eight out of 13 at session 1, but only three out of ten at session 2. IL-2 was detected in nine out of 13 at session 1, but only two out of ten at session 2. IL-6 was the least widely detected, being found in only five out of 13 at session 1 and two out of ten at session 2.

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Table 9. Cytokine gene expression in ileal biopsy samples - Session 1

Subject	IL-1β	IL-6	IFN-γ	TNF-α	IL-2
1	0.00105	0.000170	0.000332	0.00142	0.00213
3	0.00105	0.000130	0.000373	0.00121	0.000279
4	0.00109	nd	0.0000671	0.000201	0.00109
5	0.00725	0.000240	nd	0.000660	0.00134
6	nd	nd	nd	nd	nd
7	0.000668	nd	0.0000475	0.000421	0.0000855
8	0.000578	0.00015	nd	0.000608	nd
9	0.0370	nd	nd	nd	nd
10	0.00141	nd	0.000705	0.00135	0.000321
11	0.000372	nd	nd	0.000573	0.000149
13	0.00101	nd	0.00526	0.00102	0.0000920
14	0.000917	nd	nd	0.00220	nd
15	0.00697	0.00984	0.000820	0.00410	nd
16	0.000684	nd	0.00312	0.00109	0.00404

Amounts are expressed relative to cyclophilin, a housekeeping gene

Table 10. Cytokine gene expression in ileal biopsy samples - Session 2

Subject	IL-1β	IL-6	IFN-γ	TNF-α	IL-2
2	0.00326	0.000410	0.000192	0.00134	0.00292
4	nd	nd	nd	nd	nd
5	0.00355	0.0000160	0.000542	0.00110	nd
6	0.00172	nd	nd	0.000944	nd
7	0.00109	nd	nd	0.00218	nd
8	0.00613	nd	0.000452	0.00258	nd
9	nd	nd	nd	nd	nd
12	0.00765	nd	nd	0.00594	0.000119
13	nd	nd	nd	nd	nd
15	nd	nd	nd	0.00862	nd

Amounts are expressed relative to cyclophilin, a housekeeping gene

4.9   DISCUSSION

The results presented in this chapter demonstrate that, even in the absence of rejection, inflammatory mediators are significantly elevated in the first 40 days after small bowel transplantation.   In addition, these results provide evidence that some pro-inflammatory cytokines remain elevated above normal even in stable small bowel transplant recipients four months to one year post-transplant. IL-6, IL-8, IL-10, and TNF-α were significantly elevated in transplant subjects during the early post-transplant period as compared to the later period and with controls and concentrations of IL-6, IL-8, and TNF-α remained significantly higher than controls in the later post-transplant period. IL-1β, IL-2, IL-4, IL-12, and IFN-γ concentrations showed no significant difference between groups. These results, accompanied by the detection of cytokine mRNA transcripts in most of the ileal biopsy samples, provide evidence of immune activation in small bowel transplant recipients that is higher in the early post-transplant period, but that may persist in the long term, even in stable patients with no signs or symptoms of rejection or infection. Furthermore, the significant negative correlations seen between ileal *CYP3A* expression and plasma levels of IL-2, IL-4, IL-10, IL-12, and IFN-γ are consistent with previous reports of suppression of CYP3A by pro-inflammatory cytokines *in vitro* and *in vivo*, and indicate that this immune activity may significantly affect intestinal drug metabolism and transport in this study population. This will be evaluated in the following chapters.

5.0 CYTOCHROME P450 3A FUNCTION AFTER SMALL BOWEL AND MODIFIED MULTIVISCERAL TRANSPLANTATION

5.1 BACKGROUND

5.1.1 Hypothesis and objectives

Small bowel transplant recipients take numerous medications, many of them by mouth, and a significant number of them are CYP3A substrates. Ischemia-reperfusion injury and immune activation within the transplanted organ lead to inflammatory mediator release both within the graft and into the systemic circulation. *In vitro* studies have shown that pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ decrease CYP3A expression and function via suppression of gene transcription. Clinical studies in other inflammatory conditions such as cancer and sepsis have shown the clinical correlation between inflammatory mediator expression and suppression of drug metabolism. Whereas animal studies have shown suppression of CYP3A expression and function in the first 14 days after small bowel transplantation, the pharmacokinetics of the CYP3A/p-gp substrate tacrolimus appear no different in stable small bowel transplant patients months to years post-transplant.

In addition, in the previous chapter it has been shown that plasma concentrations of the pro-inflammatory cytokines IL-6 and TNF-α were significantly elevated in the early post-

transplant period (study session 1) as compared with the later post-transplant period (study session 2) and with healthy controls. Significant negative correlations were also seen between ileal *CYP3A* mRNA expression and plasma concentrations of the pro-inflammatory cytokines IL-2, IL-4, IL-10, IL-12, and IFN-γ, indicating post-transplant inflammation associated with decreases in intestinal CYP3A expression.

Based on these observations, the central hypothesis of this chapter is that intestinal CYP3A expression and function in small bowel transplant recipients will be suppressed in the early post-transplant period (estimated at approximately the first 30 to 40 days after transplant), but will be similar to that seen in healthy control subjects in stable patients without evidence of rejection in the later post-transplant period (by four to six months post-transplant). In order to examine the effects of the transplanted intestine without the confounding variable of the transplanted liver, only isolated intestinal and modified multivisceral transplant recipients were included in the study.

This chapter therefore has the following specific aim:

1. To measure intestinal CYP3A expression and function in stable small bowel transplant recipients in the early post-transplant period and late post-transplant period in comparison to CYP3A activity in healthy control subjects.

    a. This will be accomplished by characterizing the pharmacokinetics of oral and intravenous midazolam, administered as a probe drug for assessment of intestinal and hepatic CYP3A, within 40 days post-transplant and approximately four to 12 months post-transplant in transplant recipients as well as in healthy control

subjects. In addition, in transplant subjects, mRNA transcripts of *CYP3A4*, *CYP3A5*, and *NR1I2 (PXR)* will be measured in ileal mucosal biopsy samples taken within 48 hours of each midazolam study and compared between time periods.

b. It is predicted that the oral AUC and bioavailability of midazolam in the small bowel transplant recipients in the early post-transplant period will be significantly higher, and the AUC ratio of 1'hydroxymidazolam to midazolam significantly lower, than that seen in age- and gender-matched healthy control subjects and also significantly different than the same subjects in the later post-transplant period. In the later post-transplant period it is predicted that the pharmacokinetic parameters of oral midazolam will be no different than those seen in healthy controls. In addition, expression levels of *CYP3A4* and *CYP3A5* will be significantly lower in the early post-transplant period as compared with the later.

5.1.2 Use of midazolam as a probe drug for CYP3A phenotyping

Midazolam is a short-acting benzodiazepine sedative-hypnotic, approved by the FDA for use in induction of anesthesia and for sedation, especially in critical care units or prior to procedures such as endoscopy(APP, 2003). It is usually administered as an intravenous bolus or infusion, although an oral solution formulation is commercially available and is typically used in pediatric populations. Midazolam is metabolized by CYP3A4 and CYP3A5 (if present) to two primary

hydroxylated metabolites, 1'hydroxymidazolam (1'OH-MDZ) and 4'hydroxymidazolam, which

respectively account for approximately 75% and 5%, of biotransformation products(APP, 2003).

Each of these metabolites is produced by binding to a different site on the CYP3A4

enzyme(Kapelyukh et al., 2008; Khan et al., 2002). In addition, very small amounts of parent

drug are transformed to a third (dihydroxy) metabolite by CYP3A, and to a direct N-glucuronide

conjugate by UDP-glucuronosyltransferase 1A4 (UGT1A4)(Klieber et al., 2008). The hydroxy

and dihydroxy metabolites undergo Phase II biotransformation via glucuronidation and are

excreted in the urine(APP, 2003). Midazolam is not a substrate of any known transporter *in vivo*.
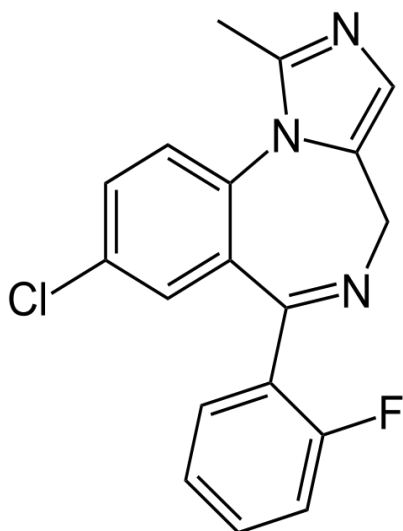
Figure 21. Chemical structure of midazolam

Midazolam is rapidly and completely absorbed via the transcellular route after oral administration(Paine et al., 2006) and undergoes significant first-pass metabolism by intestinal CYP3A(Paine et al., 1996),

leading to average bioavailability estimates of between 19% to 38% in healthy volunteers(Lee et

al., 2002; Link et al., 2008; Pentikainen et al., 1989). Midazolam is highly soluble, highly

permeable through the enterocyte membrane, and extensively metabolized; as such, it is

categorized as a class I compound in both the BCS and BDDCS(Takagi et al., 2006). Typical

volume of distribution in healthy subjects ranges from 1 to 3.1 L/kg and the average half-life is

around three hours(APP, 2003). Clearance of midazolam is diminished in patients with liver

disease(APP, 2003; Pentikainen et al., 1989; Shelly et al., 1989). Midazolam is >97% bound to

plasma proteins, mainly albumin(APP, 2003).

Because of its nearly complete metabolism by CYP3A and its lack of affinity for transporters, as well as its short half-life and favorable safety profile, midazolam has frequently been used as a probe drug to assess CYP3A function in healthy subjects(Chaobal and Kharasch, 2005; Katzenmaier et al., 2010; Kirby et al., 2006; Lee et al., 2002; Lee et al., 2006; Link et al., 2008), patients with renal failure(Dowling et al., 2003; Kirwan et al., 2009), liver cirrhosis(Chalasani et al., 2001), and in liver transplant recipients(Thummel et al., 1994a; Thummel et al., 1994b). While disposition of intravenous midazolam is used as a marker for *hepatic* CYP3A function, disposition of oral midazolam reflects both intestinal and hepatic CYP3A activity.

Although CYP3A4 and CYP3A5 are both genetically polymorphic in their expression and function, the effects of these polymorphisms on the *in vivo* disposition of CYP3A substrates such as midazolam are uncertain. Intrinsic clearance of midazolam by CYP3A5 has been shown to be essentially equal to that mediated by CYP3A4(Soars et al., 2006), so it would be reasonable to assume that the presence of genetic polymorphisms of such alleles as *CYP3A5*3* would lead to significant differences in midazolam clearance compared with carriers of the *CYP3A5*1* allele. However, this has not always or even typically been the case either *in vitro* or *in vivo*.

The relationship between 1'hydroxymidazolam formation rate and CYP3A polymorphisms was examined in human liver microsomes from 54 different donors, with no relationship found between midazolam 1'hydroxylation activity and any of the genotypes or haplotypes tested, including the relatively common *CYP3A5*1* and **3*(He et al., 2006). Studies done in healthy human subjects have shown no relationship between oral midazolam AUC$_{0-\infty}$ and the presence of *CYP3A5*3*, *CYP3A5*6*, *CYP3A4*1B*, and *CYP3A4*1F* alleles(Eap et al., 2004), between oral and IV midazolam clearance and *CYP3A5*3* and *CYP3A4*1B* alleles(Miao et al.,

2009), between oral and IV midazolam clearance and *CYP3A5*1, *3, *6,* and **7* alleles(Kharasch et al., 2007), and between oral midazolam bioavailability, oral and IV midazolam clearance, and the presence of *CYP3A5*1* and *CYP3A5*3* alleles(Tomalik-Scharte et al., 2008).

Although the relationship between genetic polymorphisms of *CYP3A4* and *CYP3A5* and midazolam disposition has not been shown consistently *in vivo*, the relationship between intestinal CYP3A4 and CYP3A5 mRNA and protein expression and disposition of CYP3A substrates has been demonstrated. 1'hydroxylation rates of midazolam were significantly correlated to hepatic microsomal CYP3A4 protein content (r_s=0.92, p<0.001) as well as CYP3A5 protein content, although to a lesser degree (r_s=0.60, p<0.001). Although homozygous carriers of the *CYP3A5*3* gene had significantly less CYP3A5 protein expression than non-carriers, this did not affect hepatic CYP3A activity(He et al., 2006).

A correlation between intestinal *CYP3A* mRNA expression and pharmacokinetic parameters of oral CYP3A substrates has been shown with budesonide, saquinavir, and felodipine. Rectosigmoidal *CYP3A4* mRNA expression levels in healthy volunteers were found to correlate significantly with the partial metabolic clearances of the two major metabolites of budesonide, 16-OH-prednisolone (r^2=0.30, p=0.010) and 6-OH-budesonide (r^2=0.25, p=0.016). *CYP3A4* expression was also significantly negatively correlated with the metabolic ratios of budesonide and 16-OH-prednisolone (r^2=0.34, p=0.006) and budesonide and 6-OH-budesonide (r^2=0.16, p=0.048), as well as with budesonide AUC_{0-24}(r^2=0.18, p=0.040)(Ufer et al., 2008).

In a study of 20 healthy volunteers administered oral saquinavir, in the six subjects in whom CYP3A5 was detected there was a significant positive correlation between saquinavir CL/F and *CYP3A5* mRNA expression (r^2=0.58, p=0.05)(Mouly et al., 2005). Similarly, a

significant negative correlation between duodenal and colonic *CYP3A4* expression and Cmax of the orally administered CYP3A substrate felodipine was observed in a study of ten healthy male volunteers(Lown et al., 1997).

5.1.2.1 Midazolam disposition in inflammation

The effects of inflammatory mediators on CYP3A expression have been discussed previously. Consistent with those results, it has also been shown that inflammation and immune activation may decrease midazolam 1'hydroxylation via CYP3A. In a study of rats administered a single dose of lipopolysaccharide (LPS) from *E. coli,* it was found that hepatic microsomal expression of CYP3A2 (the rat analog of human CYP3A4) was significantly decreased for five days. In addition, plasma levels of TNF-α and IL-1β were both significantly increased in LPS-treated rats as compared with control rats at three and six hours after administration (and also at nine hours in the case of IL-1β), but concentrations of both were similar to those found in control rats by 24 hours. In addition, the AUC of 1'hydroxymidazolam was significantly lower in LPS-treated rats on day one after treatment as compared with control rats (421 ± 105 vs. 151 ± 78.6 ng*min/mL, $p<0.01$) (Kato et al., 2008). Midazolam 1'hydroxylation activities of intestinal microsomes taken from rats with adjuvant arthritis (AA), an animal model of rheumatoid arthritis (a disease of chronic systemic inflammation), were significantly diminished as compared with those taken from healthy rats(Uno et al., 2007).

5.2   METHODS AND SUBJECT DEMOGRAPHICS

The study design, methodology and demographics of the recruited subjects have been described in detail in chapters 2 and 3 of this dissertation.   Briefly, this study was approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from all subjects before any study procedures were performed. Sixteen small bowel transplant recipients (12 isolated intestine and 4 modified multivisceral) and 16 age- and gender-matched healthy control subjects were recruited and studied. Transplant subjects were studied twice: once within the first 40 days post-transplant (median day–19, range–10 to 40 days); and again four to fourteen months post-transplant (median day–239, range–125 to 428 days). Sixteen transplant subjects (ten women and six men) underwent the first study session and ten (five women and five men) returned for the second sesson. Control subjects underwent one study session.

At each study session, following an overnight fast, the subject was administered 5mg/2.5mL of midazolam syrup orally using an oral syringe. The used oral syringe was then flushed twice with 2.5 mL of water, which was administered to the subject, and the subject was immediately given an additional four ounces of water to drink. In the four transplant subjects who were receiving medications by jejunostomy tube (J-tube) at the time of the first study session, the syrup was administered through the J-tube, together with the two tube rinses and an additional four ounces of water. The J-tube was then clamped. Seven hours after the oral dose, 2 mg of midazolam in 150 mL of normal saline was administered intravenously over 30 minutes. Blood samples were taken at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 7.25, 7.5, 8, 10, 12, and 20 hours after administration of oral midazolam. Subjects remained in bed for six hours after each midazolam administration, with the head of the bed at a 60-degree angle.

In addition, tissue samples from the transplanted ileum of the transplant subjects were obtained during routine intestinal biopsies performed to monitor the graft for rejection. They were typically taken on the same day as the pharmacokinetic study session, although they were allowed to be within ±48 hours of the study session. These mucosal biopsy samples were subsequently analyzed for *CYP3A4*, *CYP3A5*, and *NR1I2* (PXR) gene expression by RT-PCR.

Midazolam and 1'hydroxymidazolam were measured in plasma by LC-MS as previously described. The pharmacokinetic parameters of midazolam were calculated using noncompartmental analysis. Pharmacokinetic parameters and mRNA expression of genes between study sessions for transplant subjects were compared using the Wilcoxon matched pairs signed rank test. Transplant session 1 and 2 pharmacokinetic parameters were each compared with control subjects and within transplant patients by transplant type and route of administration using the Mann-Whitney U test, with a two-sided p value less than 0.05 considered statistically significant. Correlations between *CYP3A4* and *CYP3A5* ileal gene expression and pharmacokinetic parameters of oral midazolam were analyzed in transplant subjects using Spearman's rank correlations, with a one-tailed p value less than 0.05 considered a significant correlation.

5.3   RESULTS OF PHARMACOKINETIC ANALYSES

5.3.1   Composite analysis of pharmacokinetic parameters between subject groups

The pharmacokinetic parameters of intravenous midazolam are presented in Table 9. There were no significant differences in parameters between subject groups.

Table 11. Pharmacokinetic parameters of midazolam after IV administration

	Transplant Session 1 (n=16)	Transplant Session 2 (n=10)	Control Subjects (n=16)	P value* Tx1 vs. Tx2	P value[§] Tx1 vs. C	P value[§] Tx2 vs. C
MDZ AUC_{IV} (mcg*hr/L)	54.4 (41.4,74.7)	84.6 (56.0,182)	76.3[a] (61.3,124)	0.074[b]	0.093	0.68
CL_{IV} (L/hr/kg)	0.480 (0.290,0.580)	0.210 (0.190,0.425)	0.420 (0.230,0.500)	0.068	0.34	0.15
Vz (L/kg)	1.40 (1.13,1.83)	1.62 (1.38,2.15)	1.36 (0.860,2.21)	0.077	0.71	0.55
1'OHMDZ AUC_{IV} (mcg*hr/L)	8.22 (4.42,10.6)	9.08 (5.08,11.3)	8.41[a] (4.97,19.6)	1.0[b]	0.51	0.85
1'OHMDZ/ MDZ AUC $ratio_{IV}$	0.117 (0.0875,0.134)	0.0860 (0.0615,0.123)	0.0990[a] (0.0600,0.135)	0.48[b]	0.77	0.47

Parameters are median (25th, 75th percentile). *Comparisons between transplant sessions 1 and 2 made using the Wilcoxon matched-pairs signed rank test on ten matched pairs. Numbers in first column are median (25th, 75th percentile) for all 16 transplant session 1 subjects. Median concentrations in the 10 Tx1 subjects used in the paired analysis only are reported in the text as appropriate. [§]Comparisons between transplant session 1 and controls and transplant session 2 and controls made using the Mann-Whitney U test. Two-tailed p values reported. [a]n=15 for controls (one subject was not given midazolam IV). [b]Test performed on 9 pairs because one subject only received 1 mg midazolam IV.

The pharmacokinetic parameters of midazolam after oral administration are presented in Table 10. Midazolam oral solution was rapidly absorbed in all subject groups. However, control subjects reached Tmax significantly more quickly than transplant subjects at session 1 (Tx1) or session 2 (Tx2), with a median of 0.5 hr in controls compared with 1.0 hr in Tx1 (p=0.020) and 0.75 hr in Tx2 (p=0.0055). There was no significant difference in median oral Cmax between the three groups, but the AUC of midazolam was significantly higher in Tx1 compared with Tx2 (65.4 mcg*hr/L vs. 46.4 mcg*hr/L, p=0.0020), as well as Tx1 compared to controls (52.1 mcg*hr/L vs. 39.3 mcg*hr/L, p=0.037). There was no significant difference in AUC between Tx2 and controls (46.4 mcg*hr/L vs. 39.3 mcg*hr/L, p=0.48). A similar trend was observed for bioavailability (F), which was significantly higher in Tx1 than in Tx2 (0.600 vs. 0.285, p=0.0059) and Tx1 compared with controls (0.565 vs. 0.220, p=0.0010), but not significantly different between Tx2 and controls (0.285 vs. 0.220, p=0.45). Weight-adjusted apparent oral clearance (CL/F) was not significantly different between Tx1 and Tx2 (1.07 L/hr/kg vs. 1.12 L/hr/kg, p=0.38) nor between Tx2 and controls (1.12 L/hr/kg vs. 1.85 L/hr/kg, p=0.14). However, CL/F was significantly higher in control subjects as compared with Tx1 (1.85 L/hr/kg vs. 1.07 L/hr/kg, p=0.019). Similarly, weight-adjusted apparent volume of distribution (Vz/F) was significantly higher in controls than in Tx1 (7.10 L/kg vs. 4.78 L/kg, p=0.050). Moreover, Vz/F came close to statistical significance in a comparison of controls with Tx2 (7.10 L/kg vs. 4.53 L/kg, p=0.054) but not in a comparison of Tx1 with Tx2 (4.32 L/kg vs. 4.53 L/kg, p=0.065).

Table 12. Pharmacokinetic parameters of midazolam after oral administration

	Transplant Session 1 (n=16)	Transplant Session 2 (n=10)	Control Subjects (n=16)	P value* Tx1 vs. Tx2	P value[§] Tx1 vs. C	P value[§] Tx2 vs. C
$Cmax_{po}$ (mcg/L)	19.8 (13.8,32.6)	16.9 (12.0,25.5)	24.7 (16.0,31.8)	0.38	0.61	0.22
$Tmax_{po}$ (hr)	1.00 (0.500,1.50)	0.750 (0.500,1.63)	0.500 (0.313,0.500)	0.68	**0.020**	**0.0055**
MDZ AUC_{po} (mcg*hr/L)	52.1 (41.5,79.5)	46.4 (30.2,64.1)	39.3 (27.1,50.7)	**0.0020**	**0.037**	0.48
F	0.565 (0.370,0.698)	0.285 (0.0850,0.360)	0.220[a] (0.150,0.290)	**0.0059**	**0.0010**	0.45
CL/F (L/hr/kg)	1.07 (0.733,1.52)	1.12 (0.655,1.99)	1.85 (1.05,2.62)	0.38	**0.019**	0.14
Vz/F (L/kg)	4.78 (2.99,6.61)	4.53 (3.76,6.34)	7.10 (4.60,9.09)	0.065	**0.050**	0.054
MRTlast (hr)	2.48 (2.02,3.00)	2.46 (1.88,2.68)	1.83 (1.51,2.08)	0.36	**0.0014**	**0.0027**
1'OHMDZ AUC_{po} (mcg*hr/L)	14.5 (11.2,19.3)	14.2 (10.4,29.8)	19.0 (13.9,26.9)	0.49	0.086	0.33
1'OHMDZ/MDZ AUC $ratio_{po}$	0.255 (0.183,0.386)	0.290 (0.243,0.843)	0.549 (0.342,0.725)	0.11	**0.00070**	0.13

Parameters are median (25th, 75th percentile). *Comparisons between transplant sessions 1 and 2 made using the Wilcoxon matched-pairs signed rank test on ten matched pairs. [§]Comparisons between transplant session 1 and controls and transplant session 2 and controls made using the Mann-Whitney U test. Numbers in first column are median (25th, 75th percentile) for all 16 transplant session 1 subjects. Median concentrations in the 10 Tx1 subjects used in the paired analysis only are reported in the text as appropriate. Two-tailed p values reported. [a]n=15 for controls (one subject was not given midazolam IV). [b]Test performed on 9 pairs because one subject only received 1 mg midazolam IV.

Figure 22. Midazolam bioavailability between transplant sessions 1 and 2
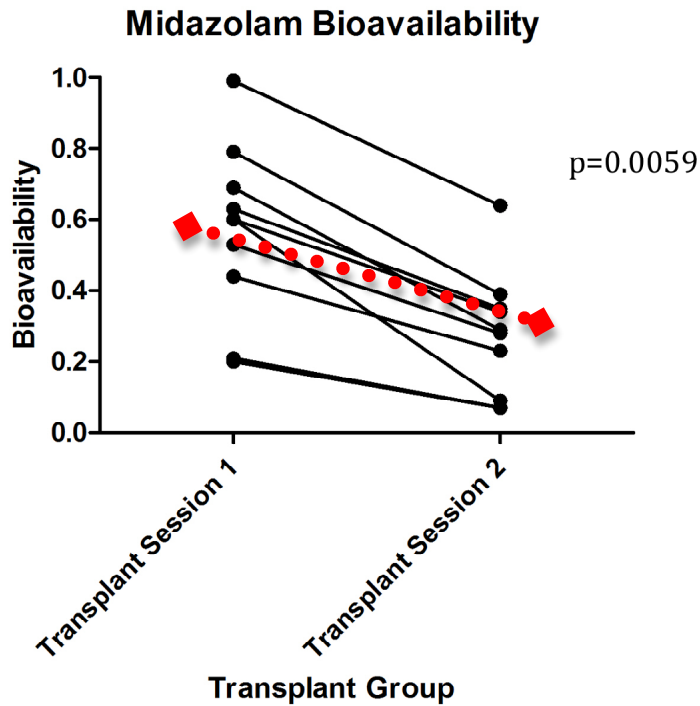
Dashed line represents medians

There was no significant difference between the AUC of 1'OHMDZ between Tx1 and Tx2 (14.5 mcg*hr/L vs. 14.2 mcg*hr/L, p=0.49); between Tx1 and controls (14.5 mcg*hr/L vs. 19.0 mcg*hr/L, p=0.086); or between Tx2 and controls (14.2 mcg*hr/L vs. 19.0 mcg*hr/L, p=0.33). However, the 1'OHMDZ/MDZ AUC ratio was significantly higher in control subjects compared to Tx1 (0.549 vs. 0.255, p=0.00070); although not between Tx1 and Tx2 (0.244 vs. 0.290, p=0.11) or between Tx2 and controls (0.290 vs. 0.549, p=0.13).

Figure 23. Midazolam concentration vs. time profiles in transplant subjects at Sessions 1 and 2
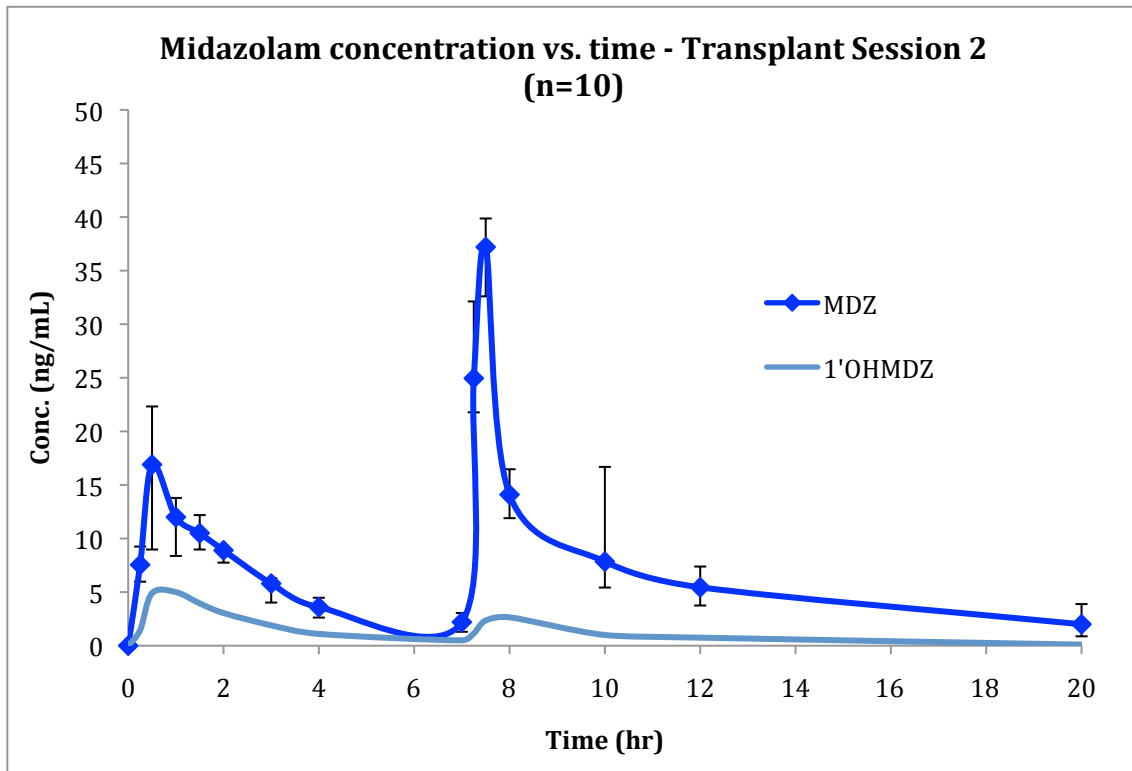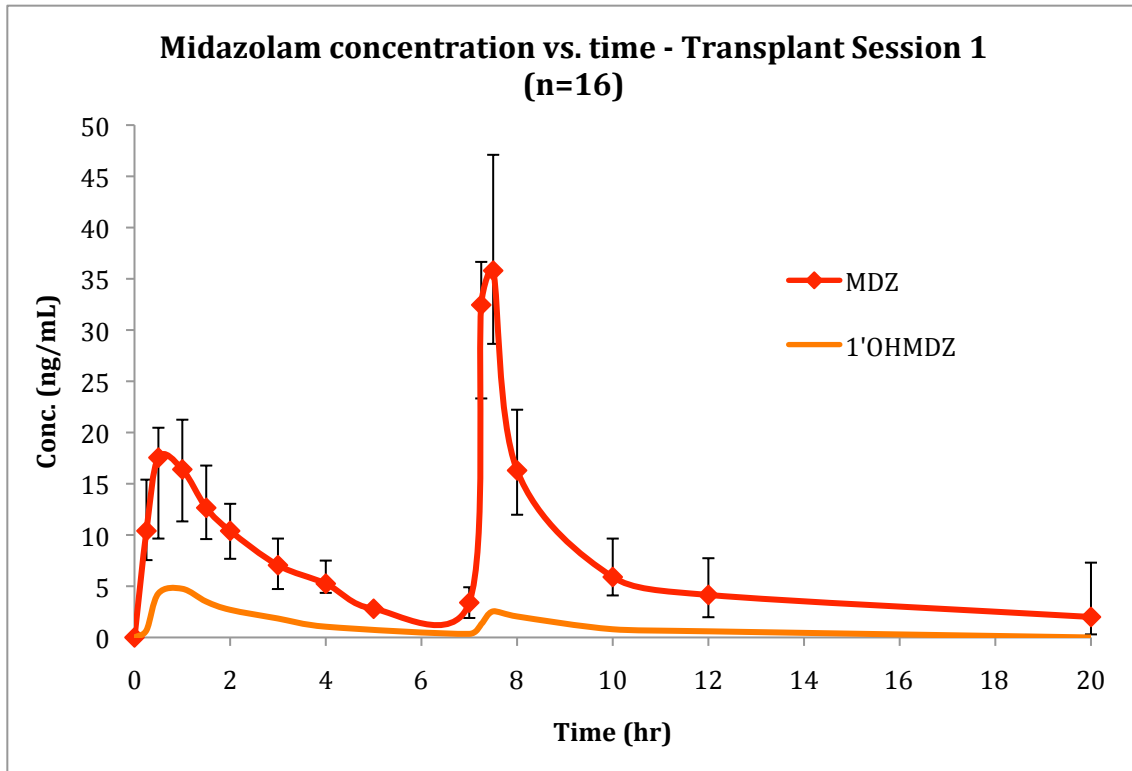
Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

Figure 24. Midazolam concentration vs. time in control subjects
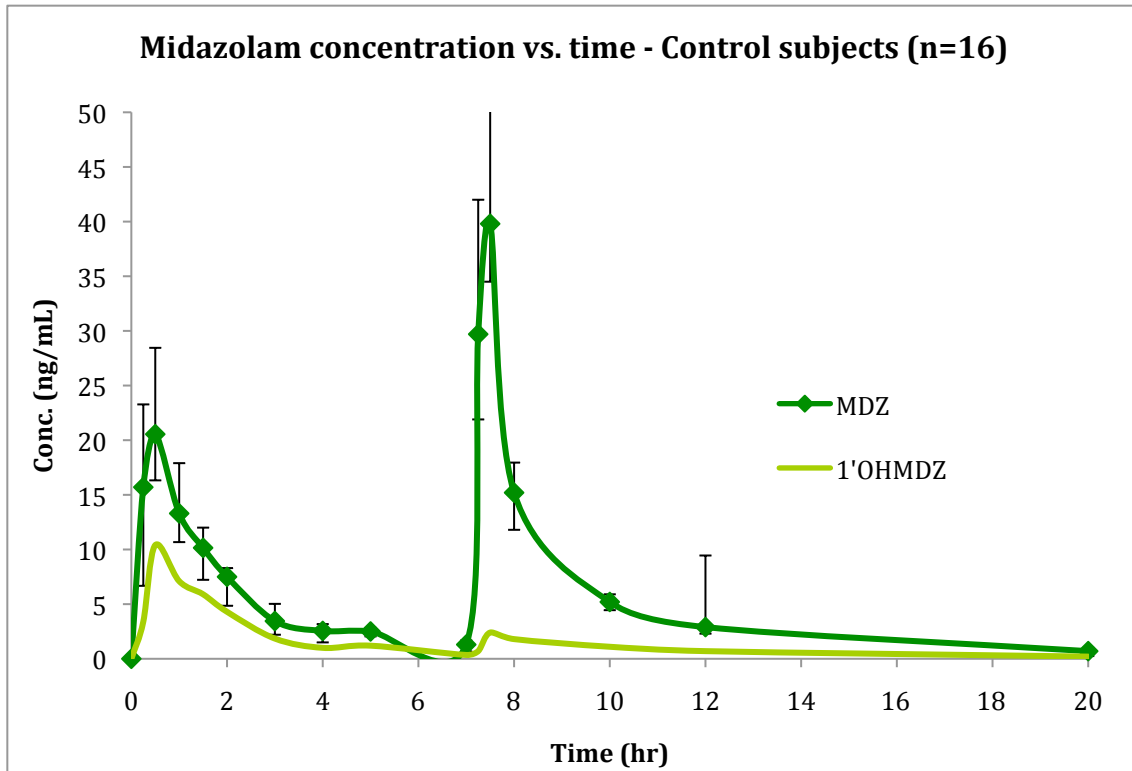
Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

Figure 25. Oral midazolam profiles in all three subject groups

Oral Midazolam in all three subject groups

Midazolam concentration (mcg/L) vs. Time (hr)

Tx Session 1
Tx Session 2
Controls
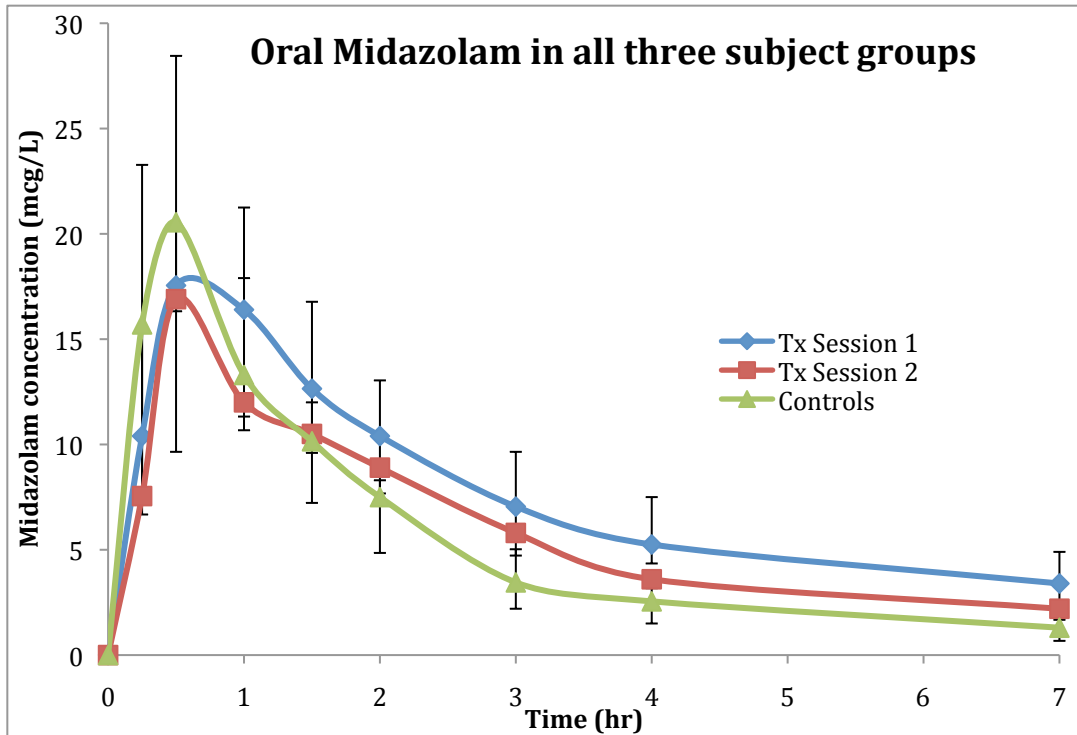
Figure 26. 1'hydroxymidazolam profiles after oral midazolam

1'OHMDZ concentration vs. time in all three study groups after oral midazolam

Conc. (ng/mL) vs. Time (hr)

Tx Session 1
Tx Session 2
Controls

121
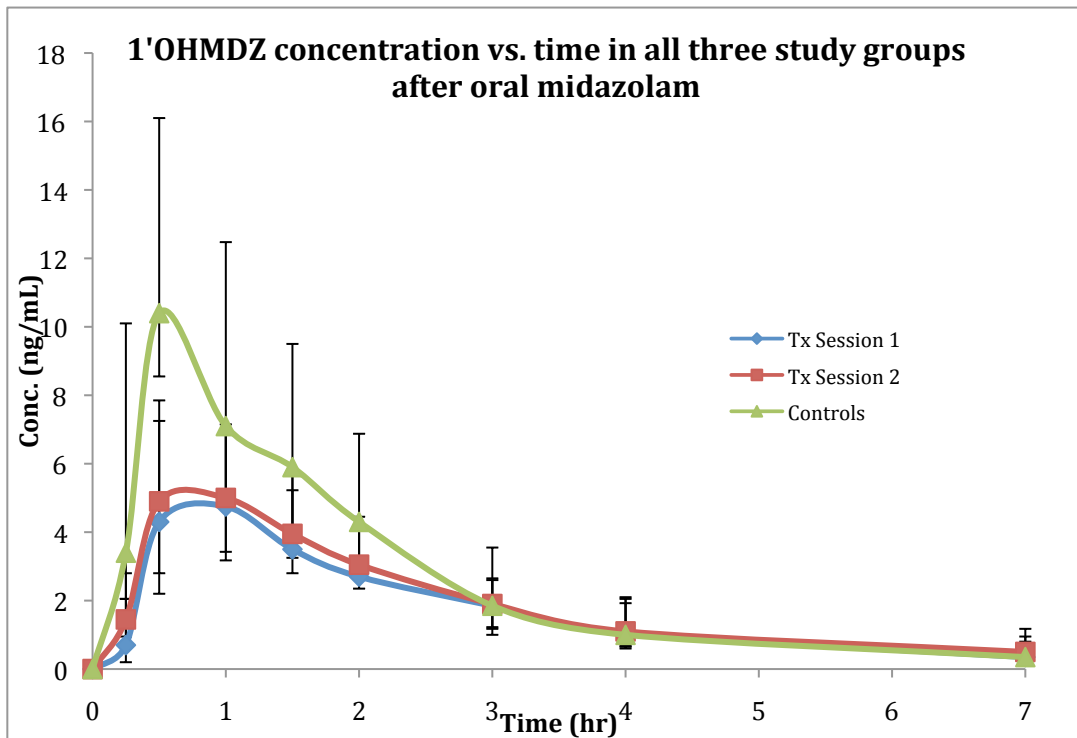
5.3.2 Comparison of J-tube and oral administration in transplant subjects at the first study session

There were no differences in the pharmacokinetic parameters of midazolam and 1'hydroxymidazolam between subjects receiving medications via jejunostomy tube (J-tube, n=4) or by mouth (n=12). There was a trend towards a higher Vz/F in the J-tube group (6.48 vs. 4.32 L/kg, p=0.060) but no significant difference in F (0.615 vs. 0.520, p=0.47); Cmax (19.5 vs. 19.8 mcg/L, p=0.95); Tmax (0.500 vs. 1.00 hr, p=0.54); oral AUC (48.5 vs. 55.6 mcg*hr/L, p=0.50); or CL/F (0.975 vs. 1.07 L/hr/kg, p=0.86) between the two routes of administration. There was also no observed difference in 1'hydroxymidazolam oral AUC (16.8 vs. 14.5 mcg*hr/L, p=0.95) or 1'hydroxymidazolam/midazolam ratio (0.338 vs. 0.215, p=0.59) between midazolam administration by the J-tube or oral route.

Table 13. Pharmacokinetic parameters of oral midazolam by route of administration

	J-tube (n=4)	PO (n=12)	P value* JT vs. PO
$Cmax_{po}$ (mcg/L)	19.5 (13.4,41.1)	19.8 (13.8, 32.6)	0.95
$Tmax_{po}$ (hr)	0.500 (0.500,1.25)	1.00 (0.313,1.88)	0.54
MDZ AUC_{po} (mcg*hr/L)	48.5 (32.8,73.8)	55.6 (41.5,93.4)	0.50
F	0.615 (0.450,0.758)	0.520 (0.248,0.698)	0.47
CL/F (L/hr/kg)	0.975 (0.780,2.02)	1.07 (0.668,1.52)	0.86
Vz/F (L/kg)	6.48 (4.89,12.6)	4.32 (2.49,5.69)	0.060
MRTlast (hr)	2.35 (1.88,2.97)	2.48 (2.07,3.08)	0.76
1'OHMDZ AUC_{po} (mcg*hr/L)	16.8 (9.43,20.4)	14.5 (11.2,18.0)	0.95
1'OHMDZ/MDZ AUC $ratio_{po}$	0.338 (0.198,0.432)	0.215 (0.183,0.363)	0.59

Parameters are median (25th, 75th percentile). *Comparisons made using the Mann-Whitney U test. Two-tailed p values reported.

Figure 27. Oral midazolam concentration-time profiles by route of administration – Session 1
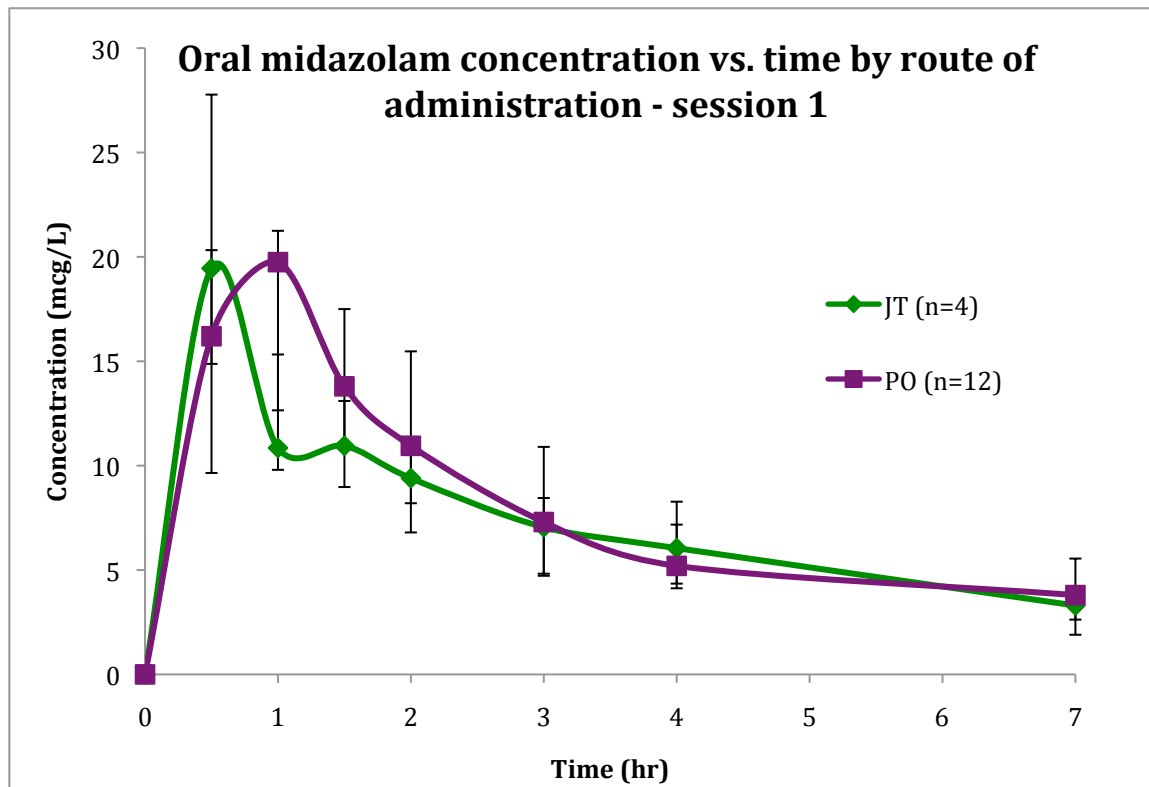
Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

5.3.3 Comparison of pharmacokinetics of oral midazolam in isolated intestinal (II) and modified multivisceral (MM) recipients

There were no significant differences in the pharmacokinetic parameters of midazolam after oral administration between modified multivisceral (MM, n=4) and isolated small bowel (II, n=12) recipients at either study session 1 or 2 (See figure 4).   There was a trend towards a lower Cmax in MM vs. II (17.6 vs. 19.8 mcg/L, p=0.50) as well as a higher Tmax (1.25 vs. 0.750 hr, p=0.71) and MRT (3.15 vs. 2.44 hr, p=0.16). At study session 2, the same patterns were observed, with a

median Cmax of 13.4 mcg/L in MM vs. 23.9 mcg/L in II (0.38).   Median Tmax was also higher

in MM vs. II (1.00 vs. 0.500, p=0.46), but the difference did not reach statistical significance.

Table 14. Pharmacokinetic parameters of oral midazolam by transplant type at Session 1

Session 1	MM (n=4)	II (n=12)	P value* MM vs. II
Cmax$_{po}$ (mcg/L)	17.6 (9.43,32.5)	19.8 (16.2,35.9)	0.50
Tmax$_{po}$ (hr)	1.25 (0.438,1.88)	0.750 (0.500,1.50)	0.71
MDZ AUC$_{po}$ (mcg*hr/L)	52.1 (43.2,152.3)	53.1 (39.2,78.5)	0.76
F	0.570 (0.458,0.750)	0.565 (0.248,0.698)	0.67
CL/F (L/hr/kg)	0.895 (0.260,1.45)	1.07 (0.780,1.52)	0.50
Vz/F (L/kg)	4.54 (2.20,6.35)	4.79 (2.99,7.31)	0.59
MRT (hr)	3.15 (2.21,3.50)	2.44 (2.02,2.58)	0.16
1'OHMDZ AUC$_{po}$ (mcg*hr/L)	16.1 (9.35,23.6)	14.5 (11.2,18.0)	0.76
1'OHMDZ/MDZ AUC ratio$_{po}$	0.298 (0.102,0.445)	0.255 (0.183,0.363)	0.86

*Comparisons made using the Mann-Whitney U test. Numbers are median (25th, 75th percentile).   Two-tailed p

values reported.

Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

Table 15. Pharmacokinetic parameters of oral midazolam by transplant type at Session 2

Session 2	MM (n=3)	II (n=7)	P value[*] MM vs. II
$Cmax_{po}$ (mcg/L)	13.4 (11.5,17.6)	23.9 (12.1,29.9)	0.38
$Tmax_{po}$ (hr)	1.00 (0.500,3.00)	0.500 (0.500,1.00)	0.46
MDZ AUC_{po} (mcg*hr/L)	43.1 (30.5,60.2)	49.6 (29.3,75.7)	1.0
F	0.350 (0.230,0.390)	0.280 (0.070,0.340)	0.36
CL/F (L/hr/kg)	0.830 (0.640,2.16)	1.40 (0.660,1.93)	1.0
Vz/F (L/kg)	3.82 (3.56,6.07)	5.05 (3.99,7.15)	0.52
MRTlast (hr)	2.64 (1.88,3.48)	2.46 (1.87,2.60)	0.36
1'OHMDZ AUC_{po} (mcg*hr/L)	12.6 (8.79,15.0)	16.9 (10.7,47.3)	0.27
1'OHMDZ/MDZ AUC ratio$_{po}$	0.288 (0.249,0.291)	0.310 (0.224,0.916)	0.67

Parameters are median (25th, 75th percentile). [*]Comparisons made using the Mann-Whitney U test. Two-tailed p values reported.

5.4   RT-PCR RESULTS

5.4.1   Biopsy tissues obtained

Two ileal mucosal biopsy samples were obtained from 15 of the 16 transplant subjects undergoing study session 1 and 10 of the 10 transplant subjects undergoing study session 2. The two samples per session (together weighing approximately 25 mg) were combined for PCR analysis. Of the samples collected from the 15 subjects at the time of study session 1, two yielded insufficient mRNA for analysis, which was consequently performed on 13 biopsy samples taken at study session 1 and 10 samples taken at study session 2.   Since the three missing values in the Tx1 group (one biopsy not done, two with insufficient RNA) belonged to subjects who returned for study session 2, paired analysis could only be performed on seven pairs.

5.4.2   Expression levels of *CYP3A4* and *CYP3A5*

Expression levels of *CYP3A4* and *CYP3A5*, and total *CYP3A* mRNA are shown in tables 16 and 17. Median (25th, 75th percentile) expression levels of *CYP3A4* relative to cyclophilin were 0.182 (0.0630,0.846) at Tx1 and 0.536 (0.300, 0.937) at Tx2 (p=0.16); levels of *CYP3A5* were 0.0360 (0.0130,0.0780) at Tx1 and 0.109 (0.0100,0.241) at Tx2 (p=0.078); and levels of total CYP3A were 0.261 (0.0990,0.956) at Tx1 and 0.561 (0.356,1.15) at Tx2 (p=0.078).

Table 16. Paired comparison of ileal *CYP3A* and *PXR* expression

mRNA	Transplant Session 1 (n=7)	Transplant Session 2 (n=7)	P value Tx1 vs. Tx2
CYP3A4	0.182 (0.0630,0.846)	0.536 (0.300,0.937)	0.16
CYP3A5	0.0360 (0.0130,0.0780)	0.109 (0.0100,0.241)	0.078
Total *CYP3A*	0.261 (0.0990,0.956)	0.561 (0.356,1.15)	0.078
NR1I2 (PXR)	0.157 (0.096,0.283)	0.183 (0.174,0.195)	1.0

Amounts are relative to cyclophilin and are expressed as median (25th, 75th percentile). Comparison made using the

Wilcoxon matched-pairs signed-rank test. Two-tailed p values reported.

Table 17. Ileal *CYP3A* and *PXR* expression in all transplant subjects

mRNA	Transplant Session 1 (n=13)	Transplant Session 2 (n=10)
CYP3A4	0.450 (0.159,0.722)	0.591 (0.272,0.898)
CYP3A5	0.0470 (0.0155,0.0945)	0.0825 (0.00975,0.190)
Total *CYP3A*	0.516 (0.200,0.848)	0.610 (0.315,1.08)
NR1I2 (PXR)	0.142 (0.0972,0.212)	0.186 (0.169,0.193)

Amounts are relative to cyclophilin and are expressed as median (25th, 75th percentile)

5.5   CORRELATION BETWEEN PLASMA CYTOKINE CONCENTRATIONS AND PHARMACOKINETIC PARAMETERS OF MIDAZOLAM

5.5.1   Study session 1

At study session 1, a strong correlation was observed between AUC of midazolam and plasma TNF-α concentrations (r_s=0.71, p=0.0021) and IL-10 concentrations (r_s=0.53, p=0.036) .   This is shown in Figures 28 and 29.

Figure 28. Correlation between oral midazolam AUC and plasma TNF-α in transplant subjects at study session 1
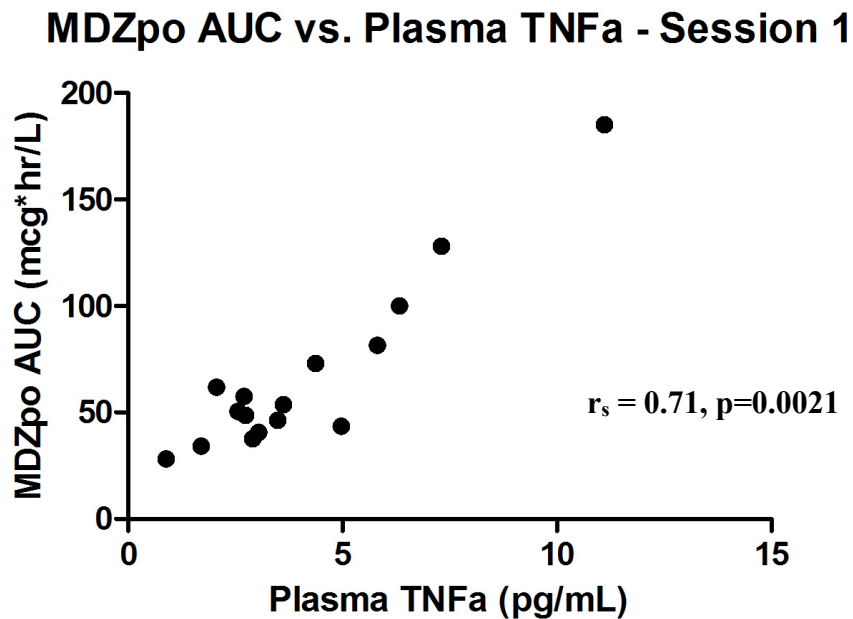
Figure 29. Correlation between midazolam oral AUC and plasma IL-10 concentrations in transplant subjects at study session 1

Midazolam oral AUC vs. Plasma IL-10 - Session 1

$r_s = 0.53$, p=0.036
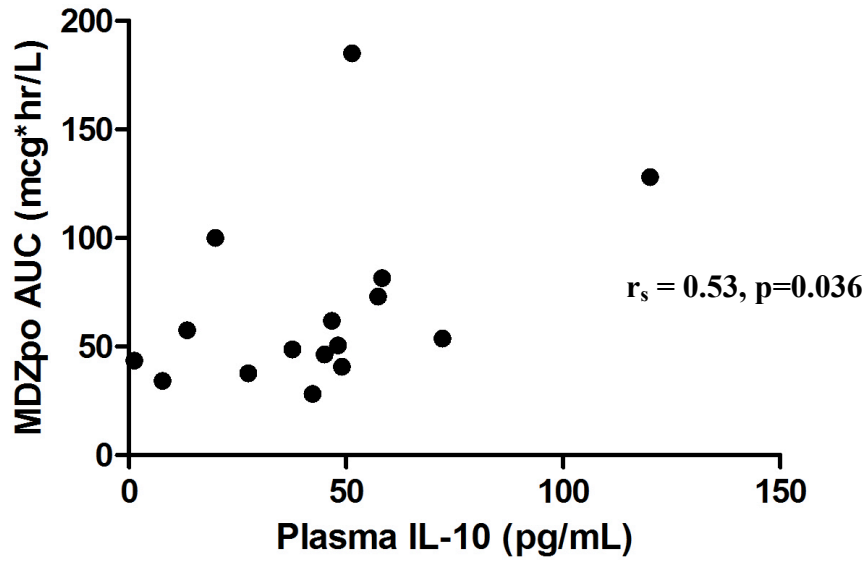
In addition, significant correlations between oral bioavailability of midazolam and plasma IL-2 (r_s=0.42, p=0.10), plasma IL-6 (r_s=0.67, p=0.0081), and plasma IFN-γ (r_s=0.56, p=0.026) were observed. These correlations are shown in figures 30, 31, and 32.

Figure 30. Correlation between midazolam bioavailability and plasma IL-2 in transplant subjects at study session 1

Midazolam Bioavailability vs. Plasma IL-2 - Session 1
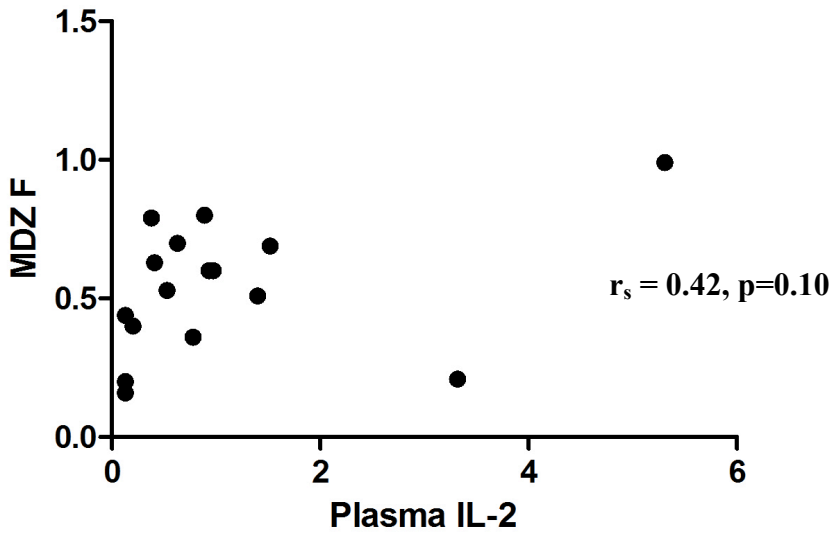
Figure 31. Correlation between midazolam bioavailability and plasma IL-6 concentrations in transplant subjects at studys session 1

Midazolam Bioavailability vs. Plasma IL-6 - Session 1
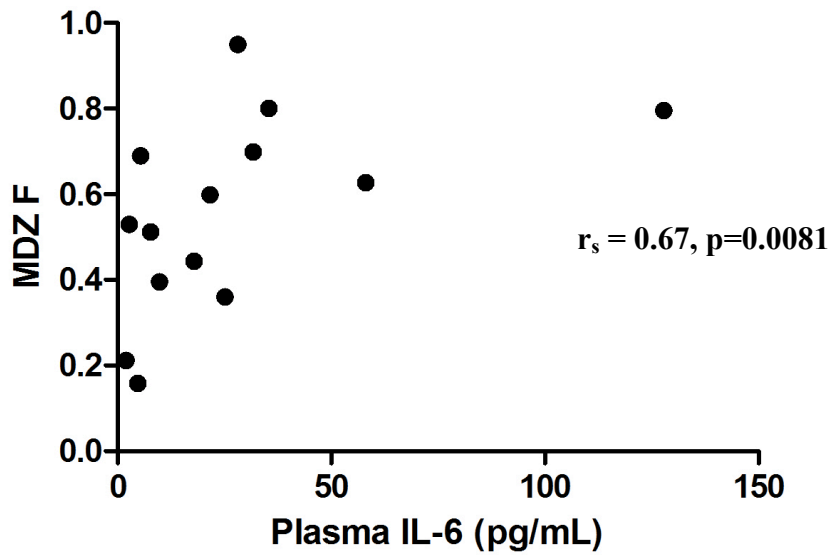
Figure 32. Correlation between midazolam bioavailability and plasma IFN-γ in transplant subjects at study session 1

Midazolam Bioavailability vs. Plasma IFNy - Session 1
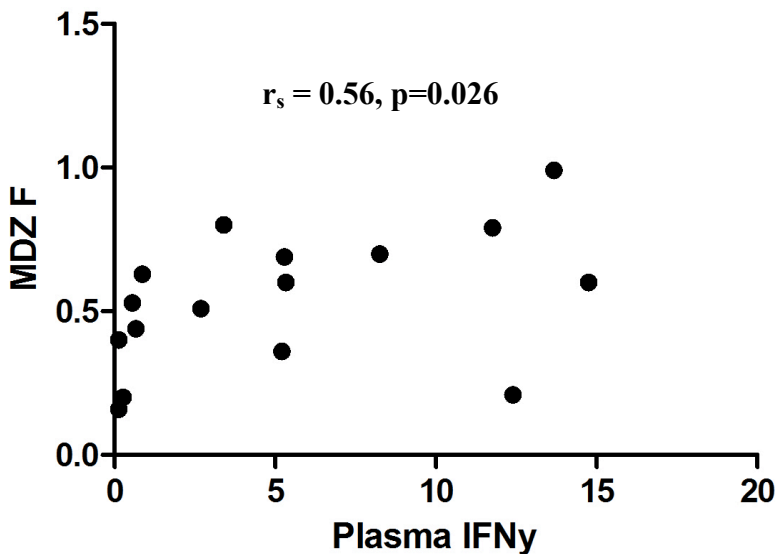
Figure 33. Correlation between 1'OHMDZ/MDZ oral AUC ratio and plasma TNF-α in transplant subjects at study session 1

1'OHMDZ/MDZpo AUC ratio vs. Plasma TNFa--Session 1
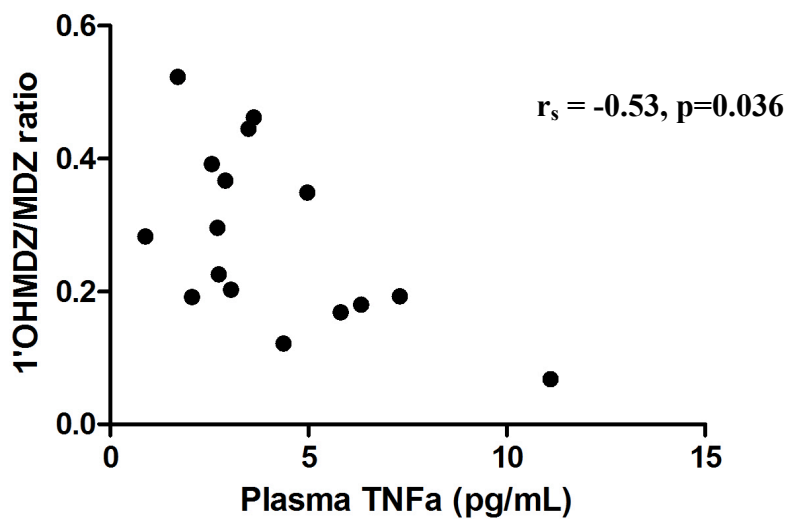
Figure 34. Correlation between midazolam CL/F/kg and plasma TNF-alpha concentrations in transplant subjects at study session 1

Midazolam CL/F/kg vs. Plasma TNFa - Session 1

$r_s = 0.57, p=0.021$
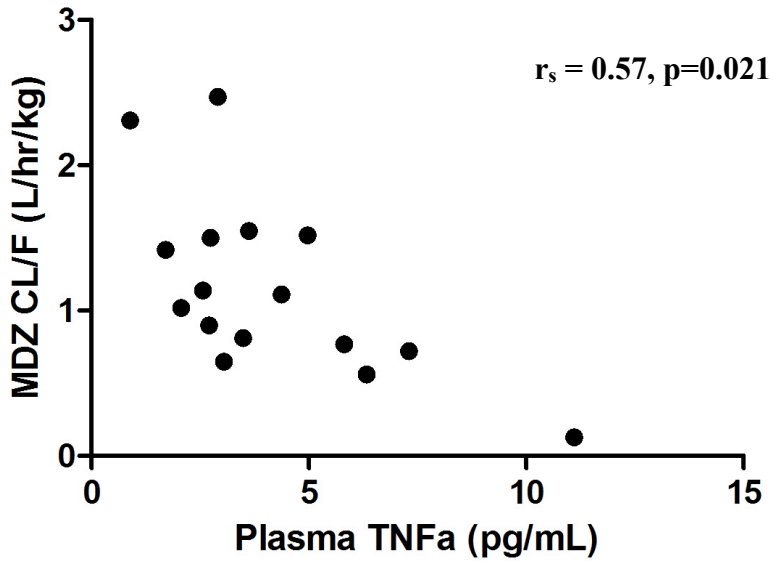
Figure 35. Correlation between difference in 1'OHMDZ/MDZ oral AUC ratio and difference in plasma IL-10 levels between study sessions in transplant subjects

**Within-subject correlation
1'OHMDZ/MDZ AUCpo ratio vs. plasma IL-10**

$r_s = 0.59, p=0.081$
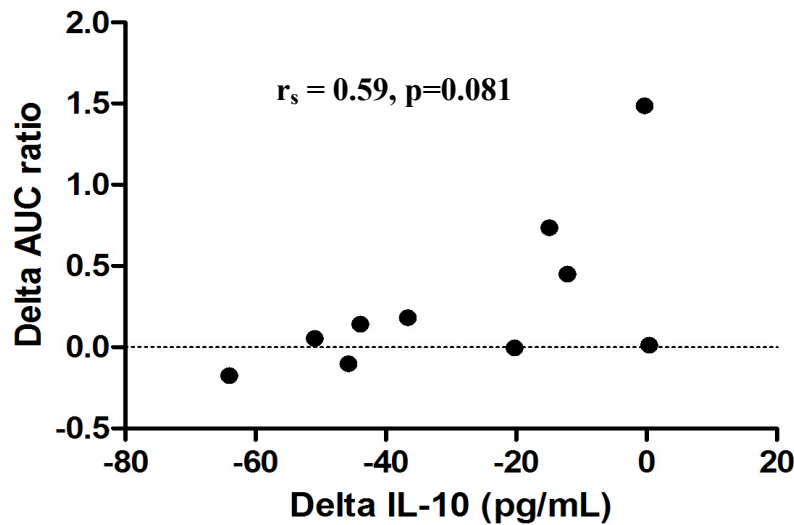
Figure 36. Correlation between difference in 1'OHMDZ/MDZ oral AUC ratio and difference in plasma IFN-γ levels between study sessions in transplant subjects

Within-subject correlation
1'OHMDZ/MDZ AUCpo ratio vs. plasma IFNy

$r_s = -0.76, p=0.015$

Delta AUC ratio (y-axis)
Delta IFNy (pg/mL) (x-axis)
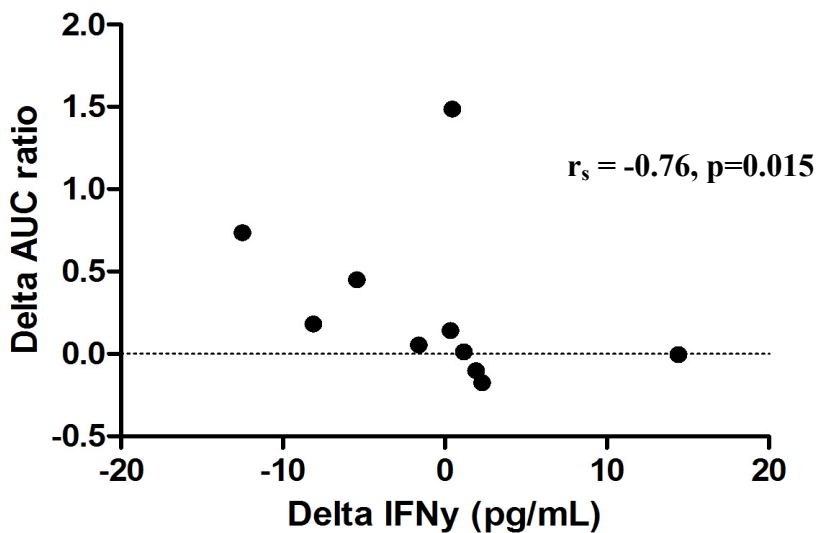
5.5.2 Relationship between % hepatic steatosis and IV AUC of midazolam

There was no correlation observed between degree of hepatic steatosis on pre-transplant liver biopsy and AUC of IV midazolam at study session 1 (Figure 36).

Figure 37. Relationship between pre-transplant % hepatic steatosis and IV midazolam AUC at study session 1
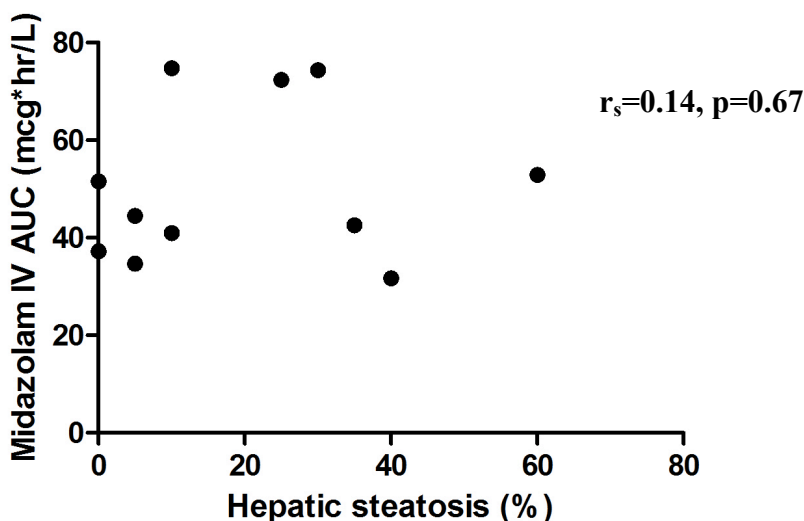
5.6   DISCUSSION

These results show distinctive differences in intestinal absorption and first-pass metabolism of oral midazolam, both between transplant sessions 1 and 2 and between transplant groups and control subjects.   The similarity in midazolam disposition between groups after IV administration indicates that the differences seen are occurring at the level of the transplanted intestine and not the native liver.   These data also show clear differences in rate and extent of absorption of midazolam as well as the extent of metabolism between transplant subjects at different timepoints and between transplant subjects and control subjects.

Midazolam is a BCS and BDDCS class I drug(Amidon et al., 1995; Benet et al., 2011), highly soluble (and in this case administered in solution form), readily absorbed through the enterocyte membrane, and extensively metabolized. Therefore, the absorption rate (reflected in

Tmax and to some extent Cmax) after oral administration will depend largely on the gastric emptying rate(Amidon et al., 1995), which under normal circumstances for a liquid is extremely rapid. Gastric emptying may be delayed in small bowel transplant recipients(Campbell et al., 1993; Furukawa et al., 1994), and this is reflected in the presented data by the significantly higher Tmax in transplant subjects – both early and late post-transplant – as compared with control subjects. This data provides evidence that slower gastric emptying persists long term even in clinically stable small bowel transplant recipients.

The area under the concentration-time curve (AUC) after oral administration and bioavailability (F) reflect the degree both of absorption and first-pass metabolism of the drug. Both AUC and F were significantly higher in the early post-transplant period as compared with the later post-transplant period, and in the early period as compared with control subjects. However, there was no significant difference in AUC or F between the later post-transplant period and healthy control subjects. This is an indication that more midazolam was reaching the systemic circulation after oral administration early post-transplant as compared with later post-transplant and with controls. Particularly striking is the fact that midazolam F was higher in the early post-transplant period as opposed to the later post-transplant in every transplant subject who underwent both study sessions as shown in Figure 14. Since Vz and CL after IV midazolam showed no difference between groups, it is likely that the lower CL/F and Vz/F values seen in transplant subjects in the early post-transplant period as compared with controls are due to the higher bioavailability (F) in transplant subjects.

Although it is possible that the increases in AUC and F of midazolam early post-transplant are at least in part a consequence of increased intestinal permeability due to altered epithelial barrier integrity or alterations in motility leading to increased contact of the drug with

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the enterocyte membrane, the additional evidence favors impairment of CYP-mediated intestinal first-pass metabolism as the most likely cause of the observation. The ratio of the oral AUC of 1'hydroxymidazolam to midazolam (a measure of the extent of first-pass metabolism by CYP3A) was significantly lower in transplant subjects early post-transplant as compared with controls. The ratio of the concentration of 1'hydroxymidazolam/midazolam at a single timepoint, usually either 20 or 30 minutes after IV administration, has been proposed by some as a marker of hepatic CYP3A metabolic activity that correlates strongly with midazolam clearance, but results have been mixed(Link et al., 2008; Rogers et al., 2002). The ratio of AUCs is presented here since it takes the entire sampling interval into account rather than simply relying on a single point estimate.

Another reason the differences in AUC and F are likely to be due to impaired metabolism is the difference in ileal mRNA expression of *CYP3A4* and *CYP3A5* between transplant subjects, early and late post-transplant. Although the difference did not reach statistical significance, probably due to the small number of paired samples available (n=7), the median *CYP3A4, CYP3A5,* and total CYP3A expression was lower at the early post-transplant session as compared with the later post-transplant session. It should also be noted that there was a significant negative relationship between *CYP3A4* expression and the AUC after oral midazolam. This is most likely the first study to assess the correlation between the pharmacokinetics of midazolam and intestinal *CYP3A* expression *in vivo*.

Interestingly, there was no significant effect resulting from differences in the route of administration (JT vs. PO) at transplant session 1 and between transplant types (MM vs. II) at either study session on the pharmacokinetics of oral midazolam. The only difference seen between routes of administration in Figure 19 is a slightly lower median Tmax in the JT group, a

difference that was not statistically significant (p=0.54). Although this may be due to the small number of subjects, especially in the JT and MM groups, the lack of difference implies that for highly soluble and highly permeable BCS/BDDCS class I drugs such as midazolam, enteral route of administration does not have a significant effect on the absorption of the drug. Administration via J-tube bypasses the stomach and thus gastric emptying has no effect on the absorption rate, but it also bypasses the duodenum and a small portion of jejunum, and therefore the available absorptive surface area is less, possibly leading to a smaller AUC for poorly absorbed drugs. However, for a highly permeable drug such as midazolam, the difference was minimal.

The pharmacokinetics of midazolam were also only minimally affected by transplant subtype in the subjects studied. Upon examination of Figure 19, a higher Tmax (i.e. decrease in rate of absorption) can be seen in the MM subjects as compared with the II at session 1, but this does not translate to any difference in AUC (p=0.76) or F (p=0.67). This higher Tmax in MM subjects could be due to a slower rate of gastric emptying in the MM subjects. Although MM patients have been demonstrated in one study to be more likely to have rapid gastric emptying due to pyloroplasty, there may still be a degree of impairment in emptying, especially in the early post-transplant period in the presence of increased immune activity. At study session 2, the median Tmax was still higher in the MM subjects as compared with II, and the median AUC and Cmax were smaller, but as with session 1, the differences did not reach statistical significance. This finding warrants further investigation with a larger group of both MM and II patients, since the small number of subjects in each group at session 2 (II=7 and MM=3) make it difficult to detect any but the most dramatic differences in parameters with any certainty. However, these results do imply motility and/or absorption differences between MM and II patients that persist into the later post-transplant period, even in clinically stable small bowel allograft recipients.

The effects of graft drainage to the caval rather than the portal circulation should be mentioned. As noted in the demographics chapter, only one of the 16 transplant subjects had this anatomical arrangement after isolated intestinal transplant, a number which did not allow for an evaluation of the differences in first-pass metabolism with caval drainage. This subject only underwent study session 1, where his pharmacokinetic profile of midazolam was unremarkable, showing a bioavailability of 0.36, midazolam oral AUC of 34.2 mcg*hr/L, and an oral 1'hydroxymidazolam/midazolam ratio of 0.523. This bioavailability and AUC are lower than the medians of 0.570 and 52.1 mcg*hr/L in II subjects at session 1, and the AUC ratio is higher than the median of 0.298. But it should also be noted that the donor of this subject's graft was African-American, and it is known that African-Americans are significantly more likely than Caucasians to be expressers of the *CYP3A5*1* allele, which leads to production of a functional CYP3A5 enzyme and is associated with enhanced CYP3A-mediated metabolism and lower bioavailability of tacrolimus.

Although the amount of tissue retrieved during ileal biopsies was not enough to do genotyping of the graft (which, it should be remembered, may be different from the genotype of the recipient), the fact that this subject's relative *CYP3A5* expression level was 0.225, nearly four times the median of 0.047 seen in session 1, is a strong indicator that the subject was at least heterozygous for the *CYP3A5*1* allele, and the presence of a functional intestinal CYP3A5 enzyme may have compensated for the absence of hepatic first-pass metabolism. The PCR primer used in this study will only amplify the functional form of the enzyme. However, homozygous carriers of the *CYP3A5*3* allele will produce very small amounts of the functional enzyme in addition to the truncated form, leading to detection of small amounts of *CYP3A5* mRNA by RT-PCR. It should also be noted that the two other transplant subjects who had

African-American donors had AUC ratios of 0.445 and 0.392, higher than the median. One of these also had a *CYP3A5* expression level of 0.111 at the first study session and 0.241 at the second, but the remaining subject had a low relative *CYP3A5* expression level of 0.018. Hence, pharmacokinetic studies should always take the graft drainage arrangement into account when analyzing small bowel transplant pharmacokinetic data, although graft genotype and intestinal CYP3A expression levels may compensate for the lack of hepatic first-pass metabolism.

This is the first study to assess intestinal CYP3A function and expression in the transplanted small bowel and it has demonstrated a significant reduction in the extent of metabolism of the CYP3A probe substrate midazolam in the early post-transplant period. These results have clinical ramifications for small bowel transplant recipients. As discussed in the introduction, small bowel transplant recipients receive numerous oral CYP3A substrates after transplant, some of which have narrow therapeutic indices and are not easily assayed in blood or plasma. These results reveal that intestinal CYP3A metabolism is impaired in the early post-transplant period, and that extra caution should be used when dosing oral CYP3A substrates.

While these results have ramifications for the dosing of all oral CYP3A substrates in small bowel transplant recipients, they are not translatable to all drugs, given that – as discussed earlier – the biopharmaceutical characteristics of the drug also play a role in oral disposition. However, these results with midazolam are highly relevant to BCS/BDDCS class I CYP3A substrates, for example amlodipine, clonazepam, diltiazem, estradiol, nicardipine, oxybutynin, quetiapine, and sildenafil, that undergo rapid absorption and extensive metabolism. These results indicate that disposition of highly soluble and highly permeable drugs such as midazolam will be only minimally affected by enteral route of administration and transplant subtype, but highly affected by intestinal CYP3A expression and function. If there is a concern for about

excessive bioavailability of these drugs, especially in the early post-transplant period, clinicians should dose cautiously and drug concentrations should be measured in blood or plasma whenever assays are available, and pharmacodynamic monitoring (i.e. blood pressure and pulse for antihypertensives, level of sedation for clonazepam) should performed more frequently in these patients until the desired pharmacologic effects are attained in the absence of toxicity.

6.0   INTESTINAL DRUG TRANSPORTER EXPRESSION AND FUNCTION AFTER SMALL BOWEL AND MODIFIED MULTIVISCERAL TRANSPLANTATION

6.1   BACKGROUND

6.1.1   Objectives of this study

Small bowel transplant recipients take numerous medications, many of them by mouth, and a significant number of them are substrates for intestinal transporters such as p-glycoprotein (p-gp). Ischemia-reperfusion injury and immune activation within the transplanted organ lead to inflammatory mediator release both within the graft and into the systemic circulation. *In vitro* studies have shown that pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ decrease p-gp expression and function via suppression of gene transcription. That these effects of cytokines on transporters may have clinical relevance in a transplant setting is further evidenced by animal studies of small bowel transplantation that shown not only impaired enzyme and transporter expression and function, but also impairments of intestinal first pass metabolism and transport of the widely-used immunosuppressive drug tacrolimus, in the first two weeks after transplant, returning to normal approximately one month after surgery. Whether

or not these changes are seen in intestinal transplant patients and when the functional recovery occurs are key questions. As mentioned earlier, the pharmacokinetic parameters of tacrolimus, a CYP3A and p-gp substrate, in clinically stable small bowel transplant recipients several months to years after transplant are similar to those seen in other transplant populations, implying eventual recovery of intestinal CYP3A and p-gp function.

Based on these observations, the central hypothesis of this chapter is that intestinal transporter expression and function in small bowel transplant recipients will be suppressed in the early post-transplant period (estimated at approximately the first 30 to 40 days after transplant), but will be similar to that seen in healthy control subjects in stable patients without evidence of rejection in the later post-transplant period (by four to six months post-transplant). In order to examine the effects of the transplanted intestine without the confounding variable of the transplanted liver, only isolated intestinal and modified multivisceral transplant recipients were included in the study. The specific aim of this study is as follows:

1. To measure intestinal transporter expression and function in stable small bowel transplant recipients in the early post-transplant period and later post-transplant period in comparison to transporter activity in normal healthy control subjects.

   a. This will be accomplished by the characterization of the pharmacokinetics of oral fexofenadine as a probe drug for transporter function assessment, within 40 days post-transplant and approximately four to 12 months post-transplant. In addition, in transplant subjects, mRNA transcripts of *ABCB1* (p-gp), *SLCO1A2* (OATP1A2), and *SLCO2B1* (OATP2B1) will be measured in ileal

mucosal biopsy samples taken within 48 hours of each fexofenadine study and compared between study sessions in the transplant subjects.

b. It is predicted that the oral AUC of fexofenadine in small bowel transplant recipients in the early post-transplant period will be significantly higher than that seen in age- and gender-matched healthy control subjects and also significantly different than the same subjects in the later post-transplant period. In the later post-transplant period, it is predicted that the pharmacokinetic parameters of fexofenadine will be no different from those seen in healthy controls. In addition, expression levels of the above-mentioned transporters will be significantly lower in the early post-transplant period as compared with the later.

6.1.2 Oral fexofenadine as a probe drug to assess transporter function

Fexofenadine is a histamine H_1receptor antagonist that is FDA-approved for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria(sanofi-aventis, 2007). It is a moderately lipophilic zwitterionic molecule that is an active metabolite of terfenadine, another antihistamine no longer marketed in the US. Fexofenadine has good water solubility (0.81 mg/mL in phosphate buffer) but a low partition coefficient (LogP) of approximately 0.3 at neutral pH. The carboxylic acid functional group on the molecule has a pKa of 4.25 and the piperidine moiety a pKa of 9.53(Chen, 2007).

Figure 38. Chemical structure of fexofenadine
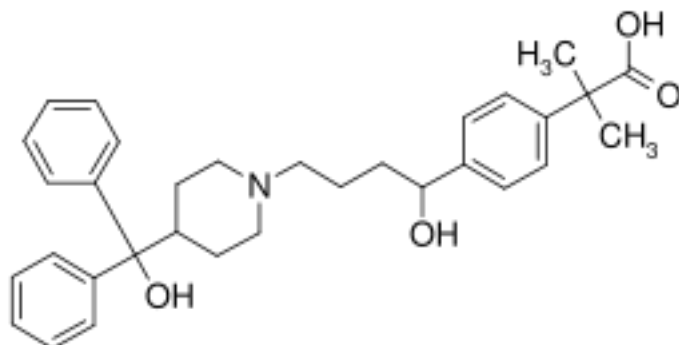
After oral administration of the immediate release tablet formulation, average Tmax estimates of fexofenadine in healthy volunteers range from 1.42(Robbins et al., 1998) to 2.7 hours(Lappin et al., 2010). Mean elimination half-life is 14.4 hours, and most of the drug is eliminated unchanged in the urine (8 to 18%)(Russell et al., 1998) and feces (80%, mainly due to biliary secretion), with less than 5% undergoing metabolism(sanofi-aventis, 2007). Binding to plasma proteins is low at 60 to 70%(sanofi-aventis, 2007). Although not marketed in an intravenous formulation, the mean bioavailability of fexofenadine was calculated at 0.30 in a study in which an intravenous microdose of 100 μg was administered to healthy volunteers. A mean volume of distribution of 116 L and clearance of 13 L/hr were reported after IV administration(Lappin et al., 2010). In a study of 24 healthy male volunteers, a single oral dose of 60 mg fexofenadine resulted in a mean $AUC_{0-\infty}$ of 1348 ng*hr/mL, Cmax of 209 ng/mL, Tmax of 1.42 hours, CL/F of 50.6 L/hr, and half life of 13.1 hours(Robbins et al., 1998).

Fexofenadine is a substrate for several drug transporters found in the intestine, including p-gp, OATP2B1, and OATP1A2(Cvetkovic et al., 1999; Ma et al., 2010; Shimizu et al., 2005). Fexofenadine is also a substrate for hepatic transporters, including the uptake transporters

OATP1B1 and OATP1B3(Shimizu et al., 2005). In addition, the bile salt export pump (BSEP) and MRP3 play a role in efflux of fexofenadine into bile(Matsushima et al., 2008). Fexofenadine is most likely not a substrate of BCRP(Matsushima et al., 2008; Shimizu et al., 2005; Swift et al., 2009), MRP4(Matsushima et al., 2008), or OCT1(Cvetkovic et al., 1999), and the role of MRP2 in its intestinal efflux and biliary excretion is under debate(Matsushima et al., 2008; Petri et al., 2004; Tahara et al., 2005). It is preferentially absorbed in the proximal small intestine (duodenum and upper jejunum) as compared with the distal (lower jejunum and ileum), probably due to the increased p-gp-mediated efflux in the distal intestine(Ujie et al., 2008). Overall effective permeability of fexofenadine from the lumen to the bloodstream in the jejunum is poor at a mean of 0.11±0.11 cm/second(Tannergren et al., 2002).

Although fexofenadine is a substrate for OATP uptake transporters as well as the p-gp efflux transporter, animal studies have demonstrated that efflux transporter effects predominate over uptake during the absorption process in the intestine. Rat studies have shown that oatp2b1 (the rat analog to the human uptake transporter OATP2B1) expression level patterns in the intestinal segments are very similar to those of p-gp, with lowest levels in the duodenum, followed by jejunum, ileum, and highest levels in the colon(MacLean et al., 2010). Another study found that permeability of fexofenadine from the serosal to mucosal side of rat ileal segments was 18-fold greater than its mucosal to serosal permeability, also indicating predominance of efflux over uptake(Ujie et al., 2008). It is possible that given the much lower relative expression of OATP transporters as compared with p-gp(Glaeser et al., 2007) that OATP transporters become saturated at clinically relevant luminal concentrations of fexofenadine. It is likely that passive diffusion also plays a role in its absorption.

Fexofenadine has been used in a number of studies as a p-gp phenotyping probe drug to assess p-gp function(Dresser et al., 2002; Glaeser et al., 2007; Kharasch et al., 2005; Xie et al., 2005). The advantages of fexofenadine as a probe drug for p-gp include its very favorable safety profile(sanofi-aventis, 2007), lack of metabolism by intestinal or hepatic enzymes(Miura et al., 2007b; sanofi-aventis, 2007), easy detection in plasma by LC-MS even at the lowest commercially available dose of 60 mg, and linear pharmacokinetics up to doses of 240 mg(Robbins et al., 1998). Drawbacks to its use as a probe include the lack of an FDA-approved intravenous formulation (without which, true oral bioavailability cannot be calculated), and its lack of selectivity for p-glycoprotein. However, evidence suggests that p-gp does not play a significant role in the biliary excretion of fexofenadine(Tahara et al., 2005), indicating that with regards to p-gp, its disposition after oral administration largely reflects that of the intestine, with a lesser contribution from intestinal OATP1A2 and OATP2B1 due to their lower expression and saturability. Disposition of fexofenadine also reflects the function of BSEP, MRP3, and OATP1B1 and OATP1B3 in the liver, as well as transporter function in the kidneys.

6.1.3 Effects of inflammation on fexofenadine pharmacokinetics

It has been previously noted in the introduction that inflammatory mediators secreted during immune activation may have suppressive effects on p-gp expression and function(Belliard et al., 2004; Belliard et al., 2002) as well as increase intestinal permeability(Kuebler et al., 2003; Nagpal et al., 2006; Welcker et al., 2004). Administration of lipopolysaccharide (LPS) to rats found a higher $AUC_{0-\infty}$ (13.9 ±9.76 vs. 5.53 ± 1.12 mcg*hr/mL, p<0.05) and bioavailability (0.030 ± 0.021 vs. 0.012 ± 0.002) of oral fexofenadine as compared with control rats, but no significant effects on the pharmacokinetics of IV fexofenadine (mainly a reflection of biliary

excretion and some renal elimination)(Jaisue et al., 2010). Although cytokines were not measured in the animals, the systemic response to LPS is well characterized, and includes increases in circulating IL-1β and IL-6. The increase in fexofenadine bioavailability was not accompanied by a decrease in clearance, indicating a likely localization of the LPS effects to the level of the intestine, possibly via suppression of p-gp efflux, loosening of tight junctions, or both.

6.2   METHODS AND SUBJECT DEMOGRAPHICS

The study design and methodology as well as demographics of the recruited subjects have been described in detail in chapters 2 and 3. This study was approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from all subjects before any study procedures were performed. Sixteen small bowel transplant recipients (12 isolated intestine, 4 modified multivisceral) and 16 age and gender matched healthy control subjects were recruited and studied. Transplant subjects were studied twice; once within the first 40 days post-transplant (median day 19, range days 10 to 40) and again four to fourteen months post-transplant (median day 239, range days 125 to 428). Sixteen transplant subjects (ten women, six men) participated in the first study session and ten (five women, five men) returned for the second. Control subjects participated in one study session.

At each study session, after an overnight fast, the subject was administered a single 60 mg immediate-release tablet of fexofenadine with four ounces of water. In the four transplant subjects who were receiving medications by jejunostomy tube (J-tube) at the time of the study session, the tablet was crushed completely to a powder, mixed with approximately 10 mL of

water and drawn up into an oral syringe. The syringe contents were then administered through the J-tube and followed by two tube rinses of water and another four ounces of water. The J-tube was then clamped. Blood samples (3 mL) were drawn into EDTA tubes at 0, 0.5, 1, 2, 3, 4, 6, 6.25, 6.5, 7, 9, 11, and 19 hours after administration of fexofenadine. Subjects remained in bed for five hours after fexofenadine administration, with the head of the bed at a 60-degree angle.

In addition, in the transplant subjects, mucosal biopsy samples from the transplanted ileum were analyzed for *ABCB1*, *MRP2, ABCG2, SLCO1A2, SLCO2A1,* and *SLCO2B1* gene expression by RT-PCR. Tissue samples were obtained during routine intestinal biopsies done to monitor the graft for rejection.   They were typically taken on the same day as the pharmacokinetic study session, although they were allowed to be within ±48 hours of the study session.

Fexofenadine was measured in plasma by LC-MS as described previously. Pharmacokinetic parameters of fexofenadine were calculated using noncompartmental analysis. Parameters and mRNA expression of genes were compared between study sessions for transplant subjects using the Wilcoxon matched pairs signed rank test, and transplant session 1 and 2 results, including biopsies, were each compared with control subjects and transplant patients by transplant type and route of administration using the Mann-Whitney U test. A two-sided p value <0.05 was considered statistically significant.   Correlations between ileal transporter gene expression and pharmacokinetic parameters of fexofenadine were analyzed in transplant subjects using Spearman's rank correlations, with a one-tailed p value less than 0.05 considered a significant correlation.

6.3 RESULTS OF PHARMACOKINETIC ANALYSES

6.3.1 Composite analysis of pharmacokinetic parameters between subject groups

Concentration-time profiles of fexofenadine in the three study groups are shown in Figures 31 to 34. The most striking differences between the groups were seen in Tmax, with both transplant groups showing a higher median Tmax than controls. Median Tmax of subjects at study session 1 (Tx1) was 10.0 hours in the paired analysis compared with 3.00 hours for those at study session 2 (Tx2) (p=0.050) and compared with 2.00 hours for controls (p=0.0007). Tx2 was also significantly higher than controls (p=0.0021). Median Cmax of fexofenadine was not significantly different between Tx1 and Tx2 (80.3 vs. 122 mcg/L, p=0.16) nor between Tx2 and controls (122 vs. 119 mcg/L, p=0.87) and Tx1 vs. controls (86.5 vs. 119 mcg/L, p=0.052). There were no significant differences in median AUC_{0-11} or AUC_{0-19} between the three groups, although there was a trend towards a lower AUC_{0-11} in the Tx1 subjects as compared with Tx2 (492 vs. 738 mcg*hr/L, p=0.16). CL/F and Vz/F could only be calculated in seven Tx1 subjects because the elimination phase could not be clearly characterized in nine of the subjects. Of the seven subjects in whom these parameters could be calculated, there was no difference between Tx1 and Tx2 (1.12 vs. 1.04 L/hr/kg, p=0.49), Tx1 and controls (1.08 vs. 1.23 L/hr/kg, p=0.57) and Tx2 and controls (1.04 vs. 1.23 L/hr/kg, p=0.29). Vz/F was not significantly different between Tx1 and Tx2 (9.61 vs. 7.14 L/kg, p=0.38) nor between Tx1 and controls (7.96 vs. 10.3 L/kg, p=0.19). However, median Vz/F values were significantly lower in Tx2 compared with controls (7.14 vs. 10.3 L/kg, p=0.043). MRTlast was significantly higher in Tx1 as compared with controls (7.46 vs. 4.80 hr, p=0.012).

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Table 18. Pharmacokinetic parameters of oral fexofenadine

Parameter	Transplant Session 1 (n=16)	Transplant Session 2 (n=10)	Control Subjects (n=16)	p value Tx1 vs. Tx2	p value Tx1 vs. C	p value Tx2 vs. C
Cmax (mcg/L)	86.5 (50.4,130)	122 (81.7,196)	119 (91.6,186)	0.16	0.052	0.87
Tmax (hr)	6.00 (3.00,11.0)	3.00 (2.75,6.00)	2.00 (1.25,2.75)	**0.050**	**0.0007**	**0.0021**
AUC$_{0-11}$ (mcg*hr/L)	496 (284,823)	738 (401,941)	618 (375,838)	0.16	0.49	0.65
AUC$_{0-19}$ (mcg*hr/L)	604 (333,1140)	871 (456,1160)	692 (415,965)	0.84	0.88	0.44
CL/F (L/hr/kg)	1.08* (0.910,1.16)	1.04 (0.630,1.48)	1.23 (0.960,1.93)	0.49	0.57	0.29
V/F (L/kg)	7.96* (4.30,10.2)	7.14 (4.12,8.78)	10.3 (7.40,13.3)	0.38	0.19	**0.043**
MRTlast (hr)	7.46 (4.92,11.3)	6.49 (4.61,7.59)	4.80 (4.60,5.23)	0.065	**0.012**	0.068

Parameters are median (25th, 75th percentile) *CL/F and V/F could only be calculated in 7 out of 16 transplant subjects at session 1. *Comparisons between transplant sessions 1 and 2 made using the Wilcoxon matched-pairs signed rank test on ten matched pairs, except for CL/F and V/F. Numbers in first column are median (25th, 75th percentile) for all 16 transplant session 1 subjects. Median concentrations in the 10 Tx1 subjects used in the paired analysis only are reported in the text as appropriate. §Comparisons between transplant session 1 and controls and transplant session 2 and controls made using the Mann-Whitney U test. Two-tailed p values reported.

Figure 39. Fexofenadine concentration vs. time in transplant subjects at session 1
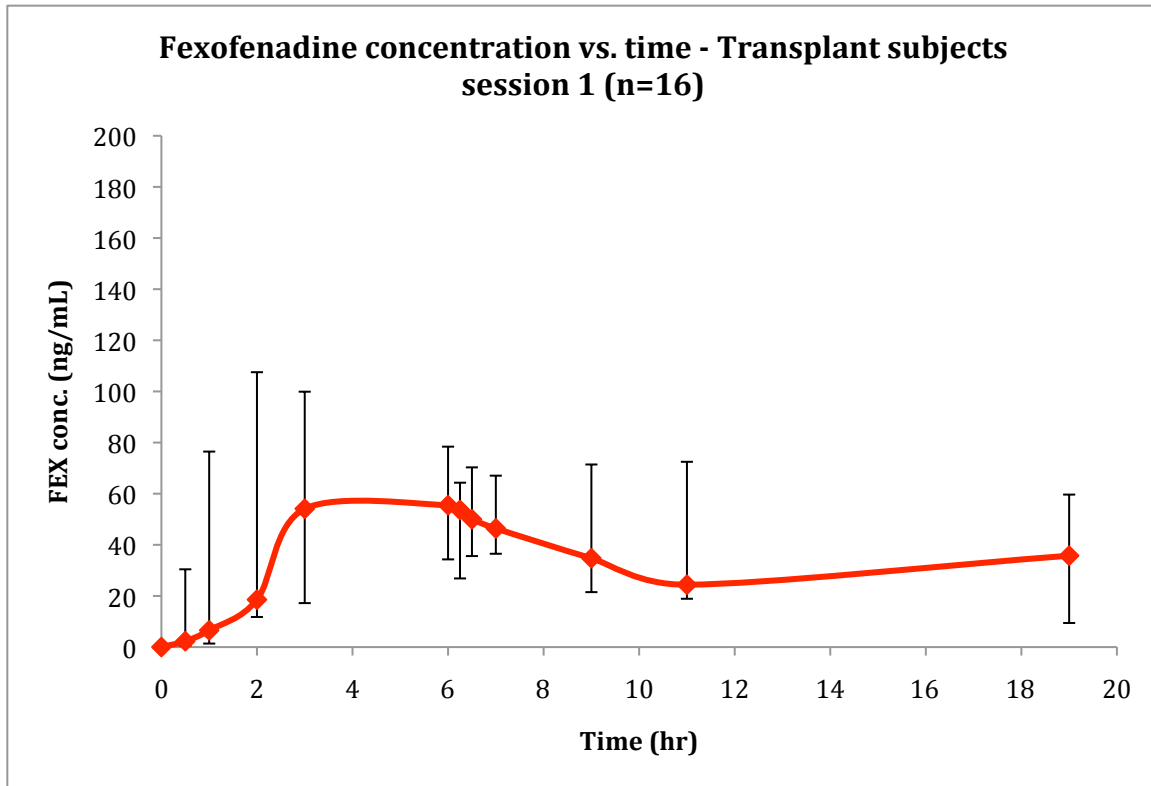
Fexofenadine concentration vs. time - Transplant subjects session 1 (n=16)

Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

Figure 40. Fexofenadine concentration vs. time in transplant subjects at Session 2
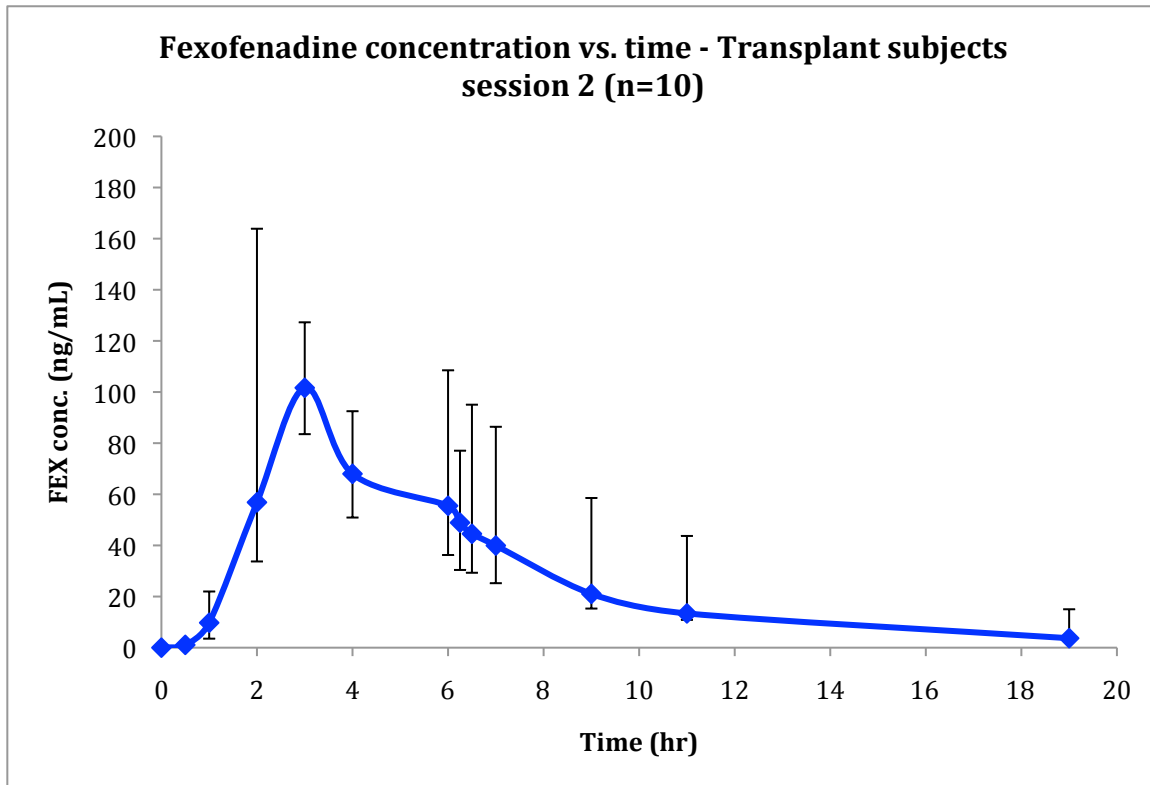
Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

Figure 41. Fexofenadine concentration vs. time in control subjects
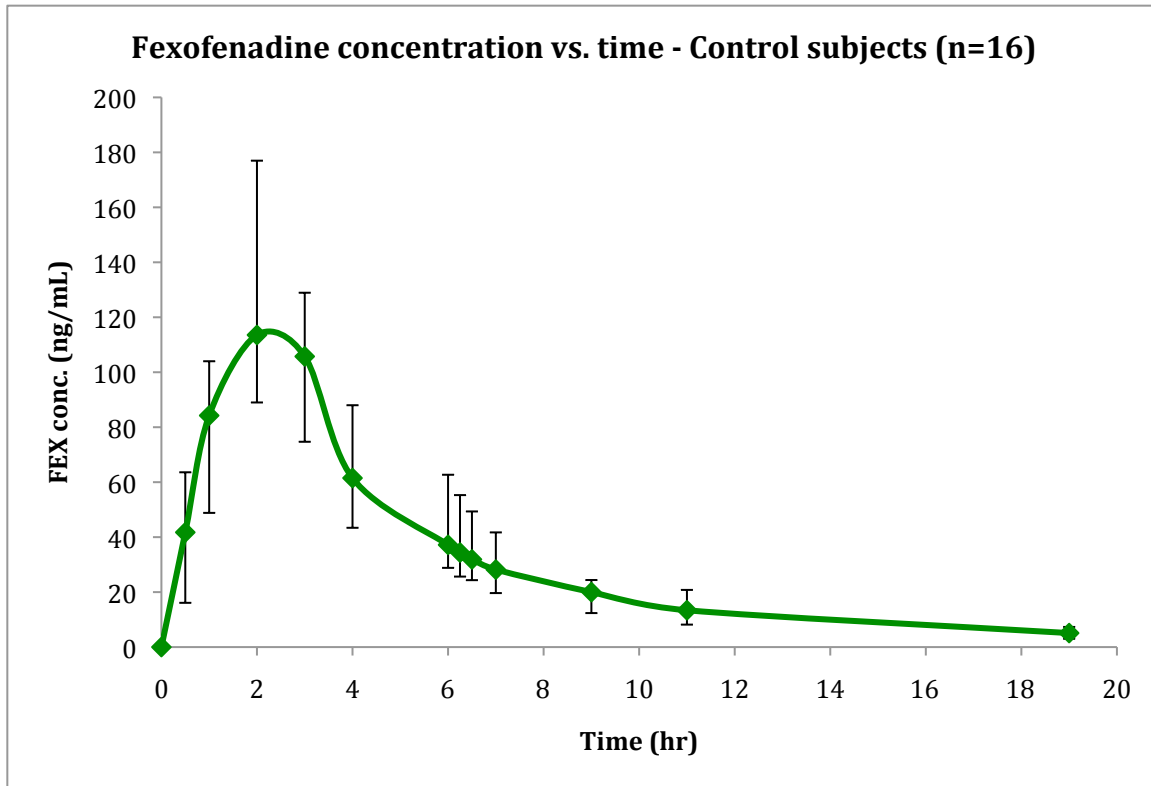
Fexofenadine concentration vs. time - Control subjects (n=16)

Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

Figure 42. Fexofenadine concentration vs. time - all subject groups
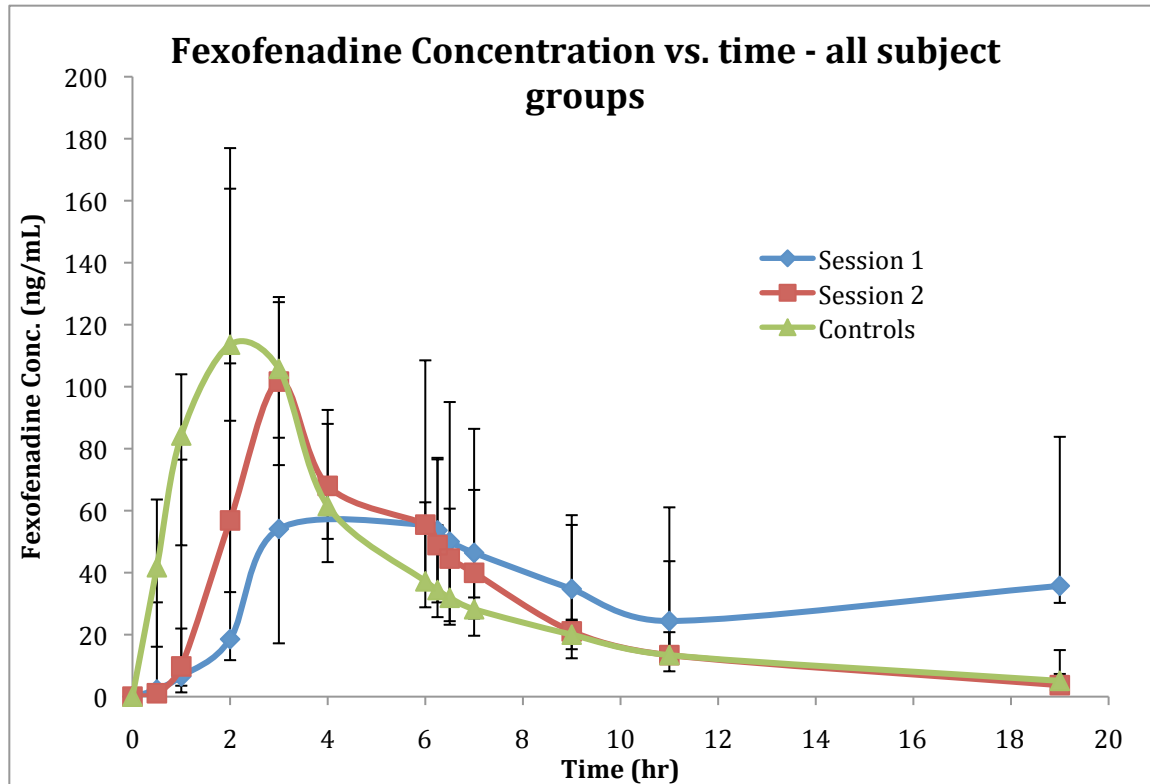
6.3.2 Comparison of J-tube and oral administration in transplant subjects at the first study session

Pharmacokinetics of fexofenadine compared between those who received the medication via jejunostomy tube (JT, n=4) and by mouth (PO, n=12) are shown in Table 17 and the concentration-time profiles in Figure 35. There was a trend towards a higher Cmax (123 vs. 80.3 mcg/L, p=0.25) and AUC_{0-11} (786 vs. 492 mcg*hr/L, p=0.16) in the JT group, as well as a trend towards a lower Tmax (3.00 vs. 10.0, p=0.26) and Vz/F (4.30 vs. 9.61 L/kg, p=0.057). MRT was significantly lower in the JT group as compared with PO (4.48 vs. 10.3 hours, p=0.034).

Table 19. Pharmacokinetic parameters of fexofenadine in transplant subjects by route of administration at session 1

Parameter	J-tube (n=4)	PO (n=12)	p value JT vs. PO
Cmax (mcg/L)	123 (71.2,260)	80.3 (48.2,125)	0.25
Tmax (hr)	3.00 (1.50,9.00)	10.0 (3.00,17.0)	0.26
AUC_{0-11} (mcg*hr/L)	786 (439,1270)	492 (186,552)	0.16
CL/F (L/hr/kg)	1.12* (1.07,3.22)	0.910§ (0.680,1.12)	0.23
V/F (L/kg)	4.30* (3.08,7.12)	9.61§ (8.23,16.8)	0.057
MRTlast (hr)	4.48 (3.75,6.45)	10.3 (6.53,11.6)	**0.034**

Parameters are median (25th, 75th percentile). Medians were compared using the Mann-Whitney U test. A two-sided p value was considered statistically significant.

Figure 43. Fexofenadine concentration vs. time profile by route of administration - transplant session 1
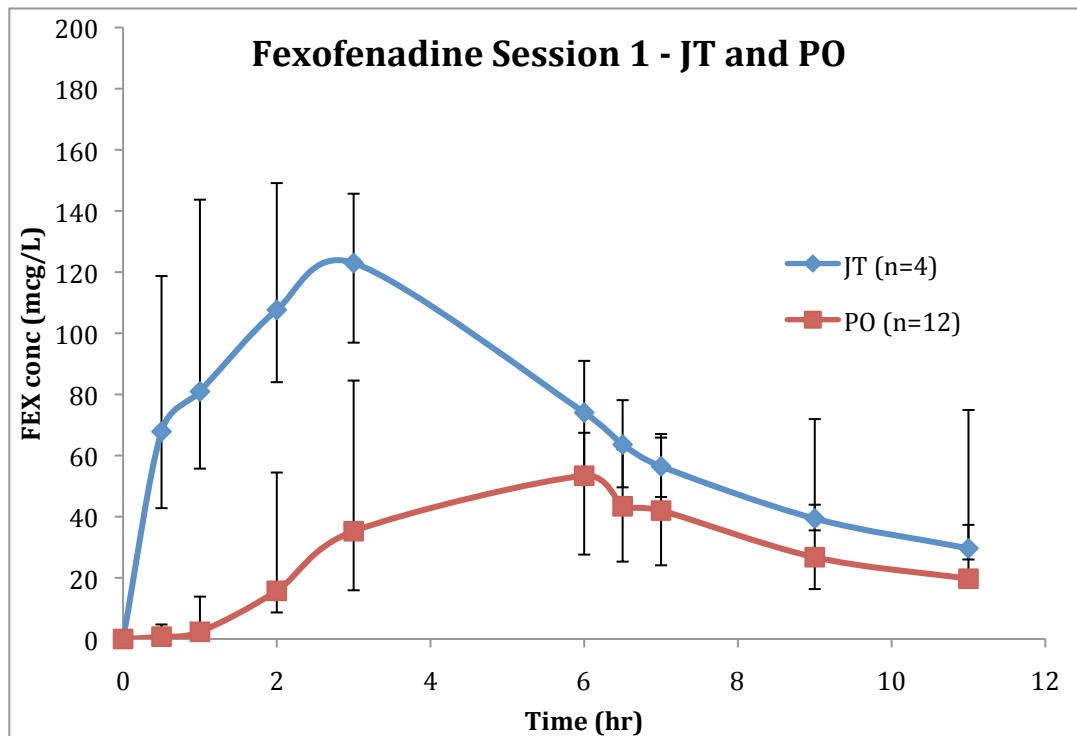
Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

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6.3.3 Comparison of pharmacokinetics of oral fexofenadine in isolated intestinal (II) and modified multivisceral (MM) recipients

6.3.3.1 Study session 1

Pharmacokinetic parameters of fexofenadine in modified multivisceral (MM) transplant subjects as compared with isolated intestinal (II) transplant subjects at study session 1 are listed in Table 18 and the concentration-time profile is shown in Figure 36. Median Cmax was significantly lower in MM as compared with II (35.0 vs. 114 mcg/L, p=0.0064), as were AUC_{0-11}(150 vs. 534 mcg*hr/L, p=0.0064) and AUC_{0-19}(282 vs. 1040 mcg*hr/L, p=0.018). Tmax was significantly higher in MM as compared with II (19.0 vs. 3.00, p=0.0062). There was a trend towards a longer MRT in MM as compared with II (12.0 vs. 6.55, p=0.060). CL/F and Vz/F could not be compared between transplant groups at this session because those parameters could not be calculated in any of the Tx1 MM patients due to the difficulty in characterizing an elimination phase within the 19 hour sampling period. Comparisons of II with controls, however, showed no significant differences between parameters except for Tmax, which was still higher in Tx1 (p=0.0071).

Table 20. Pharmacokinetic parameters of fexofenadine by transplant type at session 1

Session 1	MM (n=4)	II (n=12)	Control Subjects (n=16)	p value MM vs. II	p value II vs. C
Cmax (mcg/L)	35.0 (19.8,52.6)	114 (79.1,137)	119 (91.6,186)	**0.0064**	0.37
Tmax (hr)	19.0 (13.0,19.0)	3.00 (2.25,10.5)	2.00 (1.25,2.75)	**0.0062**	**0.0071**
AUC$_{0-11}$ (mcg*hr/L)	150 (85.5,301)	534 (490,912)	618 (375,838)	**0.0064**	0.66
AUC$_{0-19}$ (mcg*hr/L)	282 (254,428)	1040 (594,1370)	692 (415,965)	**0.0182**	0.19
MRTlast (hr)	12.0 (8.03,13.6)	6.55 (4.48,10.9)	4.80 (4.60,5.23)	0.060	0.11

Parameters are median (25th, 75th percentile) Comparisons between MM and II and and II and controls made using the Mann-Whitney U test. Two-tailed p values reported.

Figure 44. Fexofenadine concentration vs. time profiles by transplant type - session 1
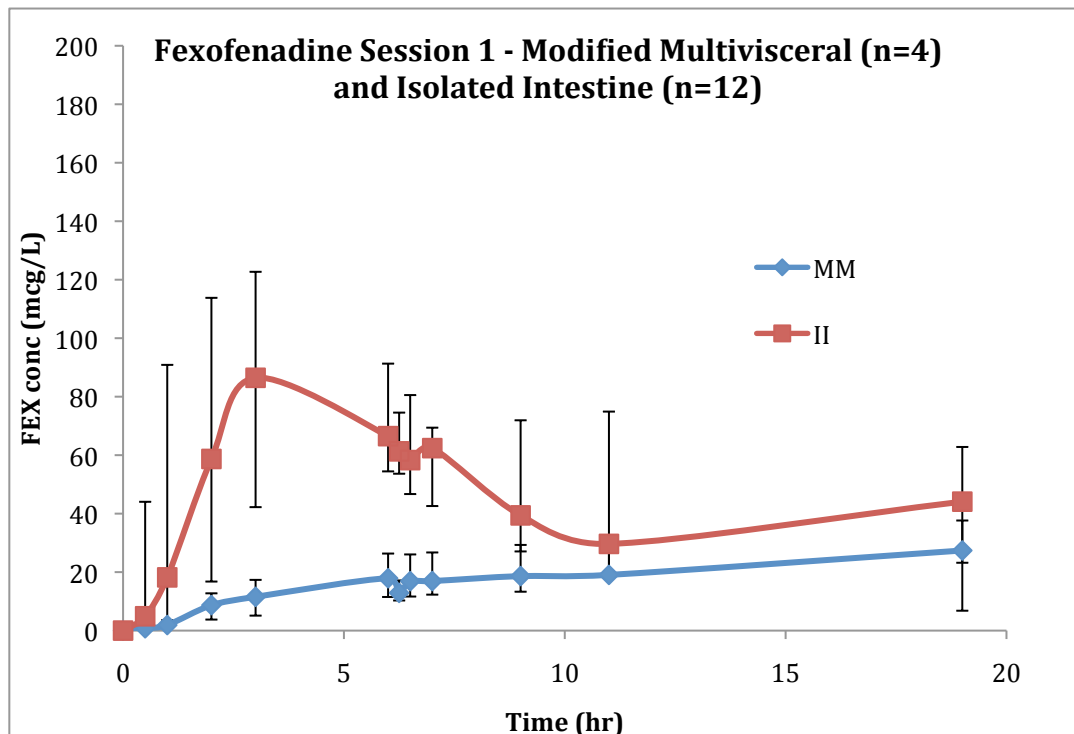
Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

6.3.3.2 Study session 2

Pharmacokinetic parameters of fexofenadine in modified multivisceral (MM) transplant subjects as compared with isolated intestinal (II) transplant subjects at study session 2 are listed in Table 19 and the concentration-time profiles are shown in Figure 37. At this later session, no significant differences in Tmax (3.00 vs. 3.00 hours, p=0.90) or MRT (5.29 vs. 6.78 hours, p=0.52) were seen. However, differences in AUC_{0-11} and AUC_{0-19} between the two groups remained. Both AUC_{0-11} (385 vs. 831 mcg*hr/L, p=0.033) and AUC_{0-19} (417 vs. 1160 mcg*hr/L, p=0.017) were still significantly lower in MM than II. In addition, both CL/F (2.03 vs. 0.720 L/hr/kg, p=0.033) and Vz/F (9.75 vs. 6.60 L/kg, p=0.017) were significantly higher in MM as compared with II.

Table 21. Pharmacokinetic parameters of fexofenadine by transplant type at session 2

Session 2	MM (n=3)	II (n=7)	Control Subjects (n=16)	p value MM vs. II	p value II vs. C
Cmax (mcg/L)	88.1 (34.6,114)	158 (118,215)	119 (91.6,186)	0.067	0.50
Tmax (hr)	3.00 (3.00,6.00)	3.00 (2.00,6.00)	2.00 (1.25,2.75)	0.90	**0.012**
AUC_{0-11} (mcg*hr/L)	385 (205,410)	831 (687,957)	618 (375,838)	**0.033**	0.10
AUC_{0-19} (mcg*hr/L)	417 (242,468)	1160 (753,1160)	692 (415,965)	**0.0167**	**0.042**
CL/F (L/hr/kg)	2.03 (1.15,2.73)	0.720 (0.570,1.12)	1.23 (0.960,1.93)	**0.033**	**0.035**
V/F (L/kg)	9.75 (8.46,15.2)	6.60 (3.86,7.15)	10.3 (7.40,13.3)	**0.017**	**0.0068**
MRTlast (hr)	5.29 (4.71,6.72)	6.78 (4.31,8.23)	4.80 (4.60,5.23)	0.52	0.12

Parameters are median (25th, 75th percentile) Comparisons between MM and II and II and controls made using the Mann-Whitney U test. Two-tailed p values reported.

Figure 45. Fexofenadine concentration vs. time profiles by transplant type - session 2
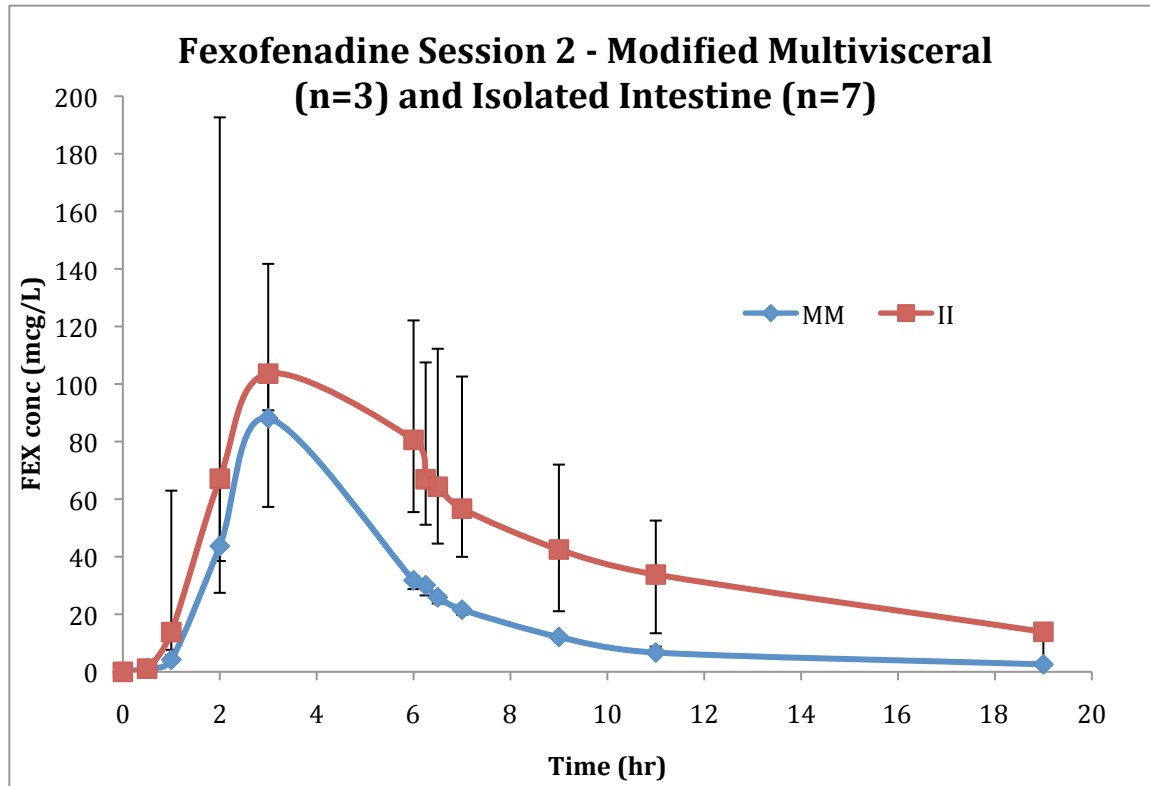
Median concentrations shown with + error bar 75th percentile, - error bar 25th percentile

6.3.4   Effects of renal function on the pharmacokinetics of fexofenadine in transplant subjects

As noted in chapter 3, creatinine clearance was significantly lower in transplant subjects at session 2 as compared with session 1 (55.5 vs. 94.0 mL/min/1.73 m^2, p=0.0098) and as compared with control subjects (55.5 vs. 99.0 mL/min/1.73 m^2, p=0.0054).   There was no difference in creatinine clearance between Tx1 and controls (91.5 vs. 99.0 mL/min/1.73 m^2, p=0.98). A decrease in creatinine clearance of greater than 50% was observed in six out of the ten transplant subjects who returned for session 2.   In the other four subjects, the creatinine

clearance had changed 15% or less from baseline. Approximately 11% of an administered dose of fexofenadine is excreted in the urine, and Cmax was 87% greater and elimination half-life 59% longer, in subjects with mild to moderate renal impairment (CrCL 41-80 mL/min)(sanofi-aventis, 2007). Therefore, the relationship between creatinine clearance and fexofenadine AUC, CL/F, MRT, and Cmax was examined in transplant subjects at session 1, session 2, and both sessions combined. There was no correlation observed between creatinine clearance and fexofenadine AUC, CL/F, and Cmax. However, there was a significant negative correlation observed between creatinine clearance and MRT at session 1. Results of correlation analysis are listed in Table 20.

Table 22. Relationship between creatinine clearance and fexofenadine disposition in transplant subjects

Session	Fex AUC$_{0-11}$	Fex AUC$_{0-19}$	CL/F	Cmax	MRTlast
1	r_s=0.17 p=0.27	r_s=0.021 p=0.47	r_s=-0.50* p=0.13	r_s=0.090 p=0.37	r_s=-0.50 p=0.025
2	r_s=-0.16 p=0.33	r_s=-0.079 p=0.42	r_s=0.15 p=0.34	r_s=-0.13 p=0.37	r_s=-0.018 p=0.49
All	r_s=-0.060 p=0.39	r_s=-0.071 p=0.38	r_s=0.16 p=0.27	r_s=-0.17 p=0.21	r_s=-0.16 p=0.22

*n=7 because fexofenadine CL/F could only be calculated in 7 transplant subjects at session 1

6.4   RT-PCR RESULTS

6.4.1   Ileal mRNA expression of transporters

The relative expression levels of the six transporters tested are listed in tables 21 and 22. Because of poor mRNA yield in two samples and one sample not obtainable (all from session 1) analysis was performed on seven pairs of samples. Although median *ABCB1* (p-gp) and *ABCG2* (BCRP) transcript levels were lower at session 1 as compared with session 2, the difference did

not reach statistical significance. However, *MRP2* levels were significantly lower at session 1 than session 2 in the paired analysis (0.0202 vs 0.0778, p=0.031). The expression levels of *SLCO2A1* (OATP2A1) and *SLCO2B1* (OATP2B1) were nearly identical between study sessions. mRNA expression of *SLCO1A2* (OATP1A2), thought to be the main uptake transporter for fexofenadine, was not detected in any of the 23 samples evaluated.

Table 23. Paired comparison of ileal transporter expression

mRNA	Transplant Session 1 (n=7)	Transplant Session 2 (n=7)	P value* Tx1 vs. Tx2
ABCB1 (P-gp*)*	0.114 (0.0920, 0.261)	0.226 (0.0860, 0.351)	0.30
MRP2	0.0202 (0.00397,0.0411)	0.0778 (0.0386,0.0925)	**0.031**
ABCG2 (BCRP*)*	0.334 (0.165,0.554)	0.616 (0.586,0.788)	0.078
SLCO1A2 (OATP1A2)	not detected	not detected	n/a
SLCO2A1 (OATP2A1)	0.0111 (0.00389,0.0368)	0.0108 (0.00565,0.0180)	0.58
SLCO2B1 (OATP2B1)	0.0111 (0.00710,0.0128)	0.0123 (0.00988,0.0146)	0.84

Amounts are relative to cyclophilin and are expressed as median (25th, 75th percentile) Comparisons made using the

Wilcoxon matched-pairs signed rank test. Two-tailed p values reported.

Table 24. Ileal transporter expression in all transplant subjects

mRNA	Transplant Session 1 (n=13)	Transplant Session 2 (n=10)
ABCB1 (p-gp)	0.125 (0.0960, 0.212)	0.281 (0.0733,0.361)
MRP2	0.0294 (0.0202,0.0440)	0.0844 (0.0354,0.101)
ABCG2 (BCRP*)*	0.302 (0.262,0.476)	0.661 (0.572,0.848)
SLCO1A2 (OATP1A2)	not detected	not detected
SLCO2A1 (OATP2A1)	0.0106 (0.00455,0.0221)	0.0108 (0.00580,0.0275)
SLCO2B1 (OATP2B1)	0.0120 (0.00911,0.0131)	0.0124 (0.0102,0.0157)

Amounts are relative to cyclophilin and are expressed as median (25th, 75th percentile)

6.4.2 Transporter mRNA expression and pharmacokinetic parameters of fexofenadine

There were no statistically significant correlations between the pharmacokinetic parameters of fexofenadine (Cmax, Tmax, AUC_{0-11}, AUC_{0-19}, CL/F, and MRT) and relative *ABCB1* mRNA expression in the samples taken as a whole. However, there was a statistically significant negative correlation between *ABCB1* expression and Cmax (r_s=-0.52, p=0.071) at Tx1 only. There was no relationship observed between *SLCO2B1* (OATP2B1) expression and fexofenadine AUC, Cmax, CL/F, or MRT either by study session or with both sessions combined. Correlation between fexofenadine parameters and *SLCO1A2* expression could not be performed because that transporter was not detected in any of the samples.

6.4.3 Fexofenadine pharmacokinetics and plasma cytokine concentrations

Figure 46. **Correlation between fexofenadine Cmax and plasma ileal *ABCB1* expression in transplant subjects at study session 1**

Fexofenadine Cmax vs. Rel. ABCB1 - Session 1

r_s = -0.52, p=0.071
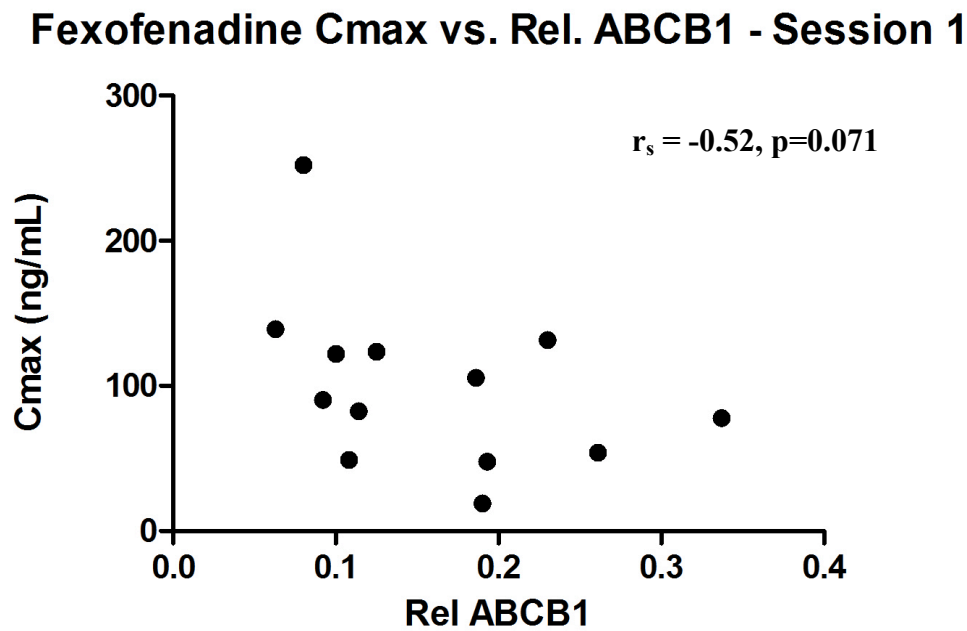
Figure 47. Correlation between fexofenadine CL/F/kg and plasma IL-12 concentration in transplant subjects at study session 1

Fexofenadine CL/F/kg vs. Plasma IL-12 - Session 1

$r_s = 0.75$, p=0.066
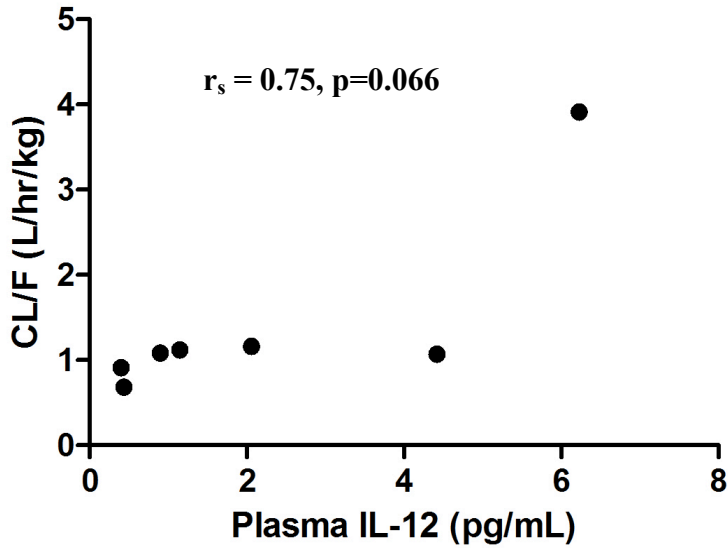
Figure 48. Correlation between fexofenadine AUC_{0-11} and plasma IL-4 concentration in transplant subjects at study session 2

AUC0-11 vs. Plasma IL4 - Session 2

$r_s = -0.67$, p=0.039
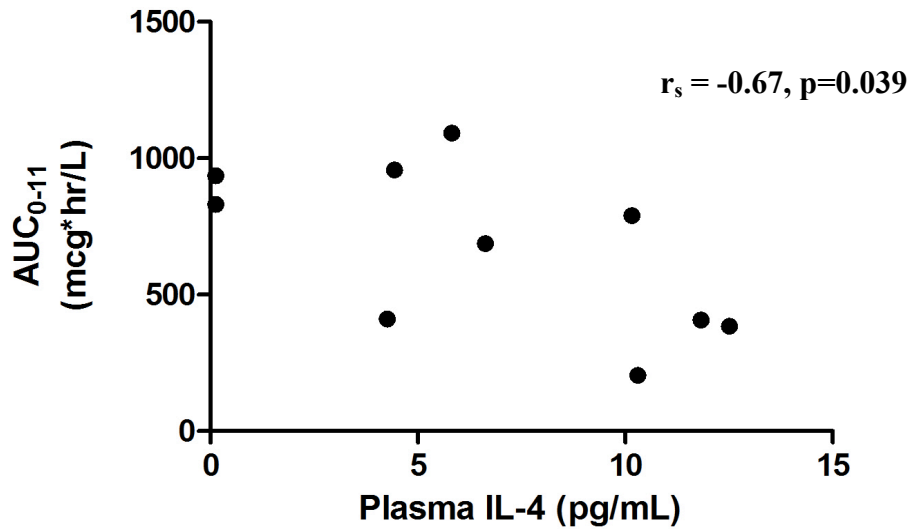
Figure 49. Correlation between fexofenadine Cmax and plasma IL-4 concentration in transplant subjects at study session 2

Fexofenadine Cmax vs. Plasma IL-4 - Session 2

$r_s = -0.58, p=0.088$
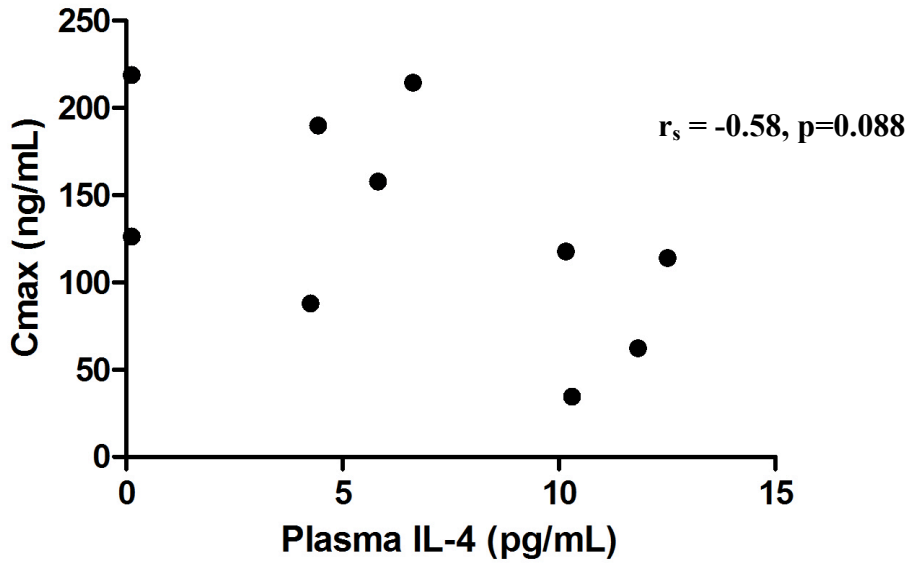
Figure 50. Correlation between fexofenadine CL/F/kg and plasma IL-8 concentration in transplant subjects at study session 2

Fexofenadine CL/F/kg vs. Plasma IL-6 - Session 2

$r_s = -0.65, p=0.049$
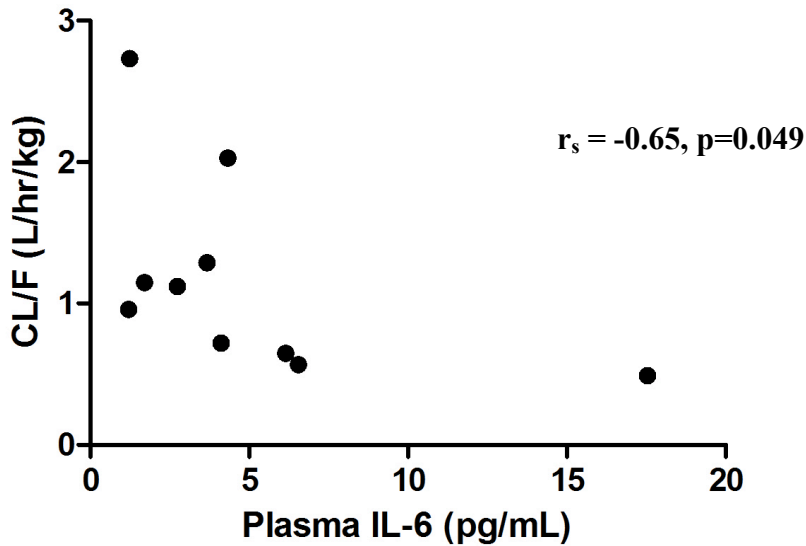
Figure 51. Correlation between fexofenadine MRTlast and plasma IL-6 concentration in transplant subjects at study session 2

Fexofenadine MRTlast vs. Plasma IL-6 - Session 2

$r_s = 0.73$, p=0.020
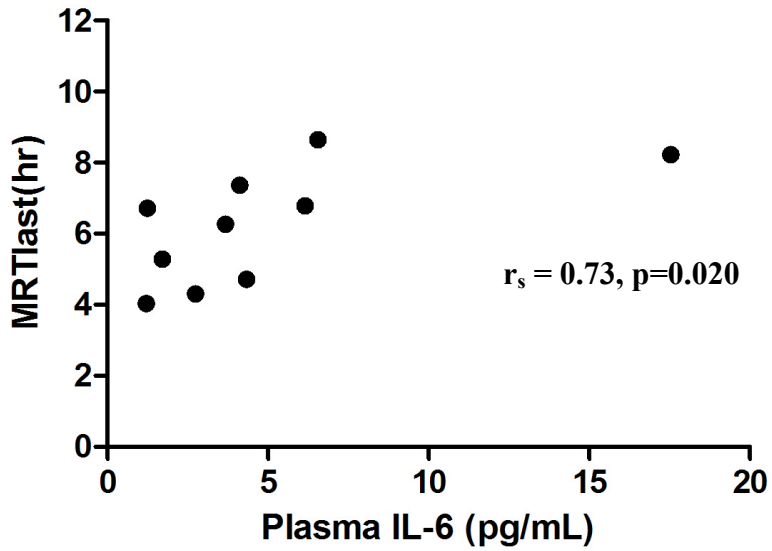
Figure 52. Correlation between difference in fexofenadine Cmax and plasma IL-4 concentrations in transplant subjects between study sessions

Within-subject correlation
Fexofenadine Cmax vs. plasma IL-4

$r_s = -0.62$, p=0.060
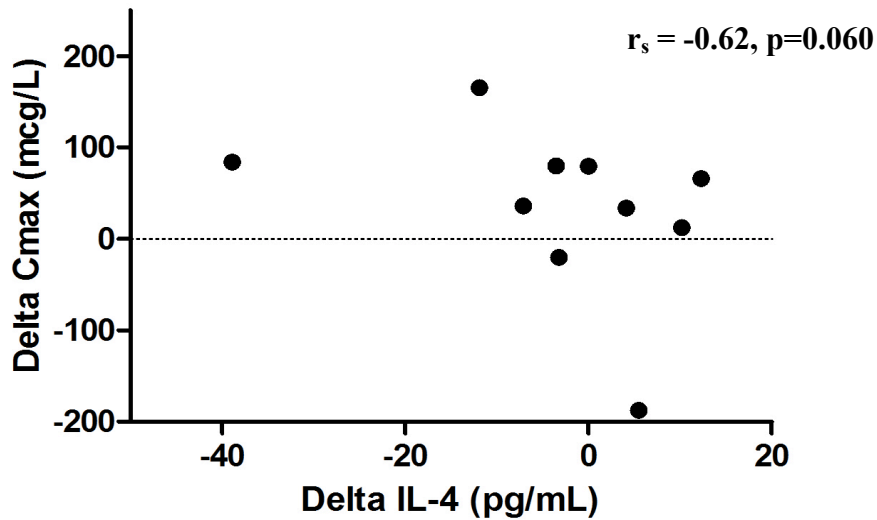
Figure 53. Correlation between difference in fexofenadine Tmax and plasma IL-6 concentrations in transplant subjects between study sessions

Within-subject correlation
Fexofenadine Tmax vs. plasma IL-6

$r_s = 0.77, p=0.013$

Delta Tmax(hr)

Delta Plasma IL-6 (pg/mL)
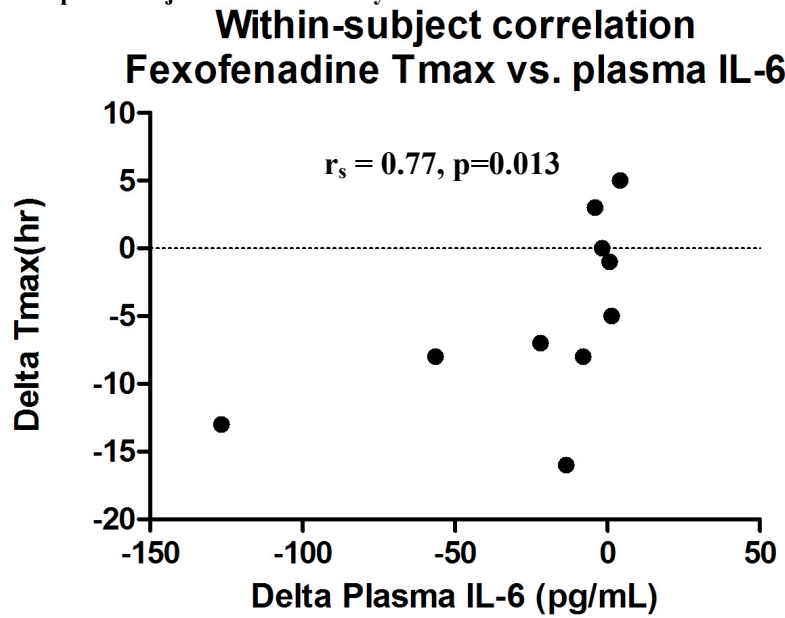
Figure 54. Correlation between difference in fexofenadine Tmax and plasma IL-8 concentrations in transplant subjects between study sessions

Within-subject correlation
Fexofenadine Tmax vs. plasma IL-8

$r_s = 0.63, p=0.060$

Delta Tmax(hr)

Delta Plasma IL-8 (pg/mL)
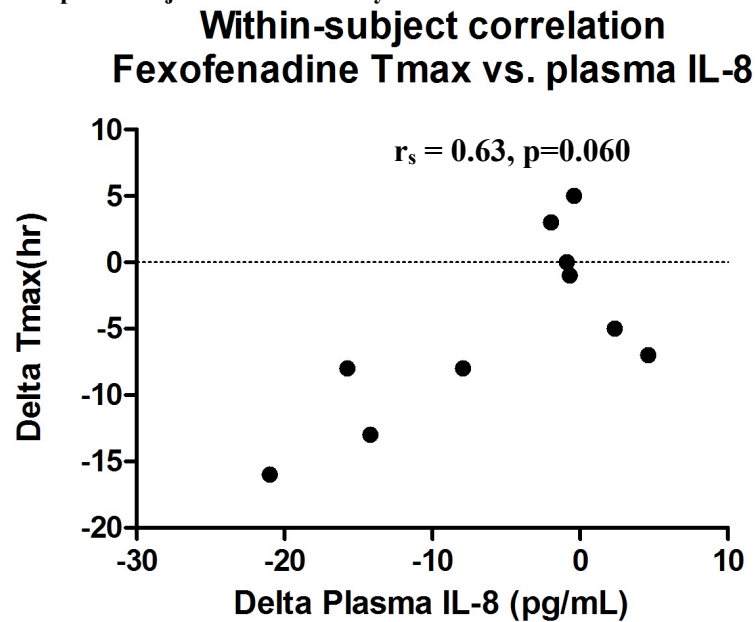
Figure 55. Correlation between difference in fexofenadine AUC_{0-19} and plasma IL-12 concentrations in transplant subjects between study sessions

Within-subject correlation
Fexofenadine AUC_{0-19} vs. Plasma IL-8

$r_s = 0.79$, p=0.028
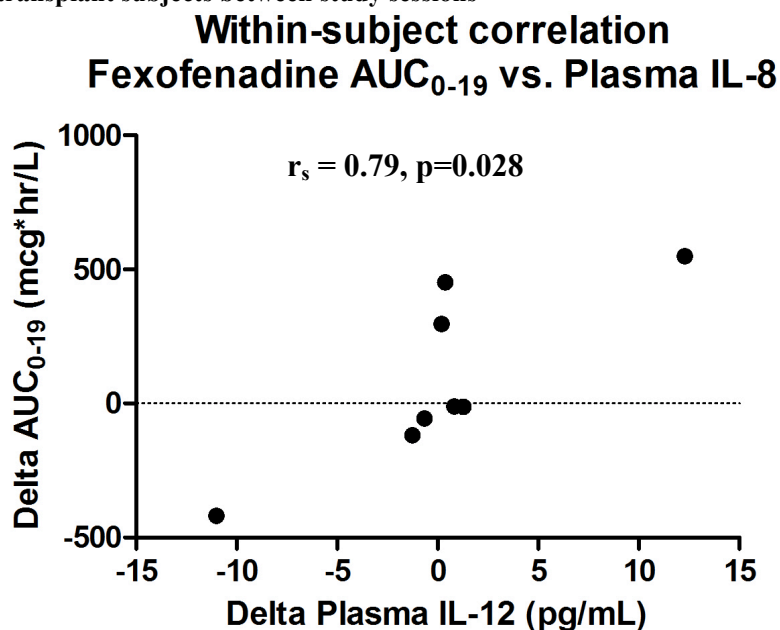
6.5 DISCUSSION

This work has examined the rate of absorption (as measured by Tmax and Cmax) and extent of absorption (as measured by AUC and also Cmax) of the BCS/BDDCS class III drug fexofenadine, a substrate for intestinal p-gp and OATP1A2 and OATP2B1 transporters, in small bowel transplant recipients and healthy control subjects.

The significantly increased median Tmax, and somewhat decreased median Cmax, seen in the transplant subjects at session 1 as compared with session 2 and with controls reflects a greatly slowed rate of absorption of fexofenadine in the early post-transplant period that has not recovered to normal even in the later post-transplant period in this group of stable transplant subjects. However, the overall extent of systemic exposure (as measured by AUC) is not significantly different between the groups. CL/F was also not different between the groups, but

could not be measured in nine out of 16 transplant subjects at session 1, and without intravenous dosing it is impossible to evaluate the relative contribution of CL and F. Despite this, given the low intestinal permeability of fexofenadine, it is possible that bioavailability (F) is reduced in these patients, especially at session 1, due to incomplete absorption. Overall, at the second study session all the parameters tended to more closely approach those of control subjects.

Unfortunately, full concentration-time profiles could not be characterized in nine of the transplant subjects at session 1 within the 19-hour sampling interval. These results were unexpected at the time of study design. While 19 hours was sufficient to evaluate the profiles of control subjects as well as transplant subjects at session 2, increasing the sampling interval to 36 or even 48 hours would have allowed for characterization of the true AUC, Cmax and clearance, especially in the transplant subjects at session 1 whose Cmax based on these profiles occurred at 11 or 19 hours post-dose. While in most of the subjects Cmax had been reached much earlier than 11 hours, in that particular group of subjects the true Cmax may even have occurred between 11 and 19 hours and been missed, leading to underestimation of the AUC. And if blood sampling had occurred until plasma concentrations were very low, the full AUC to infinity in these subjects might have been discovered to be significantly higher than those seen at session 2 and in control subjects, confirming the initial prediction of the study. Therefore, the lack of difference between fexofenadine Cmax and AUC at session 1 as compared with the other groups may have been an artifact of abnormal absorption and study design. Future pharmacokinetic studies of highly soluble but poorly permeable and slowly absorbed drugs such as fexofenadine in small bowel transplant patients should make sure that the duration of sampling is at least 36 hours.

In addition, a decreased intestinal surface area due to ileostomy may have led to decreases in absorption. A pharmacokinetic study of oral and IV azithromycin (a BDDCS class III drug that is a substrate for MRP2 efflux in the intestine and liver) in subjects with ileostomies showed that 37% of the oral dose was recovered from the ileostomy output in the six hours after dosing and 47% in the first 24 hours. After IV administration, only 13% was recovered in the ileostomy bag after 24 hours. Since azithromycin, like fexofenadine, is preferentially absorbed in the duodenum and upper jejunum, recovery of a large amount of unchanged drug after passage through this segment of intestine is likely due to incomplete absorption. This argument was further strengthened by the finding that bioavailability of azithromycin was 16.2% in the ileostomy subjects, approximately half that seen in subjects with an intact intestine(Luke and Foulds, 1997). It is possible that significant amounts of fexofenadine were washed out into the ostomy bag, especially in those subjects with an especially rapid intestinal transit time. In the case of fexofenadine, however, measurement of drug in ostomy output would have been confounded by its lack of metabolism and the high excretion of unchanged drug in the bile. It should be noted, however, that undissolved fexofenadine tablets were not observed to come out into the ostomy bag undissolved in any case, although this could have been missed amongst ostomy bag contents.

Abete et al. described the pharmacokinetics of 5 mg/kg IV ganciclovir followed by 900 mg oral valganciclovir (rapidly converted to ganciclovir, a BDDCS class III PEPT1 substrate, in the intestine) in an adult small bowel transplant recipient two months after transplant. AUC_{0-24}, Cmax, and bioavailability of ganciclovir after oral valganciclovir were similar to the values seen in other transplant populations. However, ganciclovir Tmax at 6 hours was significantly higher than the typical 1 to 3 hours expected, suggesting absorption rate differences between small

bowel transplant recipients and other patients(Abete et al., 2004). Whether the difference is due to impaired intestinal hydrolysis to ganciclovir by esterases, impaired PEPT1 uptake, or delays in gastric emptying is unknown, but this report also provides evidence of delayed absorption of a highly soluble, poorly permeable drug in the setting of small bowel transplantation.

Small bowel transplant recipients often display delays in gastric emptying combined with reduced intestinal transit times. Once the stomach empties into the proximal small intestine (the optimal site of fexofenadine absorption) if transit time is rapid the exposure of the drug to the enterocyte membrane will be limited. The drug compound will also spend longer in the jejunum and ileum than the duodenum due to the greater length and although fexofenadine absorption is normally lower in those segments due to p-gp efflux, p-gp expression may be suppressed leading to increased absorption from the ileum. It is also possible that the absence of ileal OATP1A2 expression observed in all the transplant subjects, thought to be the primary uptake transporter for fexofenadine, in the ileal biopsy samples may have also played a role in reducing fexofenadine absorption in the proximal small intestine.

The significantly higher Tmax after oral as compared with J-tube administration also suggests delays in gastric emptying in the transplant subjects in the early post-transplant period. J-tube administration bypasses the effects of the stomach (and gastric emptying times) and although it also bypasses some of the supposed optimal absorption window for fexofenadine, the reduced expression of p-gp (and MRP2, if it is indeed an efflux transporter of fexofenadine) in the transplanted ileum may lead to improved absorption there. It is also possible that crushing the fexofenadine tablet prior to J-tube administration improved the dissolution profile of the drug thus leading to more rapid absorption. However, given that it was an immediate-release formulation of a drug with high solubility, the effects of dissolution time should have been

negligible unless intestinal fluid content was significantly reduced in the transplant subjects at session 1. This is a possibility, since intestinal transplant recipients are sometimes under oral fluid restrictions in the first few weeks after surgery. However, the unusual absorption patterns with extremely high Tmax were unrelated to time post-transplant at study session 1 and were observed up to studies on post-transplant days 32 and 40 in subjects who had been receiving oral medications for some time and who were eating a normal diet.

In addition to being enteral route-dependent, the disposition of fexofenadine in the transplant subjects was also strongly dependent on transplant subtype, especially at session 1, with modified multivisceral transplanted subjects showing significantly higher Tmax and significantly lower Cmax and AUC as compared with isolated intestinal transplant subjects in the early post-transplant period, showing decreased rate and extent of absorption. As with the analysis of the transplant groups as a whole, the sampling time of 19 hours is not enough in the modified multivisceral subgroup to fully characterize AUC to infinity and therefore the true difference in AUC cannot be adequately described. However, the similarities between the profiles between the 11 and 19 hour time points suggest that the main differences are occurring during the absorption phase. At session 2, the extent of absorption, as measured by AUC, is still significantly decreased in MM subjects as compared with II, although differences in median Tmax have disappeared. These results, particularly at session 2, are difficult to explain, and may relate either to a recovery of impairments in gastric emptying in the MM subjects combined with a more rapid intestinal transit time or merely to the small number of subjects in the subgroup analysis, with only three in the MM group in the later post-transplant period.

Overall, the results of this study do not provide complete support for the main hypothesis that intestinal transporter expression and function are suppressed in the early post-transplant

period as compared with later post-transplant and control subjects. Firstly, these results do not demonstrate that the AUC of fexofenadine is significantly higher in the early post-transplant period as opposed to the later, due perhaps in part to the study sampling interval of 19 hours being insufficient to characterize the elimination phase in nine out of the 16 subjects at session 1. Secondly, although relative ileal mucosal expression of *ABCB1* (although not significantly) and *MRP2* were lower in the early post-transplant period compared with later there were no significant correlations observed between the pharmacokinetic parameters of fexofenadine and either *ABCB1* and *SLCO2B1* ileal gene expression, in sharp contrast to the results seen with midazolam and *CYP3A* expression. There was no correlation between fexofenadine AUC or Cmax and plasma cytokine concentrations in the subjects taken as a whole, although there was a significant correlation between Tmax and several cytokines. Although again the lack of correlation of cytokine concentration with extent of absorption may have been influenced by the duration of sampling it could point to an effect of inflammation on motility or gastric emptying.

Overall, these results demonstrate a stronger effect of enteral route of administration and transplant subtype on fexofenadine pharmacokinetics than ileal transporter expression, a result that casts doubt on the appropriateness of fexofenadine as a p-gp probe in patient populations with impaired gastrointestinal motility, due to its poor absorption profile. These results also recommend caution on the part of clinicians when dosing poorly permeable BCS/BDDCS class III substrates in small bowel transplant patients, since they demonstrate that rate and, in some cases, extent of absorption of these drugs will be significantly decreased, even in the later post-transplant period in stable patients. This may be an especially important consideration for the use of oral antibiotics in the case of infection. Amoxicillin, azithromycin, many of the oral cephalosporins, doxycycline, fluconazole, levofloxacin, moxifloxacin, tetracycline, and

trimethoprim are all BDDCS class III drugs, and may exhibit some of the same impairments in rate and extent of absorption as fexofenadine, particularly in the early post-transplant period.

7.0 PHARMACOKINETICS OF TACROLIMUS AFTER SMALL BOWEL AND MODIFIED MULTIVISCERAL TRANSPLANTATION

7.1 BACKGROUND

7.1.1 Hypothesis and objectives of this study

Small bowel transplant recipients take numerous medications, many of them by mouth, and a significant number of them CYP3A and/or transporter substrates. Ischemia-reperfusion injury and immune activation within the transplanted organ lead to inflammatory mediator release both within the graft and into the systemic circulation. *In vitro* studies have shown that pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ decrease CYP3A and transporter expression and function via suppression of gene transcription. Clinical studies in other inflammatory conditions such as cancer and sepsis have shown the clinical correlation between inflammatory mediator expression and suppression of drug metabolism. That these effects of cytokines on drug-metabolizing enzymes and transporters have clinical relevance in a transplant setting is further evidenced by animal studies of small bowel transplantation that shown not only impaired enzyme and transporter expression and function, but also impairments of intestinal first pass metabolism of the widely-used immunosuppressive drug tacrolimus, in the first two weeks after transplant, returning to normal approximately one month after surgery. Whether or not

these changes are seen in intestinal transplant patients and when the functional recovery occurs are key questions. As mentioned earlier, the pharmacokinetic parameters of tacrolimus, a CYP3A and p-gp substrate, in clinically stable small bowel transplant recipients several months to years after transplant are similar to those seen in other transplant populations, implying eventual recovery of intestinal CYP3A and p-gp function.

Based on these observations, the central hypothesis of this chapter is that intestinal CYP3A and transporter expression and function in small bowel transplant recipients will be suppressed in the early post-transplant period (estimated at approximately the first 30 to 40 days after transplant), but will be similar to that seen in healthy control subjects in stable patients without evidence of rejection in the later post-transplant period (by four to six months post-transplant). In order to examine the effects of the transplanted intestine without the confounding variable of the transplanted liver, only isolated intestinal and modified multivisceral transplant recipients were included in the study. This chapter has the following aim:

1. To characterize the pharmacokinetics of oral tacrolimus, a CYP3A and p-gp substrate, in small bowel transplant recipients in the first 40 days post-transplant as compared with the later post-transplant period (four to 12 months post-transplant)

    a. This will be accomplished by measurement of whole blood tacrolimus concentrations in blood samples taken at the time of the early and late post-transplant study sessions mentioned previously. Tacrolimus dose-adjusted AUC will be compared between the early and later post-transplant periods.

It is predicted that the dose-adjusted AUC of tacrolimus will be significantly higher in small bowel transplant recipients at the early post-transplant period than the later.

Figure 56. Chemical structure of tacrolimus
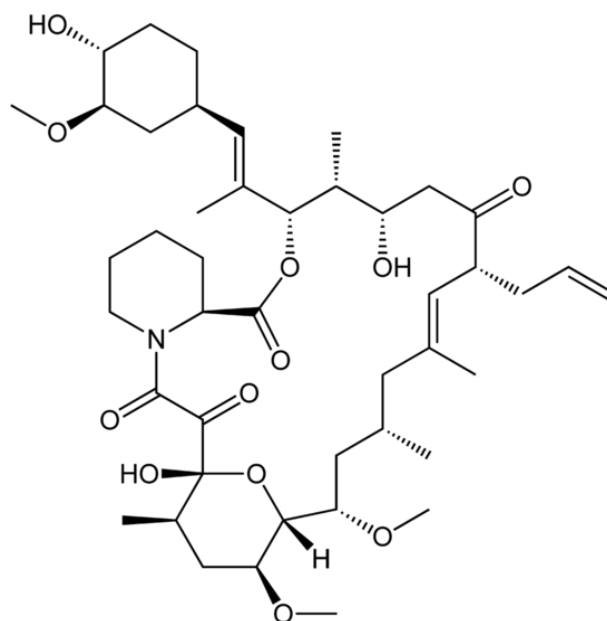
7.1.2   Pharmacokinetic properties of tacrolimus

Tacrolimus is a macrolide immunosuppressant drug produced by the actinobacterium species *Streptomyces tsukubaensis*.   It suppresses T-cell activation via calcineurin phosphatase inhibition, most likely through binding to an intracellular protein called FKBP-12. Once bound, the tacrolimus-FKBP-12 forms a complex with calcium, calmodulin, and calcineurin that inhibits the phosphatase activity of calcineurin.   This is thought to inhibit gene transcription for

production of cytokines such as IL-2 and IFN-γ. Consequently, its main effect is to suppress cell-mediated immunity, although animal studies have also shown it to have some effect on humoral immunity(Astellas, September 2011).

Tacrolimus is a BCS/BDDCS class II drug with very low solubility but good permeability(Takagi et al., 2006) and a high extent of metabolism in both the liver and intestine, where it is a substrate for both CYP3A4, 3A5, and p-glycoprotein (p-gp)(Lampen et al., 1995). Tacrolimus exhibits higher systemic absorption in the jejunum as compared to the duodenum and ileum, most likely because CYP3A is highest in the duodenum and upper jejunum, while p-gp expression is highest in the ileum(Shimomura et al., 2002; Tamura et al., 2003). Because of poor solubility and extensive first-pass metabolism, the bioavailability of tacrolimus is highly variable, with estimates ranging from 5% to 93% in small bowel transplant patients(Jain et al., 1992), while Tmax is typically between 1 and 4 hours in transplant populations generally(Venkataramanan et al., 1990). It has been noted since the earliest days of the clinical use of tacrolimus that in some patients the drug is rapidly absorbed after oral adminstration, with Tmax reached within 30 minutes, but in others the concentration-time profile is relatively flat(Venkataramanan et al., 1991a). Multiple peaks have also been noted in the concentration-time profiles in both dog and human studies(Venkataramanan et al., 1990). Figure 46 shows the unusual concentration-time profiles seen in four different liver transplant patients after an oral dose of 9 mg tacrolimus.

Figure 57. Plasma tacrolimus concentration vs. time curves after administration of 9 mg by mouth to four liver transplant patients
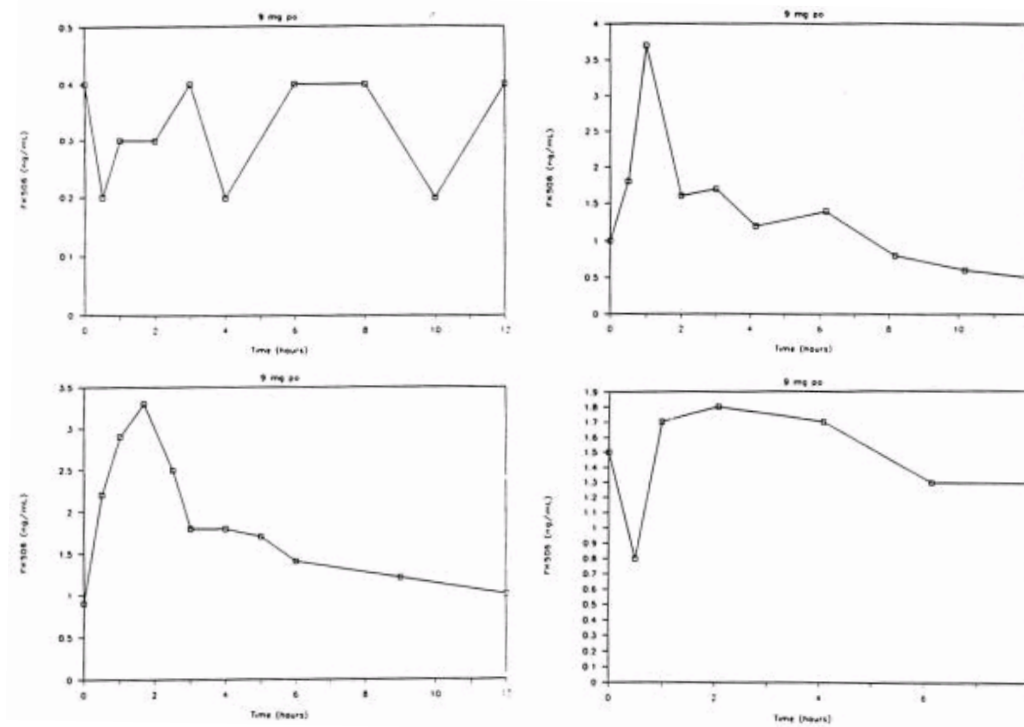
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There may be as many as 15 metabolites of tacrolimus, none of which have significant activity(Lampen et al., 1995; Staatz and Tett, 2004). Tacrolimus has also been shown to be a weak inhibitor of CYP3A4 and CYP3A5 *in vitro*(Amundsen et al., 2011), although a study in kidney transplant patients between one and five years post-transplant receiving tacrolimus reported no difference in IV clearance or apparent oral clearance of midazolam from healthy control subjects (de Jonge et al., 2011). High interindividual variability exists in the clearance of tacrolimus after both IV and oral administration, largely due to wide differences in CYP3A4 and CYP3A5 expression and function between individuals. The mean clearance of tacrolimus after IV administration has been shown to be 0.040 L/hr/kg in healthy control subjects, 0.083 L/hr/kg

in adult kidney transplant recipients, and 0.053 L/hr/kg in adult liver transplant recipients(Astellas, September 2011).

In healthy volunteers, tacrolimus has been shown to have a mean volume of distribution of 1.91 ± 0.31 L/kg after IV administration, while in a study of IV tacrolimus in 17 liver transplant recipients, the mean volume of distribution was reported to be 0.85 ±0.30 L/kg. Tacrolimus is approximately 99% bound to plasma proteins, mainly albumin and alpha-1-acid glycoprotein, and partitions into erythrocytes, with a mean whole blood to plasma ratio of 35. For this reason, it is customary to measure whole blood concentrations of tacrolimus rather than plasma concentrations(Astellas, September 2011). Tacrolimus half-life estimates have been reported to range from 11.7 to 34.8 hours(Astellas, September 2011), and, since it is typically dosed every 12 hours, significant accumulation occurs in body tissues after repeated dosing every 12 hours (see Figure 47)(Venkataramanan et al., 1990).

Figure 58. Tissue concentrations of tacrolimus in rat after 14 days of 1.28 mg/kg/day IM
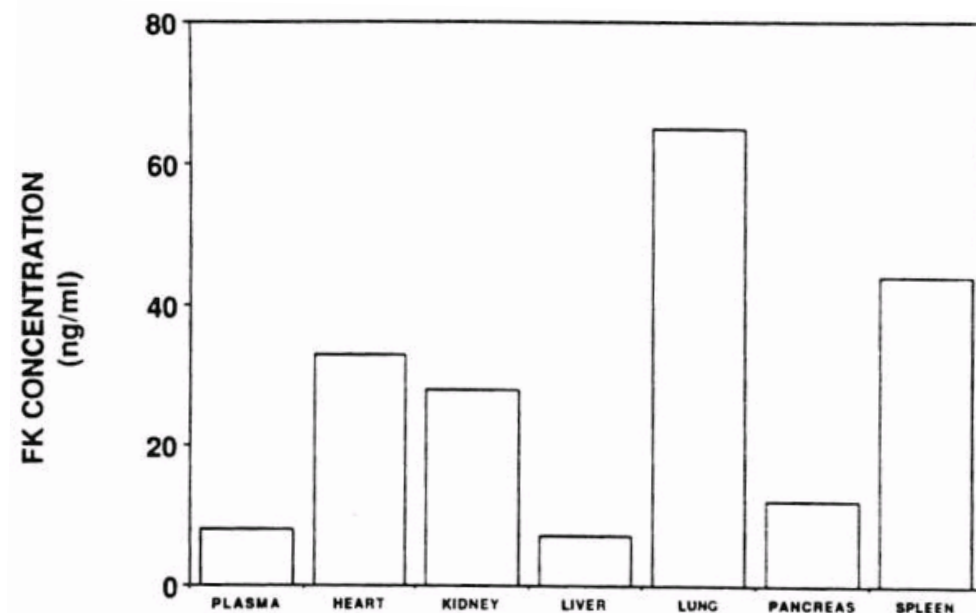
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A dose of tacrolimus undergoes almost total metabolism, with less than 5% of the drug appearing unchanged in bile, and less than 1% excreted unchanged in the urine(Venkataramanan et al., 1991a). Most metabolites of tacrolimus are excreted in bile via the feces, although small amounts of glucuronide conjugates may be excreted in the urine(Venkataramanan et al., 1991a; Venkataramanan et al., 1991b).

Despite the unpredictability of tacrolimus concentration-time profiles, many studies have found a strong correlation between tacrolimus AUC and the corresponding minimum concentration C0 or Clast. A study in kidney transplant patients found a correlation between AUC from 0 to 10 hours and Cmin or 0 to 12 hours and Cmin of 0.93(Astellas, September 2011). Another study in kidney transplant patients reported an r^2 of 0.79 (although it was only 0.67 during the first two weeks after transplant)(Scholten et al., 2005). However, in a third study there was a very poor correlation of r = 0.34, with an r^2 of 0.11 and a study in liver transplant

patients found an r^2 of 0.639.(Dansirikul et al., 2004). Overall, however, the correlation between tacrolimus AUC and trough has been found to be high, and for this reason trough concentration monitoring alone (as a surrogate for AUC) is typically performed for tacrolimus in clinical settings.

7.1.3   Tacrolimus use in small bowel transplant populations

Tacrolimus is a mainstay of immunosuppression after small bowel transplantation at many transplant centers(Fishbein, 2009). At the University of Pittsburgh, goal whole blood trough concentrations of tacrolimus for the first three months postoperatively are set at 10-15 ng/mL. Subsequently, and in the absence of rejection, concentrations are titrated to between 5 and 10 ng/mL. In stable patients, weaning of immunosuppression – involving reduction of the tacrolimus dose to daily, every other day, three times a week, or twice a week, is attempted(Abu-Elmagd et al., 2009).

The first study describing the pharmacokinetics of IV and oral tacrolimus in small bowel transplant recipients was a case series of five patients (two adult and three pediatric), four of whom had also received a liver transplant in conjunction with small bowel. Intensive pharmacokinetic sampling was done between 2 and 12 months post-transplant, with reported bioavailabilities ranging from 0.05 to 0.93, and lowest in the two patients with open ostomies. In those patients in whom the ostomy had been closed and the intestine reconnected to colon, the bioavailability of the drug ranged from 0.16 to 0.93 (mean 0.43) and Tmax from 0.5 to 5 hours (mean 2.8 hours). The investigators concluded that in their sample of patients the pharmacokinetic parameters of tacrolimus were not significantly different from those seen in other transplant populations(Jain et al., 1992). This was confirmed in a subsequent study of 21

small bowel transplant recipients (both with and without transplanted liver) also between 2 and 12 months post-transplant. The mean tacrolimus bioavailability in the adult subjects was 0.19 ±0.087 and time to maximal concentration (Tmax) was 2.9 ±2.2 hours(Jain et al., 1994).

A study comparing the steady-state pharmacokinetics of oral tacrolimus between clinically stable pediatric small bowel transplant recipients and pediatric liver transplant recipients observed no significant differences in the half-life, mean residence time (MRT), AUC, or oral clearance (CL/F) of tacrolimus between groups. The only significant difference between transplant groups was that the small bowel transplant recipients had a lower mean daily dose requirement than the liver transplant recipients (4.8±3.3 mg vs. 8.0 ±5.2, p=0.01), indicating a possible higher bioavailability of tacrolimus through the transplanted small bowel, but the influence of post-transplant time on dose requirement was not formally evaluated(Schubert et al., 2004).

7.2   METHODS AND SUBJECT DEMOGRAPHICS

As described in chapter 2, daily tacrolimus doses and whole blood trough concentrations were recorded in sixteen small bowel transplant patients (12 isolated intestinal and 4 modified multivisceral) starting on the day of transplant and continuing up to 40 days post-transplant. Subjects were started on tacrolimus continuous IV infusion (Prograf® injection, Astellas Pharma, Deerfield, IL) at a rate of 41.3 mcg/hr (approximately 1 mg over 24 hours) immediately upon arrival in the transplant ICU after surgery and infusion rates were increased if necessary to achieve target blood concentrations within the first few days after surgery. Subjects were begun on a low dose (typically 1 mg or 2 mg twice a day) regimen tacrolimus suspension administered

via jejunostomy tube on a median of post-transplant day three (range–three to six days), with the IV infusion discontinued from 12 to 24 hours later. This suspension was compounded by the hospital pharmacy by opening tacrolimus capsules (Prograf®, Astellas Pharma, Deerfield, IL) and mixing the contents with a suspending agent. A switch to the capsule formulation (Prograf® capsules, Astellas Pharma, Deerfield, IL) by mouth occurred on a median of post-transplant day 16 (range–12 to 27 days).

Doses were adjusted based on daily trough concentrations, but these concentrations were usually not true 12-hour troughs, since they were drawn between 4:00 am and 6:00 am (more typically around 4:00 am). Evening doses were typically scheduled for 9:00 pm. Therefore, even if the doses were given on time, the concentrations were no more than seven hour to 10 hour trough levels. Actual (rather than prescribed) dosing times were recorded for each day from electronic barcode administration records. The subjects, none of whom were taking aluminum or magnesium hydroxide at the time of study, took tacrolimus on an empty stomach and were not allowed grapefruit or grapefruit juice at any time during their hospitalization (and were instructed to avoid them at home).

On the day of each pharmacokinetic study session (as described in previous chapters), tacrolimus concentrations were measured in whole blood taken from each pharmacokinetic study sample before it was centrifuged to obtain the plasma. Samples were drawn at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 7.25, 7.5, 8, 10, 12, and 20 hours after oral midazolam administration. In many cases at study session 1, however, the administration of tacrolimus did not occur at time zero of the study session because the pharmacokinetic study sessions often began before the physicians had rounded on the patient and assessed whether or not the dose should be changed based on the concentration measured that morning. Therefore, intensive sampling over a full 12-hour dosing

interval and calculation of clearance by standard compartmental or noncompartmental methods was impossible in most subjects. AUC_{0-7} was estimable in all subjects and calculated by the linear trapezoidal method, and Cmax and Tmax were estimated from visual inspection of concentration-time profiles. These three parameters were compared between study sessions, route of administration, and transplant subtype using the Mann-Whitney U test. A two-tailed p value of less than 0.05 was considered statistically significant. Spearman correlations were used to examine the relationship between these three parameters and plasma cytokine concentrations and ileal *CYP3A4, CYP3A5,* and *ABCB1* gene expression. A one-sided p value of less than 0.05 was considered statistically significant for the correlations.

Since subjects were taking a range of doses, and doses were not changed for the purposes of the study, all three parameters were normalized to that morning's tacrolimus dose, Median morning dose at session 1 was 4 mg (range – 1 mg to 10 mg) and at session 2 was 6 mg (range – 3 mg to 7 mg). In two subjects, the morning tacrolimus dose was held because of high trough concentrations in the early morning. These subjects were excluded from pharmacokinetic analysis. Many subjects were not at steady state on the day of pharmacokinetic session 1, because of frequent dose changes in the first 40 days post-transplant, but assuming adherence to their prescribed regimens (they were outpatients at the time), all subjects were at steady state at session 2. For the comparisons of dose-normalized trough concentrations between IV, J-tube, and oral administration, only steady state concentrations were used (assumed if the subject had been on the same dose for at least 48 hours before the trough was drawn), with concentrations measured during biopsy-proven rejection episodes excluded from analysis. All whole blood tacrolimus concentrations were measured by LC-MS.

7.3 PHARMACOKINETIC ANALYSIS OF TACROLIMUS

7.3.1 Composite analysis of all subjects

Dose-adjusted tacrolimus concentration-time profiles for session 1 are shown in Figure 48 and for session 2 are shown in Figure 49, while pharmacokinetic parameters are shown in table 23. Tmax was significantly higher at session 1 as compared with session 2 (3.00 vs. 1.50 hours, p=0.036), as was dose-adjusted AUC_{0-7} (28.9 vs. 20.9 [mcg*hr/L]/dose in mg, p=0.0078). Although median dose-adjusted Cmax was higher at session 1 than session 2 (6.37 vs. 4.14 [mcg/L]/mg dose) the difference did not reach statistical significance.

Figure 59. Dose-adjusted concentration vs. time profiles at session 1
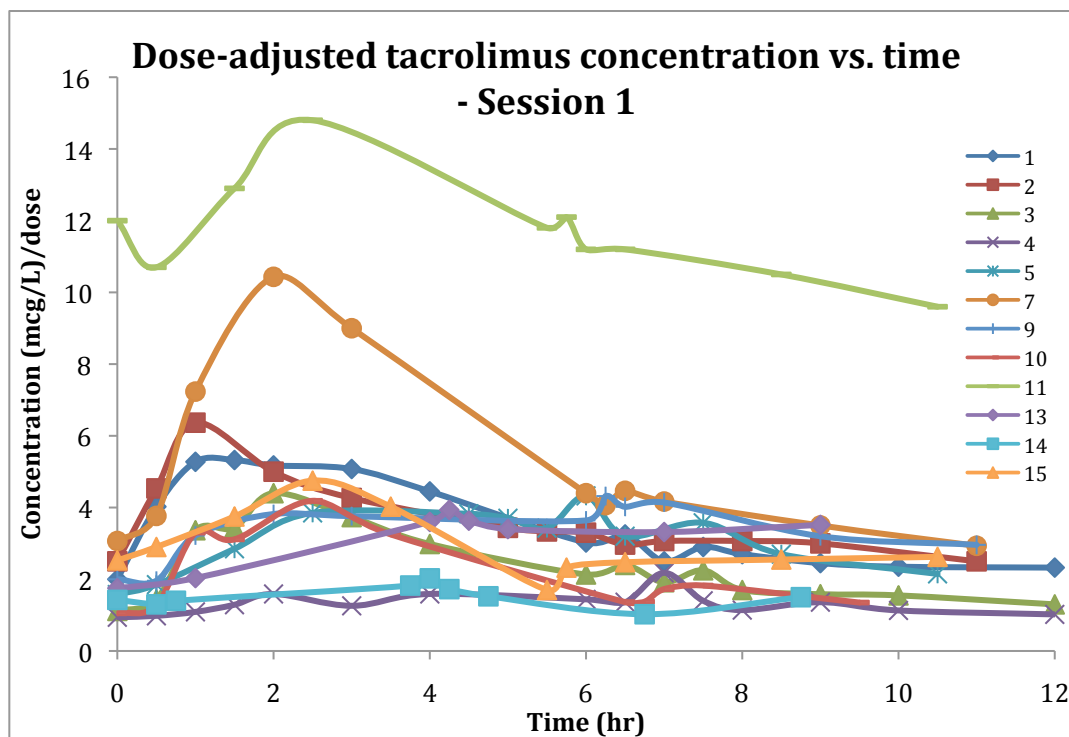
Figure 60. Dose-adjusted tacrolimus concentration vs. time profiles at session 2
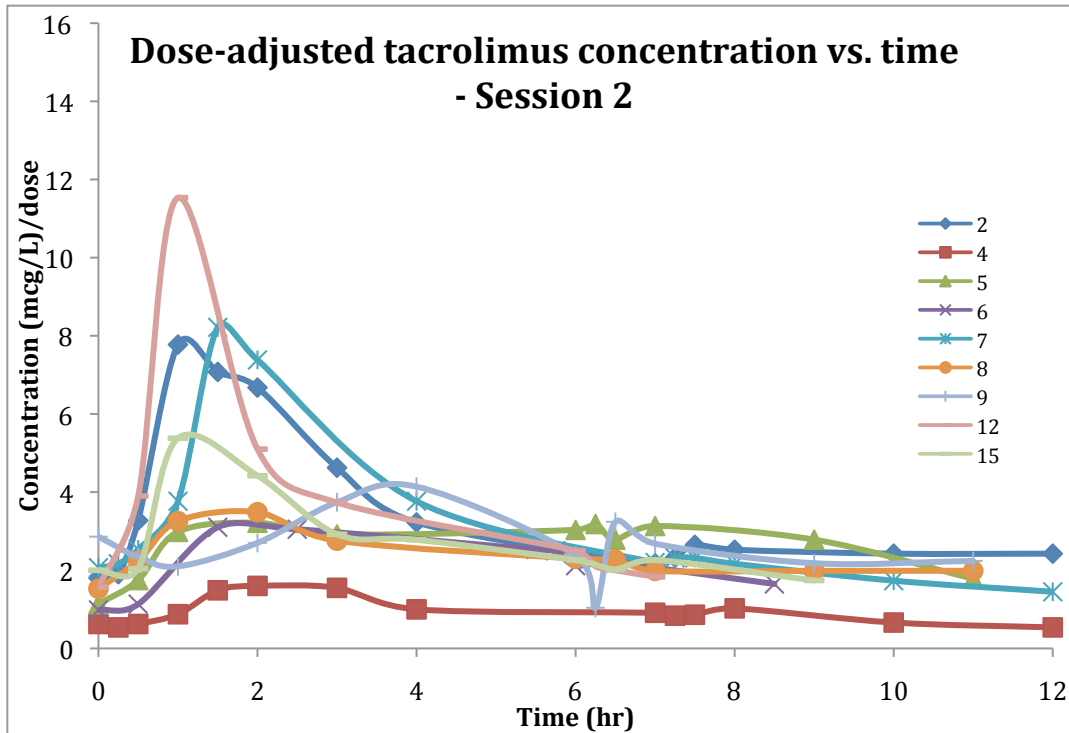
Table 25. Pharmacokinetic parameters of tacrolimus by study session

Tacrolimus parameter	Session 1	Session 2	p value Session 1 vs. 2
Dose-adjusted Cmax (mcg/L)/mg dose	6.37 (4.33,9.35)	4.14 (3.22,7.78)	0.43
Tmax (hr)	3.00 (2.25, 6.13)	1.50 (1.00, 2.00)	**0.036**
Dose-adjusted AUC_{0-7} (mcg*hr/L)/mg dose	28.9 (21.7,47.4)	20.9 (18.6,28.7)	**0.0078**

Results shown are median (25th, 75th percentile). Session 1 and session 2 were compared using the Mann-Whitney

U test. Two-tailed p values are reported.

7.3.2 Multiple peak phenomenon

All subjects showed at least two peaks in their concentration vs. time profiles at both study sessions. Five subjects at study session 1 and five at study session 2 had three peaks during the sampling interval, and four subjects – one at session 1 and three at session 2 – had four peaks. There was no apparent correlation within subjects between the number of peaks at session 1 and at session 2. Peak 1 was usually the highest and occurred at a median of 2 hours (range–1 hour to 4.25 hours), while Peak 2 occurred at a median of 6.5 hours (range–2.5 to 10.5 hours) and Peak 3 at a median of 7.5 hours (range–7 to 9 hours). If a fourth peak was present, it was reported at a median of 11 hours (range–9 to 11.5 hours). In 15 out of the 26 intensive sampling profiles of tacrolimus, a secondary peak occurred during or within 30 minutes following the midazolam IV infusion. Interestingly, this phenomenon included two subjects in whom the morning dose of tacrolimus had been held on the study day due to a supratherapeutic trough concentration that same morning. Tacrolimus concentration-time profiles for these two subjects are shown in Figure 50.

Figure 61. Tacrolimus concentration vs. time profiles in two subjects after morning dose was held
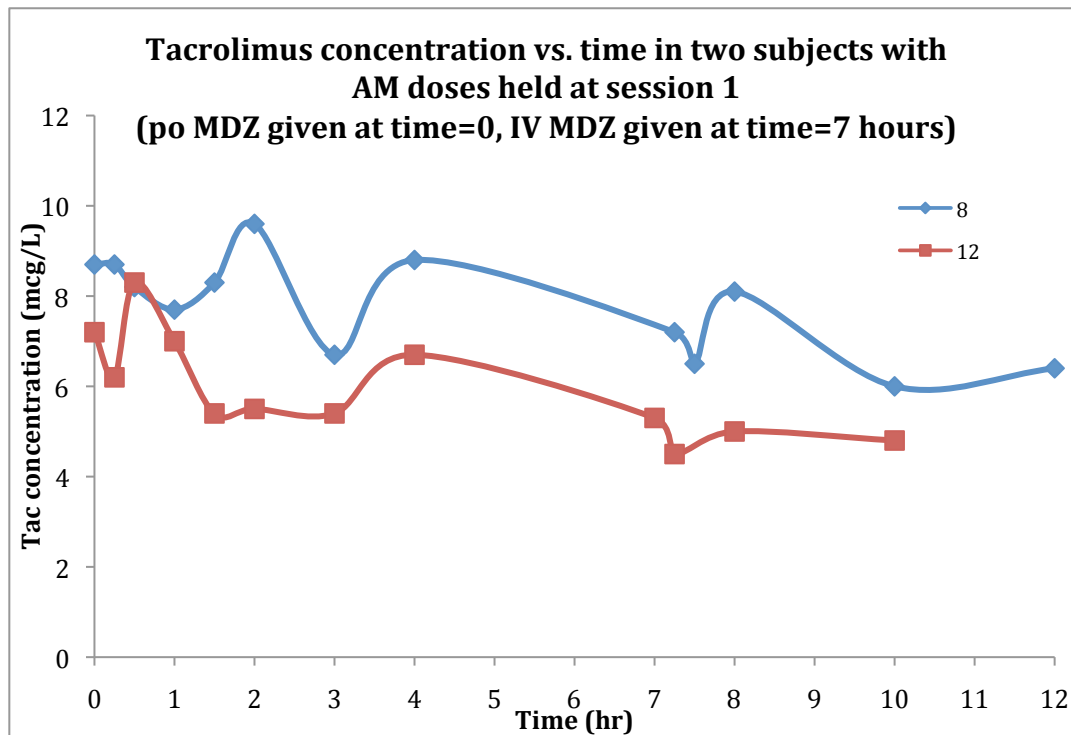
7.3.3 Effect of route of administration on tacrolimus pharmacokinetics

Dose-adjusted steady state tacrolimus trough concentrations during the period of IV, J-tube, and oral administration are shown in Table 24. Not surprisingly, concentration/dose ratios during IV infusion therapy on post-transplant days 3 to 5 were significantly higher than those observed after either J-tube or oral administration. There was no difference in dose-adjusted concentrations during J-tube administration on days 7 to 14 as compared to oral administration on days 18 to 30.

Table 26. Tacrolimus dose-adjusted trough concentrations in the first 30 days post-transplant by route of administration

	IV infusion Post-transplant days 3-5	Twice daily J-tube administration Post-transplant days 7-14	Twice daily oral administration Post-transplant days 18-30
Steady-state dose-normalized tacrolimus concentration (mcg/L)/mg	5.95 (4.68,8.18)	1.74 (1.51,1.96)	1.43 (1.05,2.03)

Results shown are median (25th, 75th percentile).

Table 25 compares the parameters of tacrolimus between the two enteral routes of administration. There was no significant difference in Tmax, dose-adjusted Cmax, or dose-adjusted AUC_{0-7} between the two groups.

Table 27. Pharmacokinetic parameters of tacrolimus by route of administration

Tacrolimus parameter	JT	PO	p value JT vs. PO
Dose-adjusted Cmax (mcg/L)/mg dose	4.86 (3.85,5.59)	4.55 (4.22,9.41)	0.76
Tmax (hr)	1.75 (1.38, 3.25)	2.50 (2.50, 4.13)	0.32
Dose-adjusted AUC_{0-7} (mcg*hr/L)/mg dose	24.6 (17.6,29.1)	23.2 (20.1,47.7)	0.43

Results shown are median (25th, 75th percentile). JT and PO were compared using the Mann-Whitney U test. Two-tailed p values are reported.

Figure 51 provides dose-adjusted tacrolimus concentration-time profiles after J-tube administration in four subjects at session 1. Three out of the four profiles are relatively smooth, with a median Tmax of 1.75 hours. One subject has the appearance of a slight double peak during the absorption phase, while another has a relatively flat profile, except for a small peak at 7 hours. In three of the four subjects, a secondary peak (preceded by a slight dip) in concentration is apparent at that same time point. In subjects 1 and 4, this peak occurred in the 30 minutes before the IV midazolam was given, while in subject 2 it occurred one hour after the end of the infusion, and in subject 3 at the end of the infusion.

Figure 62. **Dose-adjusted tacrolimus concentration vs. time profiles after J-tube administration at session 1**
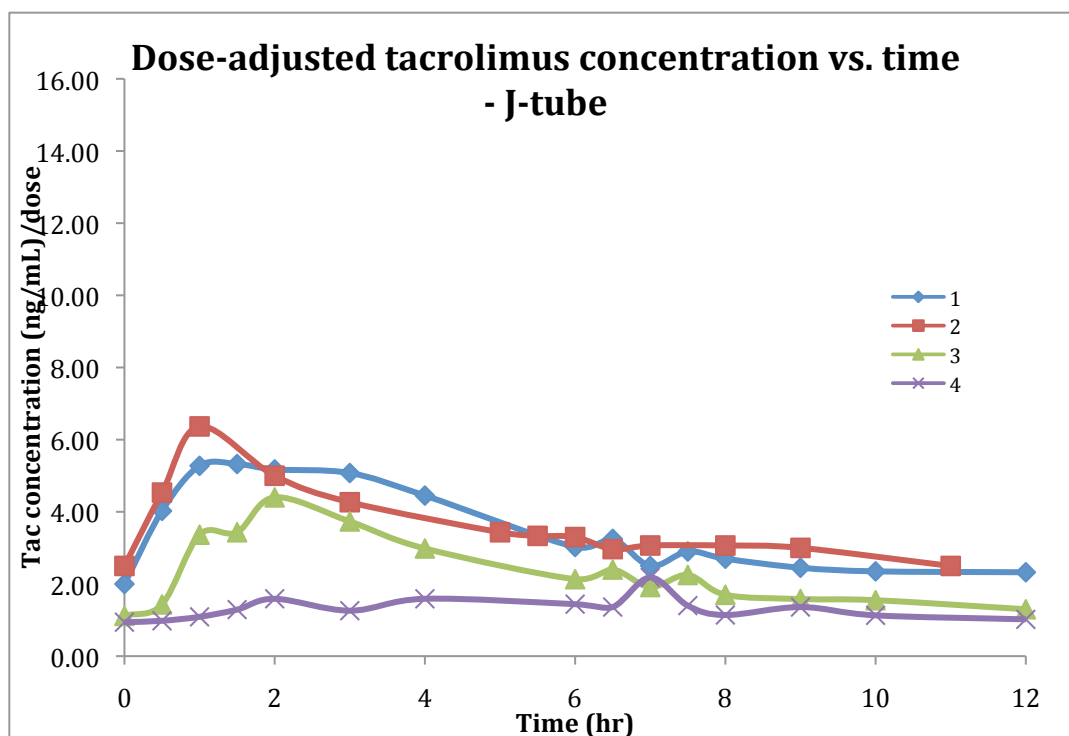
Figure 52 shows the dose-adjusted tacrolimus concentration-time profiles after oral administration in nine subjects at session 1. Five out of the nine subjects exhibited profiles with a clear Tmax (even though combined with a low Cmax in two) between 1 and 3 hours post-administration, followed by a clear elimination phase, although with one or more smaller secondary peaks. The remaining four subjects showed a relatively flat profile, punctuated by one or more small peaks. In six out of the nine subjects, small peaks occurred during or immediately after the end of the IV midazolam infusion. In the three other subjects, secondary tacrolimus peaks occurred three hours after the end of the infusion.

Figure 63. Dose-adjusted concentration vs. time profiles after oral administration at session 1
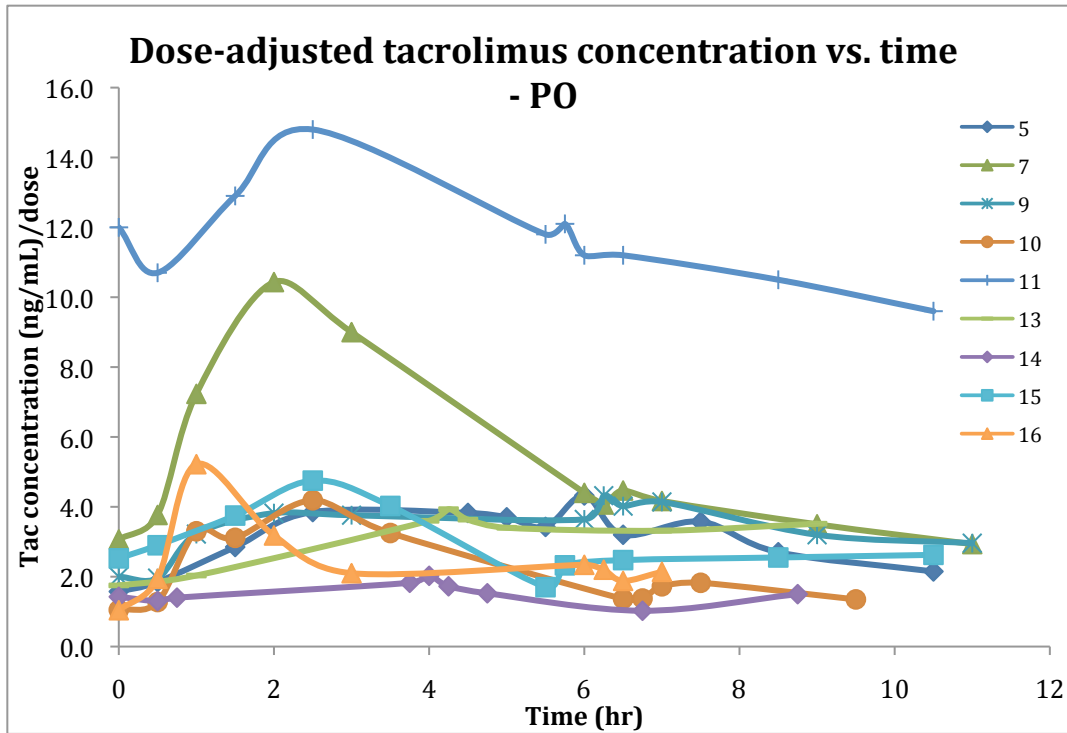
7.3.4 Effect of transplant type on tacrolimus pharmacokinetics

A comparison of pharmacokinetic parameters between transplant types is shown in table 26. Although median dose-adjusted Cmax was higher in the modified multivisceral (MM) group as compared with isolated intestinal (II) (7.38 vs. 4.58 [mcg/L]/mg dose) as was the Tmax (4.38 vs. 2.50 hr) and the dose-adjusted AUC_{0-7} (36.1 vs. 21.5 [mcg*hr/L]/dose, these differences did not reach statistical significance.

Table 28. Pharmacokinetic parameters of tacrolimus by transplant type at session 1

Session 1	MM (n=4)	II (n=12)	p value MM vs. II
Dose-adjusted Cmax (mcg/L)/mg dose	7.38 (3.79,11.5)	4.58 (4.29,6.85)	0.67
Tmax (hr)	4.38 (2.38, 6.44)	2.50 (1.63, 3.75)	0.25
Dose-adjusted AUC_{0-7} (mcg*hr/L)/mg dose	36.1 (21.0,56.2)	21.5 (19.8,32.8)	0.59

Results shown are median (25th, 75th percentile). MM and II were compared using the Mann-Whitney U test. Two-tailed p values are reported.

Dose-adjusted tacrolimus concentration-time profiles in modified multivisceral transplant subjects at session 1 are presented in Figure 53. Two out of the four subjects exhibit relatively typical drug concentration-time profiles, with a Tmax between 2 and 3 hours, and a relatively smooth elimination phase. The remaining two subjects exhibit relatively flat profiles. All subjects, however, exhibit a small peak occurring between six and seven hours post-dose. In subjects 7, 9, and 11, this peak occurred during or immediately after the end of the midazolam infusion, while in subject 4, it occurred immediately before the start of the infusion.

Figure 64. Dose-adjusted tacrolimus concentration vs. time profiles in modified multivisceral transplant recipients at session 1
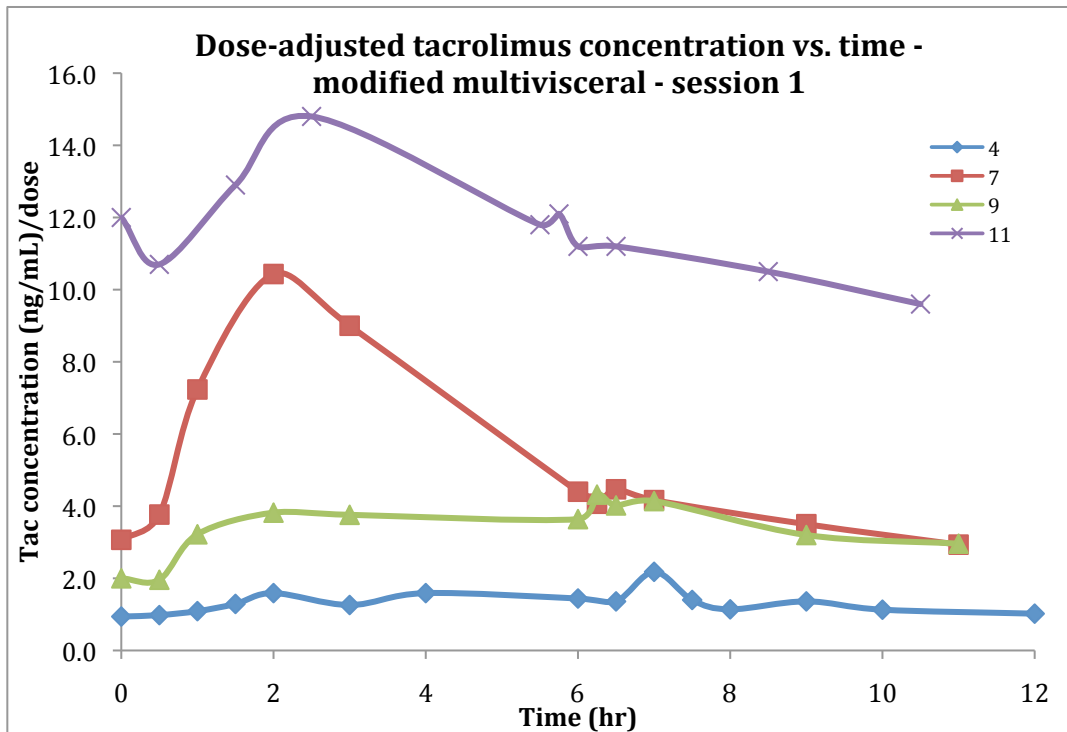
Dose-adjusted tacrolimus concentration-time profiles in isolated intestinal transplant recipients at session 1 are reported in figure 54. In this group, three out of nine subjects had relatively flat profiles, and the remaining six had a Tmax of between 1 and 4 hours. All had at least one secondary peak. In five out of the nine subjects, a small secondary peak occurred either during or immediately after the IV midazolam infusion: in one subject, this peak occurred 30 minutes before the infusion; in a second, it occurred one hour after the end of the infusion; while in three other subjects, it occurred three hours after the end of the infusion.

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Figure 65. Dose-adjusted tacrolimus concentration vs. time profiles in isolated intestine recipients at session 1
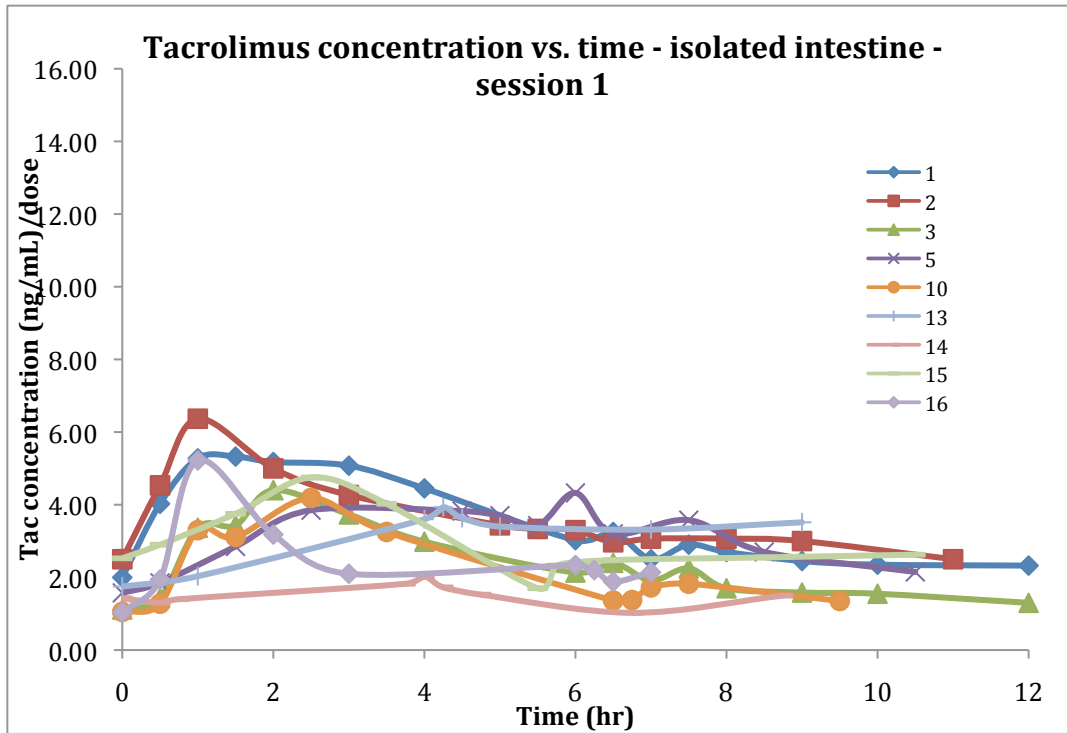
Table 27 lists the Tmax, dose-adjusted Cmax, and dose-adjusted AUC_{0-7} for both transplant types at session 2. There were no significant differences between groups with respect to any of the three parameters.

Table 29. Pharmacokinetic parameters of tacrolimus by transplant type at session 2

Session 2	MM (n=3)	II (n=7)	p value MM vs. II
Dose-adjusted Cmax (mcg/L)/mg dose	4.14 (2.87,6.18)	4.44 (3.29,7.18)	0.90
Tmax (hr)	2.00 (1.75, 3.00)	1.25 (1.00,1.88)	0.18
Dose-adjusted AUC_{0-7} (mcg*hr/L)/mg dose	20.9 (14.3,25.3)	20.7 (18.9,26.9)	1.0

Results shown are median (25th, 75th percentile). MM and II were compared using the Mann-Whitney U test. Two-tailed p values are reported.

Dose-adjusted concentration-time profiles for the three out of four modified multivisceral transplant subjects who returned for the second study session appear in Figure 55. Interestingly, each subject's profile pattern is quite similar to that from the first study session, with subject 7 showing a sharp Tmax at 1.5 hours and a smooth elimination phase, except for a very tiny peak at 7.25 hours during the IV midazolam infusion. Subject 4 again shows a fairly flat profile, although with a small peak during and immediately after the midazolam IV. Finally, subject 9, who was known to have impaired motility, even in the later post-transplant period, shows a similar irregular profile, with a decrease in tacrolimus concentration during the infusion followed by a peak at its end.

Figure 66. Dose-adjusted tacrolimus concentration vs. time profiles in modified multivisceral recipients at session 2
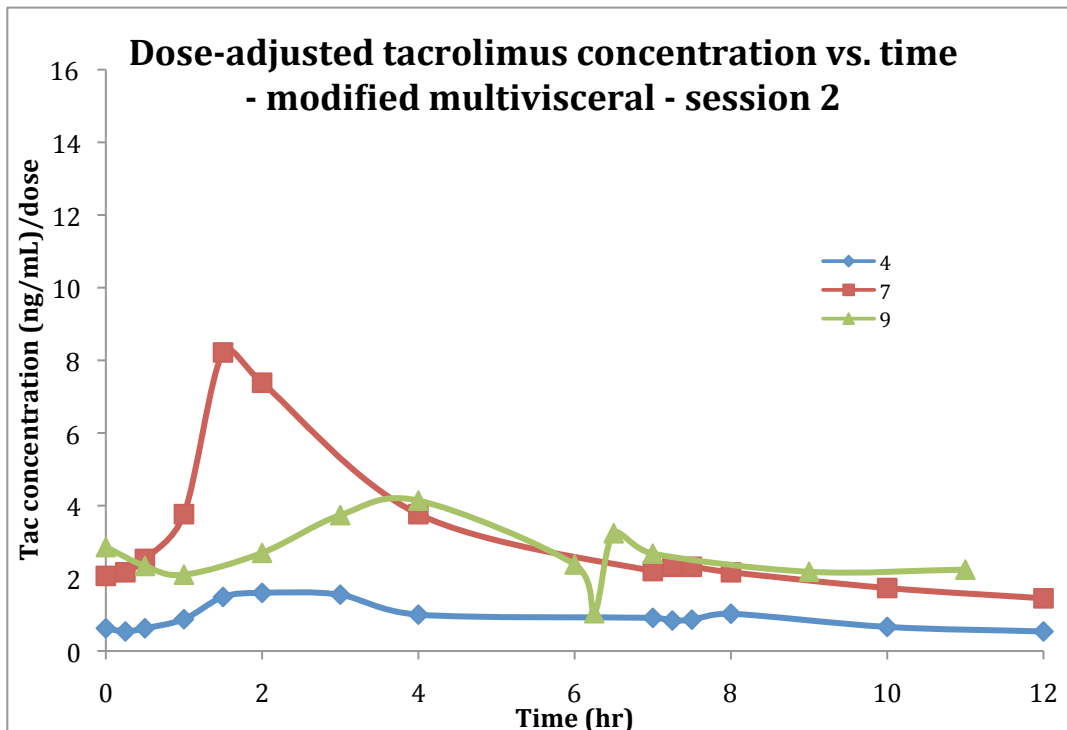
Dose-adjusted tacrolimus concentration-time profiles in isolated intestine recipients at session 2 are shown in figure 56. All subjects had an easily discernable initial peak, with a Tmax of between 1 hour and 3 hours. Dose-adjusted Cmax was widely variable, ranging from 3.10 to 11.5 [mcg/L]/mg dose, with a median of 4.44 [mcg/L]/mg dose. Four out of the six subjects exhibited a small peak during or immediately following the midazolam IV infusion, while one revealed a peak at one hour after the end of infusion, and another at three hours after the end of the infusion.

Figure 67. Dose-adjusted tacrolimus concentration vs. time profiles in isolated intestine recipients at session 2
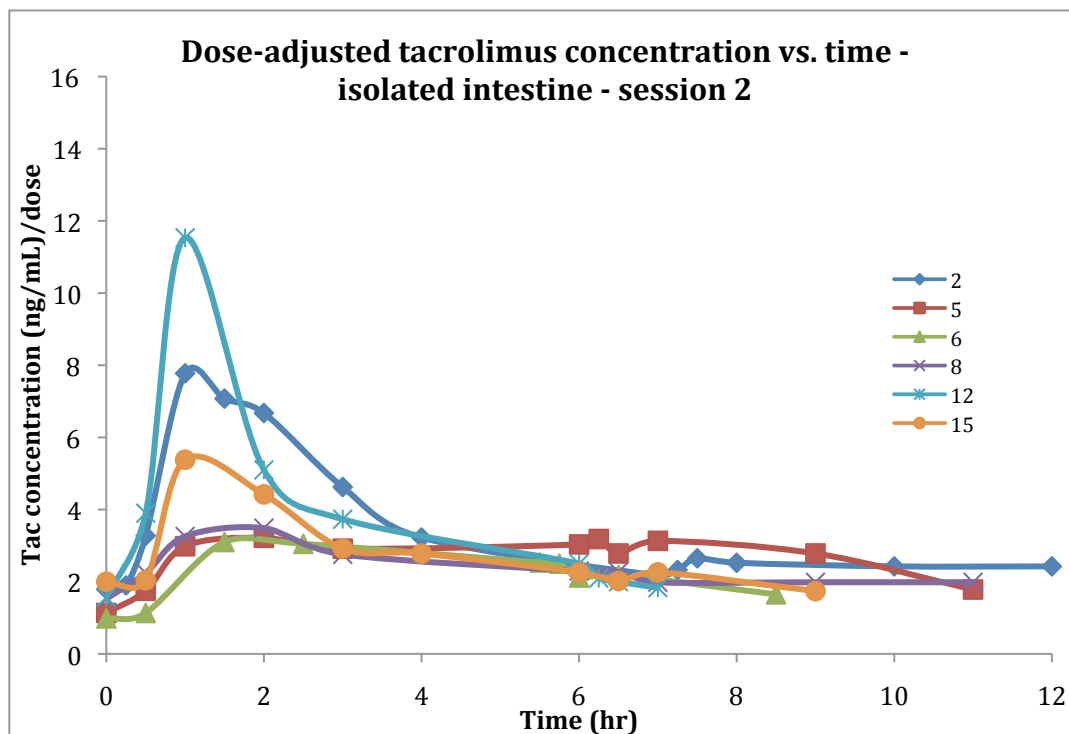
7.3.5 Correlation between tacrolimus pharmacokinetics, intestinal *CYP3A* and *ABCB1* expression, and plasma cytokine concentrations

There was no correlation observed between dose-adjusted AUC_{0-7}, dose-adjusted Cmax, or Tmax of tacrolimus and plasma concentrations of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12, TNF-α, or IFN-γ. There was also no correlation observed between pharmacokinetic parameters of tacrolimus and ileal *CYP3A4* or *ABCB1* expression. There was, however, a significant negative correlation between dose-adjusted AUC_{0-7} and *CYP3A5* expression (r_s=-0.47, p=0.015).

7.3.6 Relationship between tacrolimus trough levels and AUC

In order to compare the relationship between tacrolimus trough concentrations and AUC in this study population of small bowel and modified multivisceral transplant recipients to those seen in other transplant populations, the relationship between tacrolimus trough concentrations at the end of the sampling interval and the AUC of that interval were assessed by correlation analysis and are displayed in Figures 58, 59, and 60. There was a significant correlation between trough and AUC at session 1 (r_s=0.55, p=0.041) and in the subjects as a whole (r_s=0.57, p=0.0047).

Figure 68. Relationship between tacrolimus Cmin and AUC in transplant subjects at study session 1
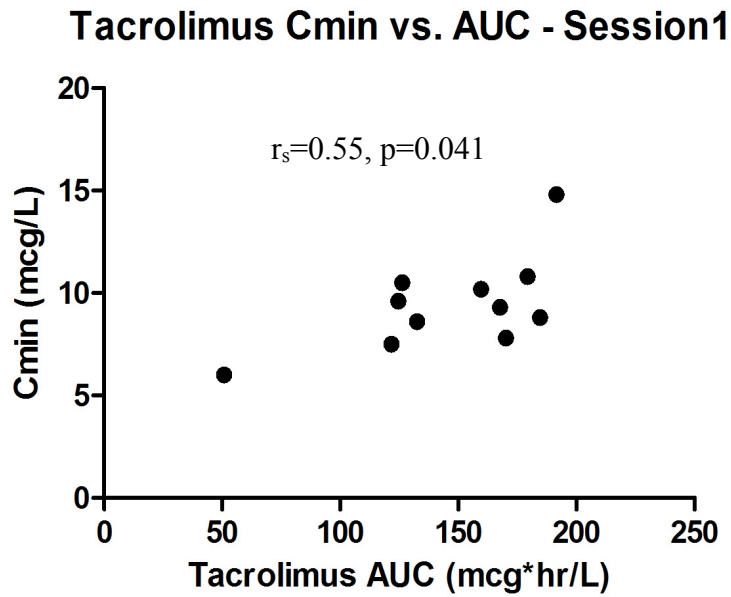
Figure 69. Relationship between tacrolimus Cmin and AUC in transplant subjects at study session 2
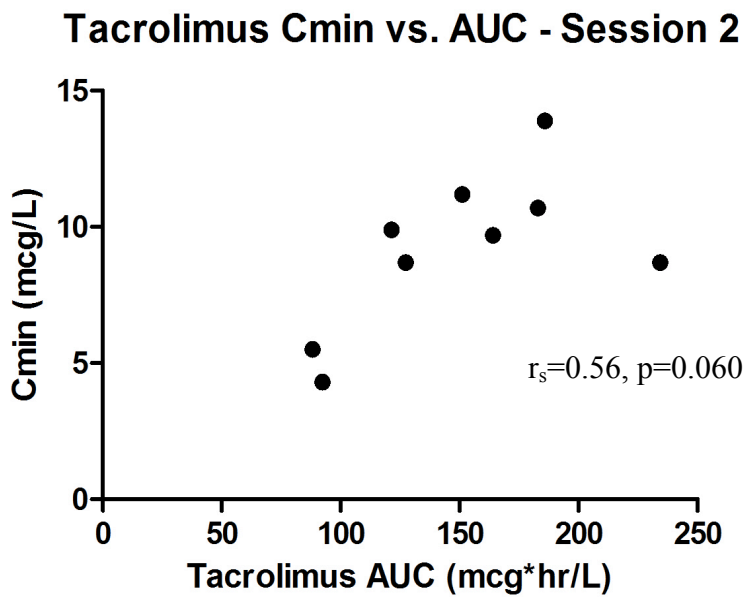
7.3.7 Relationship between midazolam and tacrolimus oral AUC

Figure 70. Relationship between midazolam oral AUC and dose-normalized tacrolimus AUC in transplant subjects at study session 1

Midazolam oral AUC_{0-7} vs. dose-normalized tacrolimus AUC_{0-7} - Session 1

$r_s=0.018, p=0.95$

Y-axis: MDZpo AUC_{0-7} (mcg*hr/L)

X-axis: Dose-normalized tacrolimus AUC_{0-7} (mcg*hr/L)
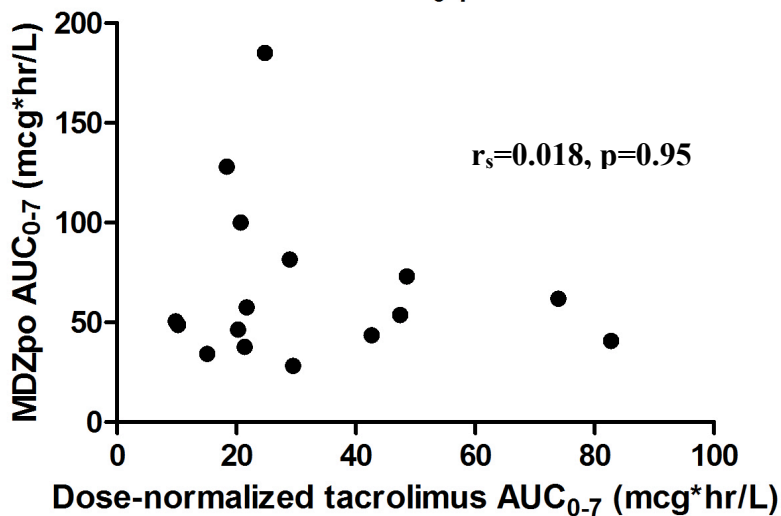
Figure 71. Relationship between midazolam oral AUC and dose-normalized tacrolimus AUC in transplant subjects at study session 2

Midazolam oral AUC$_{0-7}$ vs. dose-normalized tacrolimus AUC$_{0-7}$ - Session 2

MDZpo AUC$_{0-7}$ (mcg*hr/L) vs. Dose-normalized tacrolimus AUC$_{0-7}$ (mcg*hr/L)

r_s=-0.37, p=0.34
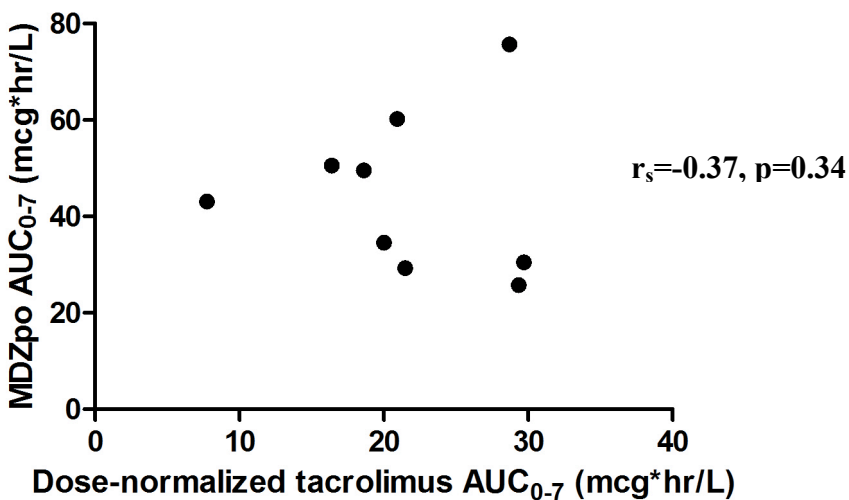
7.4   DISCUSSION

These results overall support the prediction that tacrolimus AUC will be higher in small bowel transplant recipients in the early post-transplant period as compared with later post-transplant. However, similar to studies in other transplant populations, multiple peak profiles were reported in many patients, sometimes with secondary peaks corresponding to intravenous administration of midazolam with regards to timing. The reason for these peaks is unknown but there are a number of possibilities, based on existing knowledge of tacrolimus. Multiple peaks in

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tacrolimus profiles have been noted since the drug was first in development(Venkataramanan et al., 1990), but are unlikely to be due to enterohepatic recirculation, since tacrolimus is not secreted in bile to any significant degree. Therefore, the peaks are more likely to reflect regional differences in solubility in the segments of the small intestine (most likely) and/or a pharmacokinetic interaction with midazolam (less likely).

A pharmacokinetic interaction between tacrolimus and midazolam is a possibility, but seems more likely after oral – rather than intravenous – administration of midazolam. Tamura et al. found that when midazolam was coperfused with tacrolimus in the rat intestinal lumen, tacrolimus absorption was enhanced in the jejunum but not in the ileum, suggesting some kind of inhibitory effect of midazolam on intestinal CYP3A at luminal concentrations(Tamura et al., 2003). It is possible that when the two drugs are present in the intestine at the same time, they may compete for the available CYP3A enzyme. This could also occur at the level of the liver, but is less likely, since both drugs are highly protein bound and the free fraction available to the hepatic CYP3A enzymes might be too low for this effect to occur. The sharp decrease in tacrolimus concentration that was observed in some cases before the secondary peak could also have been due to dilution of the blood samples, but this is unlikely, given that the IV was only 120 mL and was administered in the opposite arm from the site of blood draws. Furthermore, 120 mL would be unlikely to cause a significant increase in intestinal fluid content leading to improved solubility of tacrolimus in the intestine.

Instead, the poor solubility of the drug may have lead to dissolution and re-precipitation of the drug at various points in the GI tract. As has been noted previously, it is thought that much of the intestinal fluid content is contained in pockets throughout the intestine rather than being evenly distributed(Sutton, 2009). In six of the seven oral concentration-time profiles of

tacrolimus shown in the papers by Venkataramanan et al., similar multiple peaks were observed. Secondary peaks were visible at three and six hours in one patient, four and six hours in the second, four hours in the third and fourth, five hours in the fifth, and 6.5 hours in the sixth. In addition, secondary peaks were observed at five and 12 hours in a dog and at four and six hours in two human patients after intravenous dosing. It is quite possible that the timing of some of the smaller secondary peaks corresponded to the midazolam IV administration by coincidence, since the administration of the IV always occurred at the 7 hour timepoint of the study session and, for the most part, tacrolimus doses were administered in the morning, one or two hours after the oral midazolam dose.

Interestingly, multiple peaks were also observed in the profiles of two isolated intestinal transplant recipients at session 1 in whom the morning dose had been held due to high trough concentration. Not only were peaks observed, they occurred in a very similar pattern. One possible explanation for this is that small amounts of precipitated tacrolimus remained in the small intestine even after greater than 12 hours since the last dose due to slowed transit, and that these particles continued to be re-dissolved in fluid pockets at various points along the intestine and were absorbed, even in the absence of recent oral doses. Another explanation could be that as blood concentrations decline, accumulated drug begins to be released from tissues back into the systemic circulation.

The lack of correlation between ileal *ABCB1* mRNA expression and AUC, Cmax, and Tmax of tacrolimus was somewhat surprising, since a case of an adult small bowel transplant recipient (exact time post-transplant was not given) with very high dosing requirements for oral tacrolimus and cyclosporine despite apparently normal graft function demonstrated high levels of p-glycoprotein relative to duodenal mucosal samples taken from healthy subjects on Western

blot. Protein expression of CYP3A4 was also measured but found to be consistent with the amounts in the normal duodenal samples(Kaplan et al., 1999). A second study described a significant correlation between dose-normalized oral tacrolimus trough concentrations and ileal mucosal *ABCB1* mRNA expression in two pediatric small bowel transplant recipients. Similar to the results shown in this work, no significant correlation was observed between ileal *CYP3A4* expression and dose-normalized tacrolimus concentrations(Masuda et al., 2004). The finding in this study's population of small bowel recipients of a significant negative correlation between tacrolimus AUC and ileal *CYP3A5* expression is consistent with the finding that in *CYP3A5*1* expressors, CYP3A5 plays a larger role than CYP3A4 in tacrolimus metabolism.

The lack of a demonstrated dependence of tacrolimus AUC, Cmax, and Tmax on enteral route of administration or transplant subtype also points to the likelihood of the patterns seen being the result of solubility rather than motility. The reasonably high correlations seen in this study between tacrolimus AUC and trough concentrations also imply consistency in profile patterns with other transplant populations. Suppression of intestinal CYP3A function and expression in this population in the early post-transplant period was shown in chapter 5 by the increased oral bioavailability of midazolam, the decreased 1'hydroxymidazolam/midazolam oral AUC ratio, and the lower expression levels of ileal *CYP3A*. The increased AUC and Cmax of tacrolimus seen in the early post-transplant period as opposed to the later, in addition to the lack of association of absorption patterns with enteral route of administration or transplant subtype, imply that, similar to other transplant populations, solubility in the various regions of the small intestine is the main factor controlling the absorption of tacrolimus in small bowel transplant recipients, although when in soluble form, extent of absorption may be increased by lower

expression of intestinal CYP3A and p-gp, as this data has shown in the early post-transplant period.

This concept is likely translatable to other poorly soluble but highly permeable BCS/BDDCS class II substrates used in small bowel transplant recipients. Figure 61 shows concentration-time profiles of the antifungal medication voriconazole, also a class II drug, after oral dosing in four small bowel transplant recipients (three isolated intestinal and one full multivisceral) who were greater than nine months post-transplant. A second peak in the profile is observable in three of the four subjects, including one subject (an isolated intestinal recipient) in whom the profile is relatively flat(Choudry et al., 2007). Similar to the tacrolimus data presented earlier, there is no obvious association between small bowel transplant subtype and profile pattern in these voriconazole profiles.

Figure 72. Oral voriconazole concentration vs. time profiles in four small bowel transplant recipients
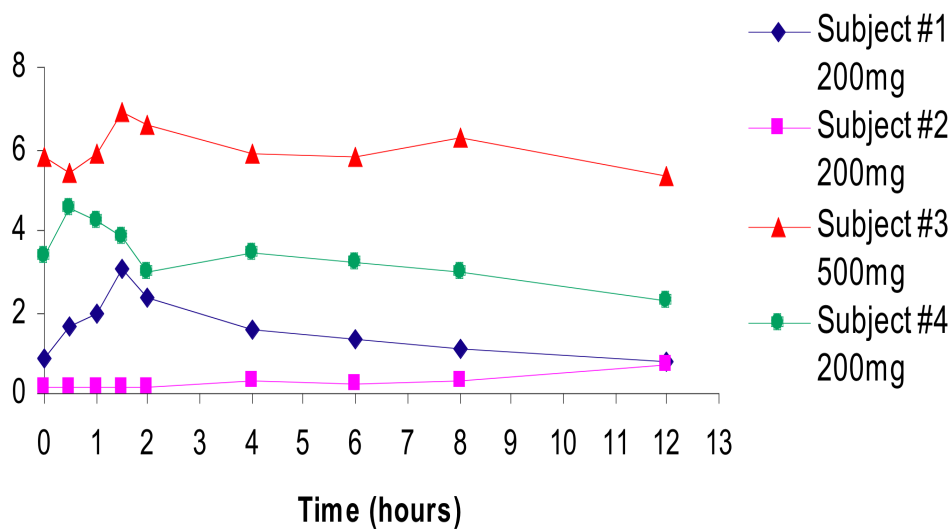
From Choudry et al. (Choudry et al., 2007)

Overall, these results suggest that overall oral absorption of poorly soluble but highly permeable BCS/BDDCS class II CYP3A/p-gp substrates may be higher in the early post-transplant period as compared with later due to initial suppression and eventual recovery of CYP3A and p-gp expression and function. However, concentration-time profiles over a dosing interval may be erratic, and this effect does not appear to depend on transplant subtype, but on solubility. These effects may be translatable in small bowel transplant recipients to other class III CYP3A/p-gp substrates such as sirolimus, dapsone, and nifedipine, but further studies with a larger number of subjects are required.

8.0   SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

8.1   SUMMARY

The transplanted intestinal graft undergoes many changes during removal from the donor and placement in the recipient, including ischemia and subsequent reperfusion injury and activation of cell-mediated immunity, leading to the local and systemic upregulation of inflammatory mediators, especially in the first few weeks after surgery. Since small bowel transplant recipients receive numerous medications after transplant, many by the oral route, the effects of transplantation of the intestine on drug absorption and intestinal first-pass metabolism and transport are of key clinical importance.

The intestine contains significant amounts of cytochrome P450 3A (CYP3A) and transporters such as p-glycoprotein (p-gp) that limit the oral bioavailability of substrate drugs. The suppressive effects of inflammation and inflammatory mediators on CYP450 and drug transporter gene expression and function have been well characterized. In addition, dog models of intestinal transplantation have shown increased availability of tacrolimus (a CYP3A and p-gp substrate) in the first 14 days post-transplant that returns to normal by day 28. Moreover, tacrolimus pharmacokinetics in stable small bowel transplant recipients months to years post-transplant are not significantly different than in other transplant populations, likely indicating eventual recovery of CYP3A and p-gp function.

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The major hypothesis of this work is that intestinal CYP3A and p-gp expression and function would be impaired in the early post-transplant period but that it will be no different than that of healthy control subjects in the later post-transplant period in stable patients without rejection. To examine this, 16 small bowel transplant recipients (12 isolated intestine and 4 modified multivisceral) were studied in the first 40 days post-transplant (median day 19, range day 10 to 40) and ten returned for a second study session between day 125 and 428 (median day 239). Sixteen age- and gender-matched control subjects were also administered study drugs for comparison purposes. The major findings of this work are as follows:

1. Midazolam (a highly soluble, highly permeable probe for CYP3A) oral bioavailability (p=0.0059) and oral AUC (p=0.0020), two measures of the extent of systemic absorption, were significantly higher in transplant subjects at the early study session, but no different from controls at the later study session. The AUC ratio of 1'hydroxymidazolam to midazolam, a measure of the extent of CYP3A-mediated metabolism, was significantly lower in the early period compared with controls (p=0.00070), but at the later period there was no difference with controls (p=0.13). There were no observed differences in the pharmacokinetics of oral midazolam between routes of administration (jejunostomy tube vs. oral) or between transplant subtype (modified multivisceral vs. isolated intestine). *CYP3A4* and *CYP3A5* gene transcripts were also lower in ileal biopsy samples in the early post-transplant period as compared with the later, and there was a positive correlation in the early post-transplant period between plasma TNF-α and midazolam oral AUC (r_s=0.71, p=0.0021). There was also a significant positive correlation at study session 1 between plasma IL-6 concentrations and midazolam bioavailability (r_s=0.67, p=0.0081). There were significant negative correlations observed early post-transplant

between ileal *CYP3A4* expression and plasma concentrations of IL-2 (r_s=-0.58, p=0.036), IL-4 (r_s=-0.53, p=0.062), IFN-γ (r_s=-0.53, p=0.062) and IL-12 (r_s=-0.51, p=0.077).

2. Oral AUC of fexofenadine (a highly soluble but poorly permeable probe for p-gp) was no different between study groups taken as a whole, but Tmax was significantly higher at the early study session as compared with the later (p=0.050), and remained higher than controls at the second study session as well (p=0.0021). There were trends towards a higher Cmax, lower Tmax, and higher AUC after administration via jejunostomy tube as compared with oral administration, but the differences did not reach statistical significance. When analyzed by transplant subtype, at the early study session modified multivisceral transplant recipients had a significantly lower fexofenadine Cmax (p=0.0064) and AUC (p=0.018) and higher Tmax (p=0.0062) than isolated intestinal transplant recipients. This difference in AUC persisted into the later post-transplant period (p=0.0167), although Tmax became similar between the two groups (p=0.90). Ileal transcript levels of the transporters *ABCB1* (p-gp), *MRP2*, and *ABCG2* (BCRP) were all lower in ileal biopsy samples in the early post-transplant period as compared with later, with a statistically significant difference in the case of *MRP2* (p=0.031). A significant positive correlation was observed between fexofenadine Cmax and ileal *ABCB1* expression (r_s= -0.52, p=0.071).

3. Tacrolimus (a poorly soluble, highly permeable CYP3A and p-gp substrate) dose-adjusted oral AUC$_{0-7}$, and Tmax were significantly higher in the early post-transplant period than in the later period (p=0.0078 and p=0.036, respectively). No significant effect of route of administration or transplant subtype on tacrolimus pharmacokinetics was observed. Absorption patterns of tacrolimus were erratic, with a range of two to four peaks seen on concentration-time

profiles. No significant effects of enteral route of administration or transplant subtype on tacrolimus dose-adjusted AUC_{0-7}, dose-adjusted Cmax, and Tmax were seen.

8.2 CONCLUSIONS

1. Intestinal CYP3A expression and function are impaired in the early post-operative period after small bowel transplantation

With regards to intestinal CYP3A expression and function after small bowel transplantation, these results clearly show diminished CYP3A activity and expression in the early post-transplant period as compared with the later period. Although there was a somewhat slower rate of absorption of midazolam seen in the transplant subjects as compared with controls, likely due to impaired gastric emptying, the pharmacokinetics of this highly soluble and highly permeable drug were not significantly affected by enteral route of administration or presence of transplanted stomach and duodenum, as in the case of modified multivisceral recipients. Therefore, the differences in pharmacokinetic parameters of oral midazolam between transplant subjects as a whole and controls may be assumed to be due for the most part to differences in CYP3A function. The decreased ileal mRNA expression of *CYP3A4* and *CYP3A5* in the early post-transplant period as compared with later, and the strong correlation between midazolam oral AUC and *CYP3A* expression provide further evidence for the suppression of intestinal CYP3A in the early post-transplant period. The strong inverse correlation between CYP3A expression and plasma cytokines, as well as the positive correlation between midazolam bioavailability and oral AUC and cytokines, provide evidence that this suppression is the result of the increased immune activity in the early post-transplant period.

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2. Intestinal CYP3A function is not significantly different from that seen in healthy persons four to 12 months post-transplant in small bowel transplant recipients

The fact that oral midazolam Cmax, AUC, bioavailability, and 1'hydroxymidazolam/midazolam AUC ratio in stable small bowel transplant recipients in the later post-transplant period were no different from those seen in healthy controls indicates that intestinal CYP3A function returned to normal in the later post-transplant period. In addition, the increased *CYP3A4* and *CYP3A5* mRNA expression levels in ileal mucosa and lower plasma cytokine concentrations in the later post-transplant period as compared with earlier support the hypothesis that CYP3A expression and function recover with time.

3. For highly soluble but poorly permeable transporter substrates such as fexofenadine, factors such as gastric and small intestinal motility may override the effects of transporters on oral absorption

These results also showed that absorption of the highly soluble but poorly permeable p-gp substrate fexofenadine was more dependent on route of administration and transplant subtype than ileal *ABCB1* expression. The significantly increased Tmax in the MM recipients as compared with II at both study sessions and the significantly improved rate and extent of absorption when the drug was administered by the jejunostomy tube, a route of administration that bypasses the effects of gastric emptying, as compared with oral administration, all indicate that alterations in gastric emptying rate and intestinal motility may reduce the time that this poorly permeable drug spends in the proximal small intestine, its optimal site of absorption. This argument is strengthened by the lack of correlation between pharmacokinetic parameters of fexofenadine and ileal *ABCB1* expression. The diminished transporter gene expression in the early post-transplant period as compared with the later, and the significant inverse correlation

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between plasma IFN-γ and *ABCB1* expression, as well as the positive correlation between plasma IL-6 and IL-8 and fexofenadine Tmax, indicate that immune activation may still be affecting absorption, but at the level of intestinal motility as much as transporter expression in the case of poorly permeable drugs.

4. *For poorly soluble but highly permeable CYP3A and p-gp substrates such as tacrolimus, solubility limitations will lead to erratic absorption patterns in small bowel transplant recipients, but when in soluble form in the intestine, systemic absorption of tacrolimus in the early post-transplant period will be increased due to impairments in intestinal CYP3A and p-gp. In the later post-transplant period, erratic absorption patterns and poor solubility persist, but intestinal CYP3A and p-gp suppression will no longer be present.*

The previous indications of the relationship between markers of inflammation and CYP3A and p-gp expression provide evidence that the increased tacrolimus dose-adjusted AUC_{0-7}, dose-adjusted Cmax, and Tmax seen in the early post-transplant period as compared with the later in the transplant groups as a whole likely reflect early immune-mediated suppression of CYP3A and p-gp. As a highly permeable but poorly soluble drug, the disposition of tacrolimus was not significantly affected by enteral route of administration or transplant subtype in this study population. Although a great deal of variability and multiple peaks were seen in concentration-time profiles, patterns could not be generalized to any particular transplant subgroup or post-transplant period with any certainty. A larger study with more subjects in the jejunostomy tube and modified multivisceral subgroups might elucidate trends further. Since the unusual and erratic concentration-time profiles seen in some of these subjects have also been seen in other transplant populations, the most likely explanation is that as a poorly soluble drug, tacrolimus undergoes repeated dissolution, absorption, and precipitation amongst the irregular

fluid volumes of the small intestine, and when in its soluble form, is highly susceptible to the amount of intestinal CYP3A and p-gp present at the site of absorption.

6. Taken as a whole, these results suggest that while CYP3A and transporter expression and function are suppressed early after transplant in small bowel transplant recipients, the solubility and permeability properties of a particular drug will determine the effect that this suppression has on the drug's disposition.

Highly soluble, highly permeable CYP3A substrate drugs such as midazolam are so readily absorbed that CYP3A metabolism will be the main determinant of their disposition. Drugs of this type, especially in solution form, can be expected to be rapidly and well absorbed by small bowel transplant patients. Clinicians taking care of these patients should choose BCS/BDDCS class I drugs whenever possible, although with the caveat that bioavailability may be enhanced in the early post-transplant period and during times of immune activation, such as rejection, if the drug is a CYP3A substrate.

This caveat also holds for poorly soluble but highly permeable BCS/BDDCS class II drugs such as tacrolimus, for although their absorption patterns will depend largely on solubility, when in soluble form their systemic absorption will also be affected by CYP3A and p-gp function, and increases in bioavailability may be seen in the early transplant period and during times of rejection, as has been shown in other studies. By contrast, factors such as gastric emptying and intestinal motility will have more significant effects on the systemic absorption of highly soluble but poorly permeable BCS/BDDCS class III drugs such as fexofenadine that are absorbed slowly even in healthy subjects. These motility effects may outweigh the effects of p-gp expression and function, a concept that casts doubt on the appropriateness of fexofenadine as a p-gp probe drug in populations with impaired intestinal motility.

8.3   FUTURE DIRECTIONS

This work offers important insights into drug absorption and intestinal first-pass metabolism and transport in small bowel transplant recipients, and offers proof that rates, patterns, and extents of absorption of midazolam, fexofenadine, and tacrolimus are different in small bowel transplant recipients than in healthy subjects. This work also offers strong evidence that immune activity is higher in the early post-transplant period after transplantation of the small bowel, and that this inflammation is at least partly responsible for the decreased CYP3A and transporter expression and the changes in pharmacokinetic parameters of the three drugs early post-transplant. However, larger studies are required to elucidate the mechanisms behind these changes and assess the relationship between the many patient variables that may affect drug disposition in this complex population and the pharmacokinetics of these or other oral drugs. Suggestions for future studies include:

- *Population pharmacokinetic analysis* The information presented in this work, especially the tacrolimus data with the large number of trough concentrations and rich but inconsistent intensive sampling, lends itself well to a population pharmacokinetic approach that examines the effects of covariates such as route of administration, transplant subtype, post-transplant day, intestinal CYP3A and transporter expression, corticosteroid dose, proton pump inhibitor use, creatinine clearance, and plasma cytokine concentrations on clearance, volume of distribution, and bioavailability. This work is currently in process in our lab, as is the genotyping of subject blood samples to assess the effects of recipient *CYP3A5* and *ABCB1* polymorphisms on the disposition of the study drugs. With a larger

group of subjects, enough carriers of the *CYP3A5*1* allele would be present to assess its effect on CYP3A-mediated metabolism.

- *Further study of gastric emptying rates and intestinal transit times after small bowel and modified multivisceral transplantation* Gastric emptying and intestinal transit times in the small bowel and modified multivisceral transplant populations need further study, both in patients with and without ileostomy. Concurrent objective measures of gastric emptying and intestinal transit times in the transplant subjects in this study would have been very valuable to the full understanding of their contribution to the absorption profiles observed, particularly of fexofenadine. In addition, a larger study at set timepoints early and later post transplant by oral administration and subsequent recovery of a non-disintegrating capsule would provide valuable insight into the fate of all orally administered medications after small bowel transplantation.

- *Assessment of the effects of ileostomy and ileostomy closure on the pharmacokinetics of BCS/BDDCS class II/III drugs* All transplant subjects were studied while the ileostomy was present, for maximum consistency. However, the effects of the presence or absence of the additional absorptive surface area of the colon would be useful to understand, especially for poorly permeable drugs such as fexofenadine. In addition, since tacrolimus bioavailability was noted in a previous study to be lower in patients with open ileostomy, and since tacrolimus is not secreted unchanged in bile to any significant degree, measurement of tacrolimus recovery in ostomy output would be a useful adjunct to future studies

of tacrolimus pharmacokinetics in small bowel transplant recipients, to assess how much drug is passing through the intestine unabsorbed.

- *Measurement of intestinal CYP3A and transporter protein expression in addition to mRNA expression* The small amount of mucosal tissue yielded by biopsy precluded the performance of Western blot analysis of CYP3A and transporter protein expression, which in some cases is a more reliable indicator of functional tissue protein content than mRNA levels. Although high correlations between *CYP3A4* mRNA and CYP3A4 protein expression have been reported, at least two studies have reported a poor correlation between *ABCB1* mRNA and p-gp protein expression. It is possible that this accounts for the lack of correlation between the pharmacokinetic parameters of fexofenadine and tacrolimus and *ABCB1* mRNA expression that were seen in this work.

- *Assessment of passive intestinal permeability in the early vs. later post-transplant periods* This work could not assess the contribution of passive permeability to the oral bioavailability of midazolam or the absorption profiles of fexofenadine and tacrolimus. As described in the introduction, pro-inflammatory mediators may loosen tight junctions between enterocytes leading to increased paracellular permeability. In addition, severe immune activation may cause mucosal sloughing and disruption of villus morphology, another mechanism of potential increased permeability. Although transplant subjects did not have biopsy-proven rejection at the time of study, it is possible that patchy areas of sloughing and blunted villi may have been present contributing to the enhanced bioavailability of midazolam in the early post-transplant period.

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Despite the continued knowledge gaps, it is to be hoped that this study provides a preliminary understanding of intestinal drug absorption, metabolism, and transport after small bowel and modified multivisceral transplantation, and will encourage further study of drug disposition in this unique and complex patient population.

APPENDIX A

CORRELATION ANALYSIS TABLES

A.1   CORRELATIONS BETWEEN CYP3A EXPRESSION AND PHARMACOKINETIC PARAMETERS OF ORAL MIDAZOLAM

Table 30. Spearman correlation coefficients for midazolam pharmacokinetics and *CYP3A* expression

		Spearman r *CYP3A4*	P value	Spearman r Total *CYP3A*	P value
MDZpo AUC (mcg*hr/L)	Tx 1 (n=13)	-0.43	0.14	-0.40	0.18
	Tx 2 (n=10)	-0.35	0.33	-0.22	0.54
	Tx 2-Tx 1 (n=7)	-0.071	0.91	-0.25	0.59
MDZ Bioavailability	Tx 1 (n=13)	-0.12	0.71	-0.23	0.45
	Tx 2 (n=10)	-0.33	0.17	-0.21	0.56
	Tx 2-Tx 1 (n=7)	-0.29	0.56	-0.32	0.50
1'OHMDZ/MDZ AUCpo ratio	Tx 1 (n=13)	0.35	0.24	0.32	0.28
	Tx 2 (n=10)	0.091	0.81	-0.0061	1.0
	Tx 2-Tx 1 (n=7)	-0.11	0.84	-0.29	0.56
MDZ CL/F (L/hr/kg)	Tx 1 (n=13)	0.23	0.46	0.32	0.28
	Tx 2 (n=10)	0.52	0.13	0.44	0.20
	Tx 2-Tx 1 (n=7)	0.14	0.78	0.36	0.44

A.2 CORRELATIONS BETWEEN MDR1 EXPRESSION AND PHARMACOKINETIC PARAMETERS OF ORAL FEXOFENADINE

Table 31. Spearman correlation coefficients for fexofenadine parameters and *ABCB1* expression

		Spearman r *ABCB1*	P value
FEX Cmax (mcg/L)	Tx 1 (n=13)	**-0.52**	**0.071**
	Tx 2 (n=10)	-0.042	0.92
	Tx 2-Tx 1 (n=7)	**-0.75**	**0.066**
FEX Tmax (hr)	Tx 1 (n=13)	**0.47**	**0.10**
	Tx 2 (n=10)	-0.078	0.84
	Tx 2-Tx 1 (n=7)	0.071	0.91
FEX AUC0-11 (mcg*hr/L)	Tx 1 (n=13)	**-0.57**	**0.040**
	Tx 2 (n=10)	-0.079	0.84
	Tx 2-Tx 1 (n=7)	-0.68	0.11
FEX AUC0-19 (mcg*hr/L)	Tx 1 (n=13)	-0.45	0.19
	Tx 2 (n=10)	-0.16	0.66
	Tx 2-Tx 1 (n=7)	-0.31	0.56
FEX CL/F (L/hr/kg)	Tx 1 (n=13)	-0.60	0.35
	Tx 2 (n=10)	0.15	0.68
	Tx 2-Tx 1 (n=3)	1.0	0.33
FEX MRT (hr)	Tx 1 (n=13)	0.23	0.44
	Tx 2 (n=10)	-0.35	0.33
	Tx 2-Tx 1 (n=7)	-0.046	0.92

CORRELATIONS BETWEEN PLASMA CYTOKINE CONCENTRATIONS AND

PHARMACOKINETIC PARAMETERS OF ORAL MIDAZOLAM

Table 32. Spearman correlation coefficients for plasma cytokines and pharmacokinetic parameters of midazolam. 2-sided p values given.

		MDZpo AUC (mcg*hr/L)	p	1'OHMDZ/ MDZ AUCpo ratio	p	MDZ F	p	MDZ CL/F (L/hr/kg)	p
IL-1β	Tx1 (n=16)	-0.28	0.30	0.27	0.32	0.31	0.25	-0.057	0.83
	Tx2 (n=10)	-0.26	0.46	0.18	0.64	-0.15	0.68	0.19	0.60
	Tx2-Tx1 (n=10)	-0.079	0.84	-0.36	0.31	0.43	0.22	0.53	0.12
IL-2	Tx1 (n=16)	-0.035	0.90	-0.13	0.64	**0.42**	**0.10**	-0.27	0.31
	Tx2 (n=10)	-0.16	0.66	-0.44	0.20	-0.46	0.18	0.049	0.90
	Tx2-Tx1 (n=10)	-0.33	0.35	-0.38	0.28	-0.055	0.89	0.44	0.20
IL-4	Tx1 (n=16)	-0.081	0.77	0.0090	0.97	0.21	0.44	-0.093	0.73
	Tx2 (n=10)	0.036	0.92	-0.16	0.66	0.21	0.56	0.018	0.97
	Tx2-Tx1 (n=10)	-0.32	0.37	-0.45	0.19	0.079	0.84	0.42	0.23
IL-6	Tx1 (n=16)	0.11	0.70	0.055	0.86	**0.67**	**0.0082**	-0.29	0.32
	Tx2 (n=10)	0.50	0.14	-0.52	0.13	0.12	0.76	-0.38	0.28
	Tx2-Tx1 (n=10)	0.37	0.30	0.042	0.92	0.31	0.39	-0.13	0.73
IL-8	Tx1 (n=16)	0.05	0.86	0.14	0.60	-0.11	0.68	-0.24	0.38
	Tx2 (n=10)	0.27	0.22	0.33	0.17	0.28	0.21	0.030	0.47
	Tx2-Tx1 (n=10)	0.31	0.37	0.52	0.13	-0.018	0.97	-0.20	0.58
IL-10	Tx1 (n=16)	**0.53**	**0.036**	-0.39	0.14	-0.043	0.88	-0.32	0.22
	Tx2 (n=10)	0.018	0.98	-0.50	0.14	-0.24	0.52	-0.13	0.74
	Tx2-Tx1 (n=10)	-0.12	0.38	**0.59**	**0.081**	-0.042	0.92	-0.018	0.97
IL-12	Tx1 (n=16)	-0.29	0.27	0.12	0.66	0.20	0.46	-0.070	0.80
	Tx2 (n=10)	0.042	0.92	-0.43	0.21	-0.15	0.68	-0.26	0.47
	Tx2-Tx1 (n=10)	0.091	0.81	-0.48	0.17	-0.33	0.35	-0.22	0.54
IFNγ	Tx1 (n=16)	0.24	0.37	-0.25	0.35	**0.56**	**0.026**	-0.27	0.32
	Tx2 (n=10)	-0.018	0.98	-0.47	0.18	-0.18	0.64	-0.21	0.56
	Tx2-Tx1 (n=10)	0.0061	1.0	**-0.76**	**0.015**	-0.067	0.87	0.054	0.89
TNF-α	Tx1 (n=16)	**0.71**	**0.0021**	**-0.53**	**0.036**	0.11	0.68	**-0.57**	**0.021**
	Tx2 (n=10)	0.44	0.20	0.10	0.78	0.21	0.56	-0.42	0.24
	Tx2-Tx1 (n=10)	0.030	0.95	-0.33	0.35	0.018	0.97	-0.19	0.61

A.4   CORRELATIONS BETWEEN PLASMA CYTOKINE CONCENTRATIONS AND

PHARMACOKINETIC PARAMETERS OF ORAL FEXOFENADINE

Table 33. Spearman correlation coefficients for plasma cytokines and pharmacokinetic parameters of fexofenadine

		FEX Cmax (mcg/L)	p	FEX Tmax (hr)	p	FEX MRT (hr)	p	FEX AUC 0-11 (mcg*hr/L	p	FEX AUC 0-19 (mcg*hr/L)	p	FEX CL/F (L/hr/kg	p
IL-1β	Tx 1 (n=16)	0.095	0.73	-0.21	0.43	-0.41	0.11	0.030	0.91	-0.12	0.71	0.43	0.35
	Tx 2 (n=10)	-0.055	0.90	-0.48	0.16	-0.14	0.70	-0.27	0.44	-0.13	0.72	0.055	0.90
	Tx2-Tx1 (n=10)	-0.33	0.35	-0.27	0.45	-0.30	0.41	0.13	0.73	0.19	0.66	0.40	0.75
IL-2	Tx 1 (n=16)	0.086	0.75	-0.24	0.37	-0.38	0.14	0.00	1.0	-0.11	0.72	0.50	0.27
	Tx 2 (n=10)	-0.073	0.84	0.23	0.52	0.22	0.54	-0.23	0.52	-0.012	0.98	-0.15	0.68
	Tx2-Tx1 (n=10)	-0.18	0.63	-0.30	0.39	-0.32	0.37	0.45	0.19	0.47	0.24	0.20	0.92
IL-4	Tx 1 (n=16)	0.087	0.75	-0.22	0.42	-0.26	0.34	-0.024	0.93	-0.0070	0.98	0.64	0.14
	Tx 2 (n=10)	**-0.58**	**0.088**	0.013	0.97	-0.030	0.95	**-0.67**	**0.039**	-0.53	0.12	-0.53	0.12
	Tx2-Tx1 (n=10)	**-0.62**	**0.060**	-0.47	0.17	0.30	0.41	-0.21	0.56	-0.071	0.88	0.80	0.33
IL-6	Tx 1 (n=16)	-0.11	0.70	0.37	0.16	0.23	0.39	-0.14	0.60	-0.049	0.88	0.11	0.84
	Tx 2 (n=10)	0.042	0.92	0.22	0.54	**0.73**	**0.020**	0.30	0.40	0.53	0.12	**-0.65**	**0.049**
	Tx2-Tx1 (n=10)	0.0061	1.0	**0.77**	**0.013**	0.45	0.19	0.10	0.79	0.48	0.24	0.0	1.1
IL-8	Tx 1 (n=16)	-0.25	0.34	**0.48**	**0.056**	**0.48**	**0.060**	-0.21	0.42	-0.084	0.80	-0.32	0.50
	Tx 2 (n=10)	0.31	0.38	-0.10	0.78	0.079	0.84	**0.55**	**0.10**	0.44	0.20	-0.20	0.58
	Tx2-Tx1 (n=10)	0.31	0.39	**0.63**	**0.060**	0.37	0.30	-0.079	0.84	0.086	0.84	0.0	1.1
IL-10	Tx 1 (n=16)	-0.076	0.78	0.14	0.60	0.28	0.30	-0.13	0.64	0.00	1.00	-0.18	0.72
	Tx 2 (n=10)	-0.21	0.56	0.27	0.44	0.26	0.46	-0.26	0.46	-0.055	0.90	0.030	0.94
	Tx2-Tx1 (n=10)	0.25	0.49	0.24	0.51	0.067	0.87	0.13	0.73	-0.40	0.33	0.20	0.92
IL-12	Tx 1 (n=16)	0.084	0.76	-0.23	0.39	-0.37	0.15	0.094	0.73	-0.064	0.84	**0.75**	**0.067**
	Tx 2 (n=10)	-0.26	0.51	0.078	0.84	0.28	0.43	-0.36	0.31	-0.12	0.76	-0.12	0.76
	Tx2-Tx1 (n=10)	-0.43	0.22	0.049	0.89	0.20	0.58	0.0061	1.0	**0.79**	**0.028**	-0.40	0.75
IFNγ	Tx 1 (n=16)	-0.0015	1.0	-0.073	0.79	-0.095	0.73	-0.13	0.62	-0.21	0.51	0.37	0.50
	Tx 2 (n=10)	-0.30	0.40	0.12	0.76	0.25	0.50	-0.42	0.24	-0.19	0.60	-0.067	0.86
	Tx2-Tx1 (n=10)	-0.26	0.47	0.018	0.97	0.12	0.76	0.15	0.68	0.43	0.30	-0.40	0.75
TNF-α	Tx 1 (n=16)	0.012	0.96	0.20	0.46	**0.44**	**0.10**	0.074	0.78	0.16	0.62	0.00	1.00
	Tx 2 (n=10)	0.26	0.46	0.11	0.76	0.32	0.38	0.38	0.28	0.38	0.28	-0.41	0.24
	Tx2-Tx1 (n=10)	0.16	0.66	0.27	0.45	0.19	0.61	0.26	0.47	0.45	0.27	0.0	1.1

A.5 CORRELATIONS BETWEEN PLASMA CYTOKINE CONCENTRATIONS AND CYP3A EXPRESSION

Table 34. Spearman correlation coefficients for plasma cytokines and *CYP3A* expression

		Spearman r *CYP3A4*	P value	Spearman r Total *CYP3A*	P value
IL-1β	Tx 1 (n=13)	-0.22	0.48	-0.33	0.27
	Tx 2 (n=10)	0.042	0.92	-0.19	0.61
	Tx2-Tx1 (n=7)	0.32	0.50	0.59	0.16
IL-2	Tx 1 (n=13)	**-0.48**	**0.10**	**-0.58**	**0.036**
	Tx 2 (n=10)	-0.48	0.17	-0.49	0.15
	Tx2-Tx1 (n=7)	0.39	0.40	0.54	0.24
IL-4	Tx 1 (n=13)	**-0.49**	**0.088**	**-0.53**	**0.062**
	Tx 2 (n=10)	-0.13	0.73	-0.34	0.33
	Tx2-Tx1 (n=7)	0.21	0.66	0.39	0.40
IL-6	Tx 1 (n=13)	0.41	0.17	0.33	0.28
	Tx 2 (n=10)	-0.079	0.84	-0.042	0.92
	Tx2-Tx1 (n=7)	0.61	0.17	0.50	0.27
IL-8	Tx 1 (n=13)	**0.62**	**0.025**	**0.62**	**0.026**
	Tx 2 (n=10)	0.44	0.20	0.47	0.18
	Tx2-Tx1 (n=7)	0.036	0.96	-0.11	0.84
IL-10	Tx 1 (n=13)	0.18	0.55	0.12	0.70
	Tx 2 (n=10)	**-0.65**	**0.049**	**-0.59**	**0.081**
	Tx2-Tx1 (n=7)	0.14	0.78	-0.036	0.96
IL-12	Tx 1 (n=13)	-0.39	0.19	**-0.51**	**0.077**
	Tx 2 (n=10)	**-0.58**	**0.088**	**-0.62**	**0.060**
	Tx2-Tx1 (n=7)	-0.11	0.84	-0.18	0.71
IFN-γ	Tx 1 (n=13)	**-0.48**	**0.098**	**-0.53**	**0.062**
	Tx 2 (n=10)	-0.49	0.15	-0.54	0.11
	Tx2-Tx1 (n=7)	0.25	0.59	0.39	0.40
TNF-α	Tx 1 (n=13)	-0.16	0.60	-0.19	0.53
	Tx 2 (n=10)	-0.33	0.35	-0.30	0.41
	Tx2-Tx1 (n=7)	0.32	0.50	0.29	0.56

A.6 CORRELATIONS BETWEEN PLASMA CYTOKINE CONCENTRATIONS AND ABCB1 EXPRESSION

Table 35. Spearman correlation coefficients for plasma cytokines and *ABCB1* expression

		Spearman r *ABCB1*	P value
IL-1β	Tx 1 (n=13)	-0.34	0.26
	Tx 2 (n=10)	0.36	0.31
	Tx2-Tx1 (n=7)	0.14	0.78
IL-2	Tx 1 (n=13)	-0.40	0.18
	Tx 2 (n=10)	-0.22	0.54
	Tx2-Tx1 (n=7)	0.29	0.56
IL-4	Tx 1 (n=13)	-0.18	0.56
	Tx 2 (n=10)	0.11	0.76
	Tx2-Tx1 (n=7)	0.54	0.24
IL-6	Tx 1 (n=13)	0.033	0.91
	Tx 2 (n=10)	-0.055	0.89
	Tx2-Tx1 (n=7)	-0.36	0.44
IL-8	Tx 1 (n=13)	0.23	0.46
	Tx 2 (n=10)	0.37	0.30
	Tx2-Tx1 (n=7)	-0.64	0.14
IL-10	Tx 1 (n=13)	0.16	0.59
	Tx 2 (n=10)	-0.54	0.11
	Tx2-Tx1 (n=7)	0.036	0.96
IL-12	Tx 1 (n=13)	-0.26	0.38
	Tx 2 (n=10)	-0.37	0.30
	Tx2-Tx1 (n=7)	0.25	0.59
IFN-γ	Tx 1 (n=13)	-0.41	0.16
	Tx 2 (n=10)	-0.32	0.37
	Tx2-Tx1 (n=7)	0.61	0.17
TNF-α	Tx 1 (n=13)	-0.27	0.37
	Tx 2 (n=10)	-0.14	0.71
	Tx2-Tx1 (n=7)	0.57	0.20

PLASMA CYTOKINE CONCENTRATIONS IN THE FIRST 21 DAYS POST-TRANSPLANT

B.1   PLASMA CYTOKINE TRENDS IN THE FIRST 21 DAYS POST-TRANSPLANT

Figure 73. Plasma IL-1beta vs. time
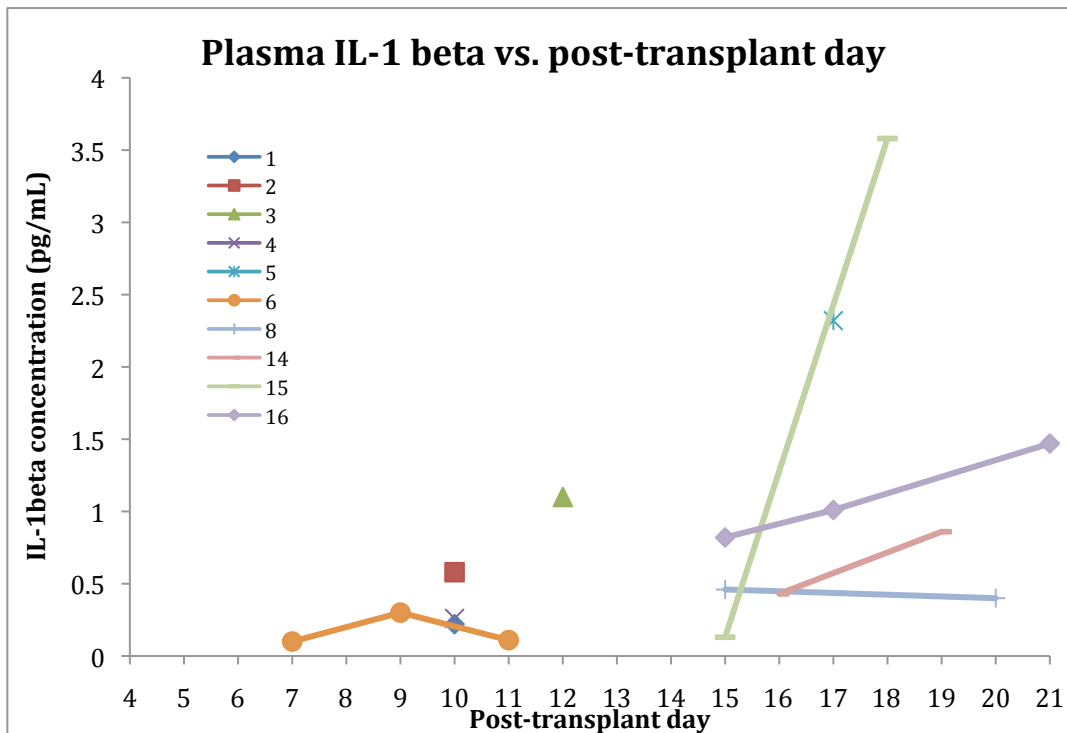
Figure 74. Plasma IL-2 vs. time

Plasma IL-2 vs. post-transplant day

Figure 75. Plasma IL-4 vs. time
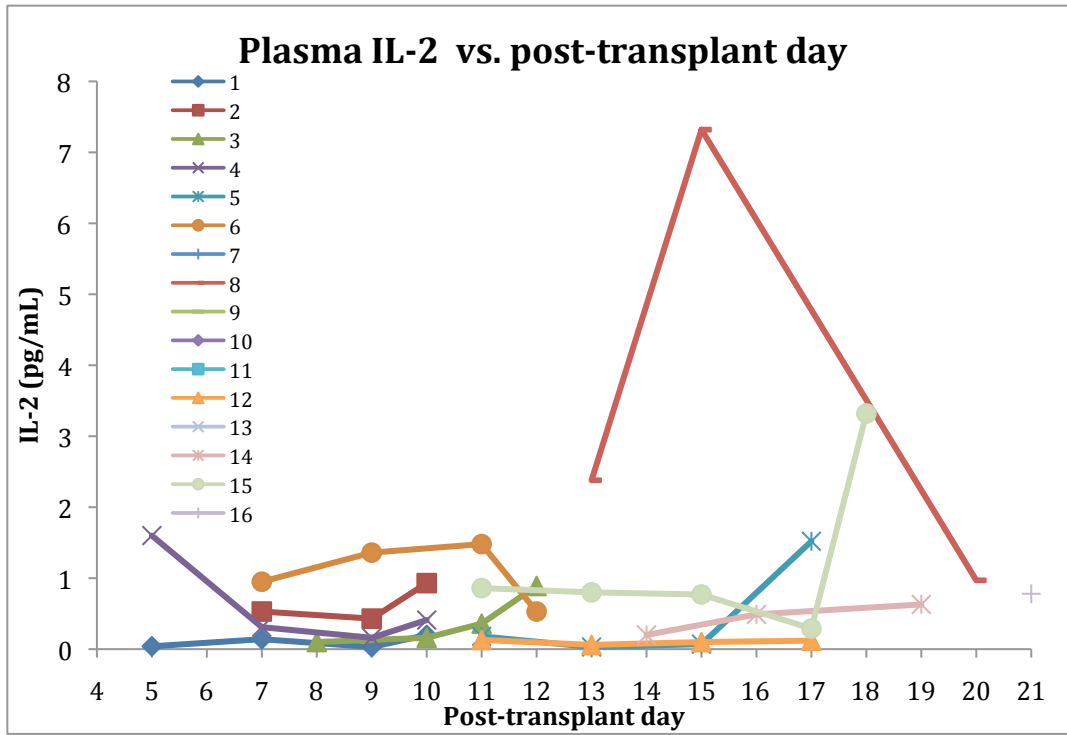
Figure 75. Plasma IL-4 vs. time
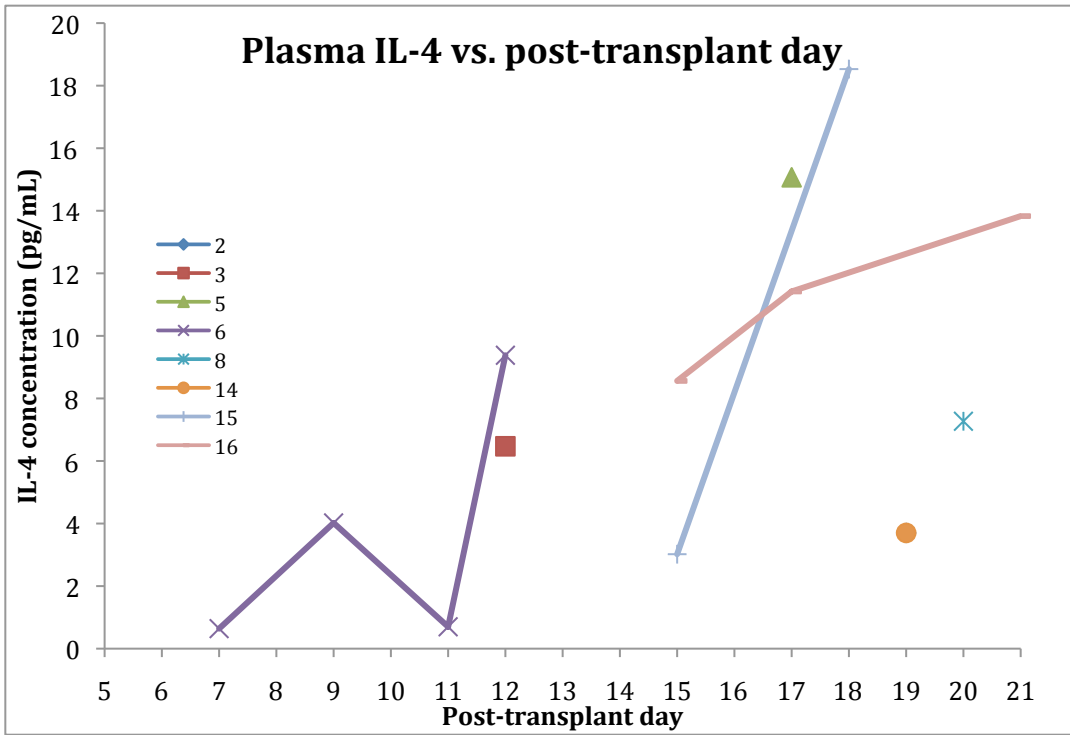
Figure 76. Plasma IL-6 vs. time

Plasma IL-6 vs. post-transplant day

Figure 77. Plasma IL-8 vs. time
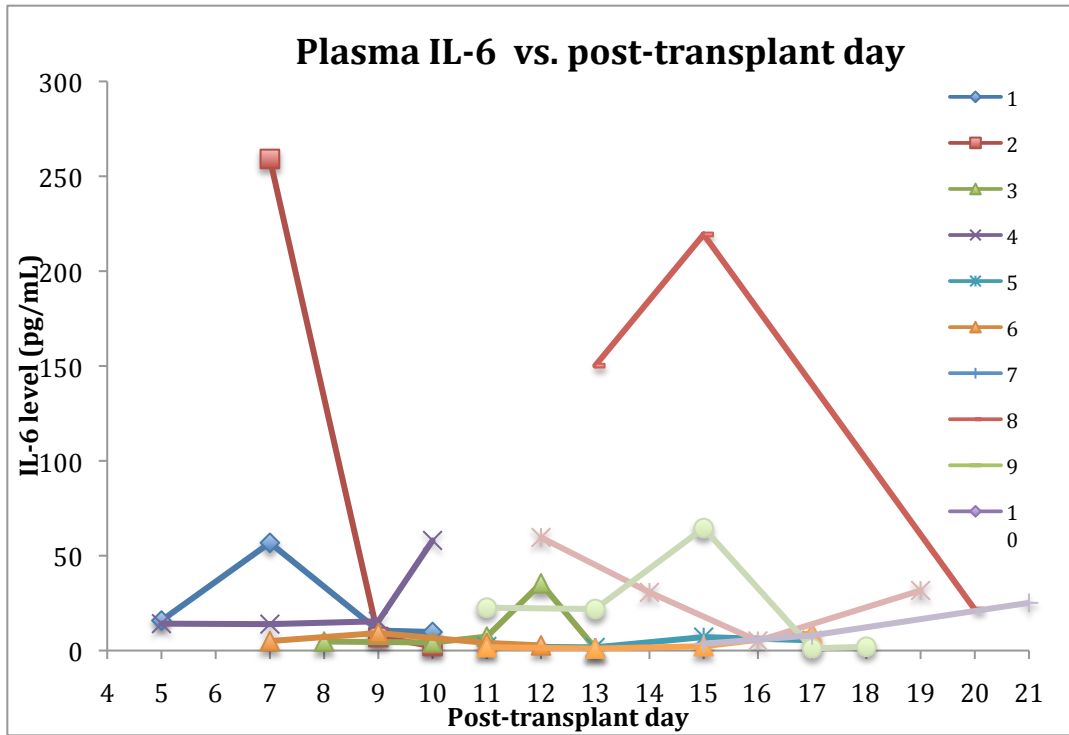
Figure 77. Plasma IL-8 vs. time
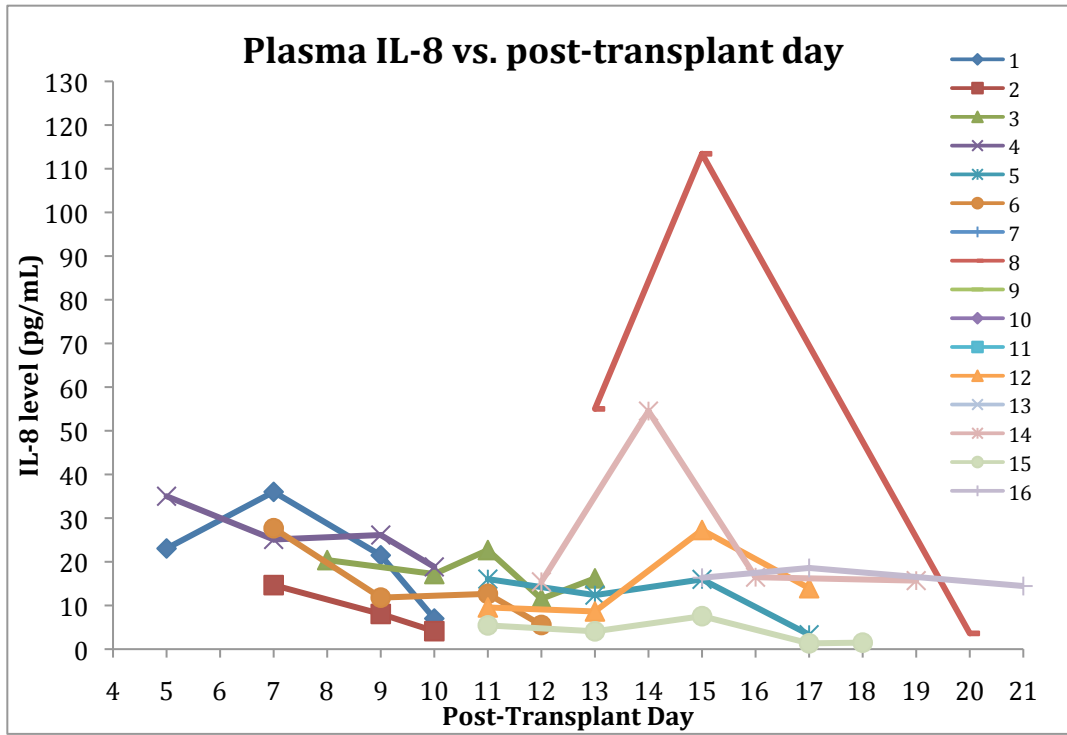
Figure 78. Plasma IL-10 vs. time

Plasma IL-10 vs. post-transplant day

Figure 79. Plasma IL-12 vs. time
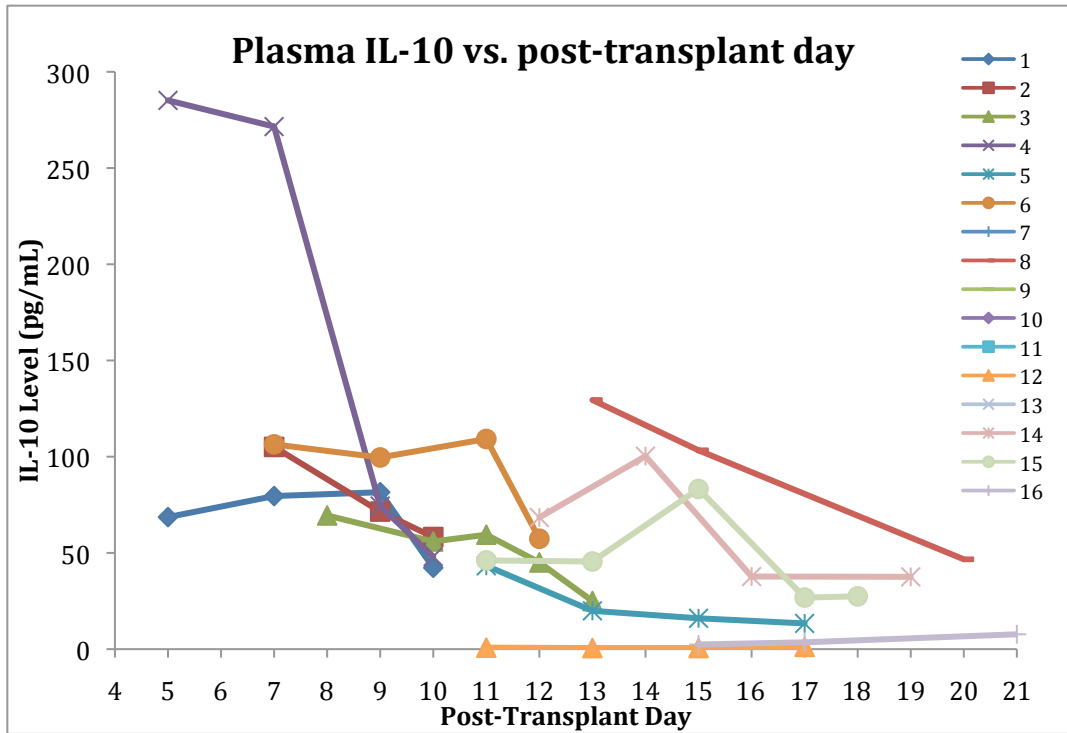
Figure 79. Plasma IL-12 vs. time
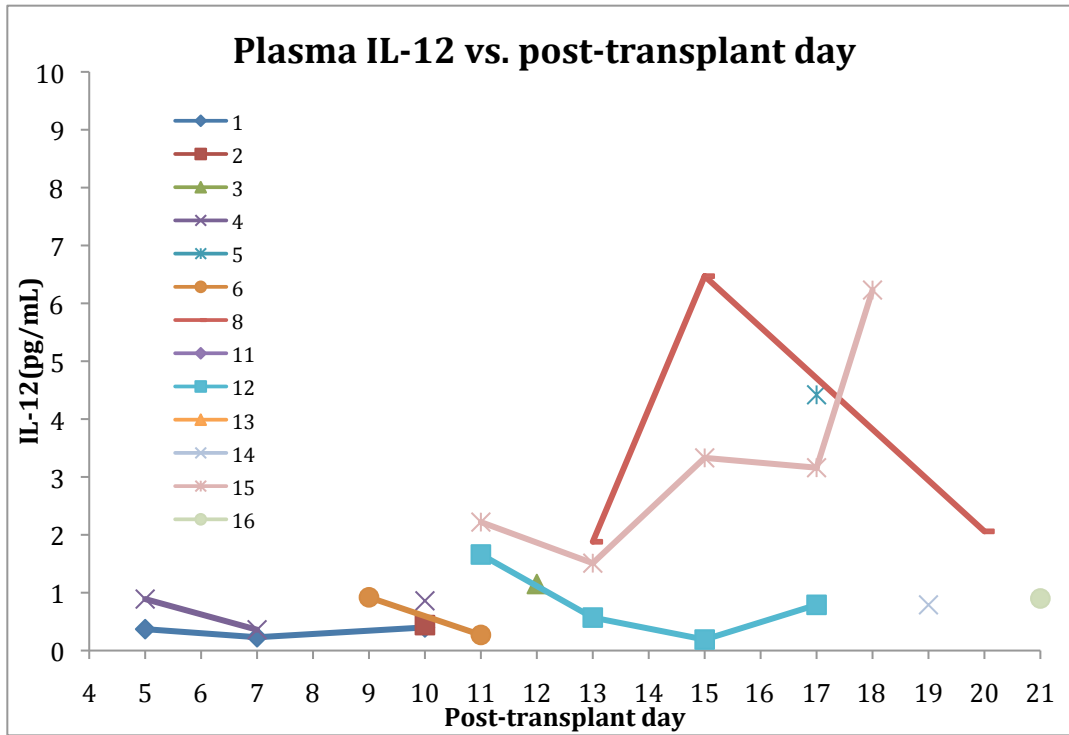
Figure 80. Plasma TNF-alpha vs. time

Plasma TNF-alpha vs. post-transplant day
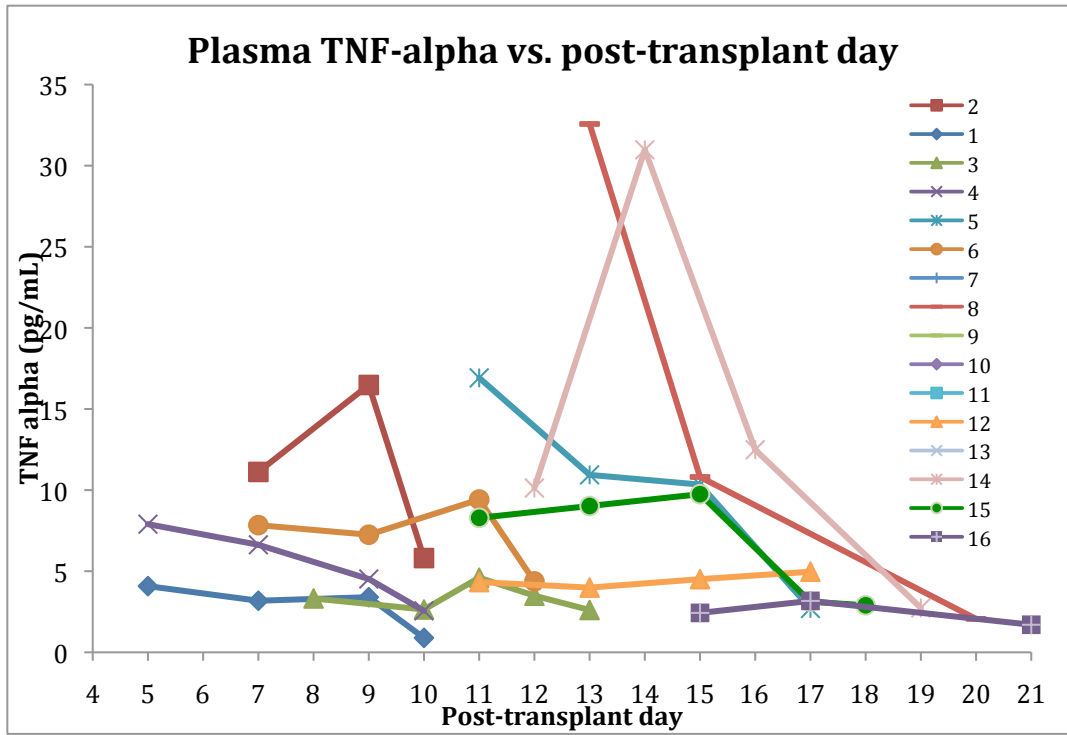
Figure 81. Plasma IFN-gamma vs. time
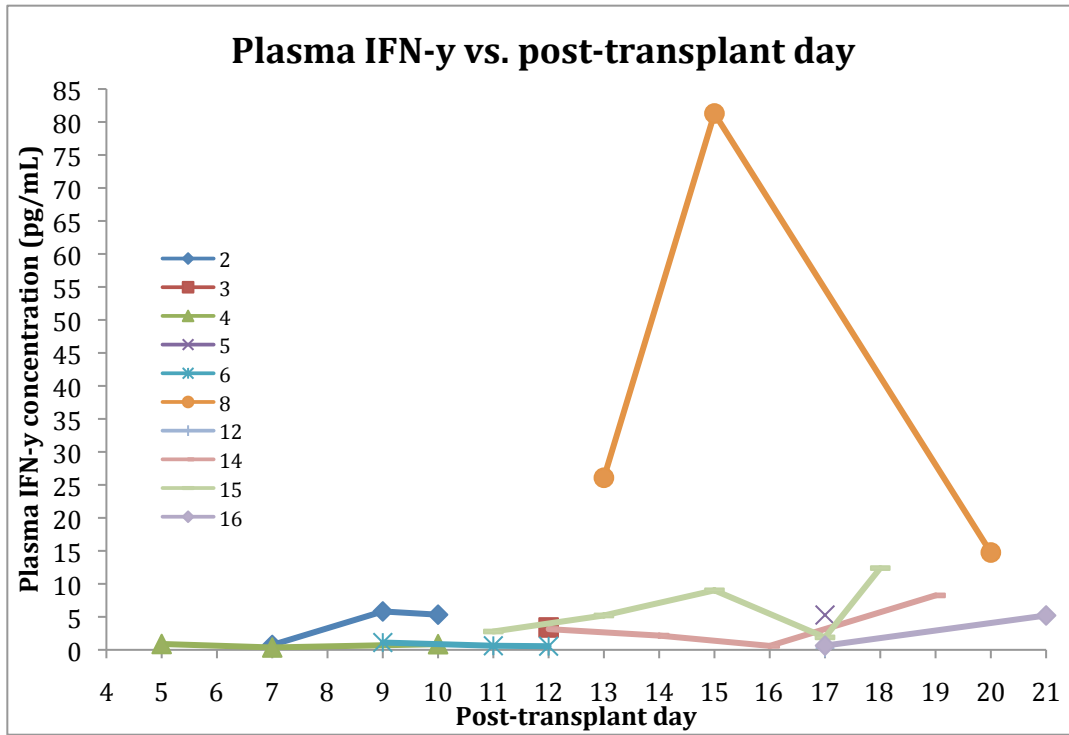
APPENDIX C

PROTOCOL AND CONSENT FORMS

C.1   PROTOCOL

TITLE: EVALUATION OF INTESTINAL CYP3A4/5 AND P-GLYCOPROTEIN IN SMALL BOWEL TRANSPLANT RECIPIENTS (IRB#PRO07050246)

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Abstract:

The small intestine is involved in uptake, metabolism and efflux of many endogenous and exogenous compounds. Drug uptake transporters, drug metabolizing enzymes such as cytochrome P450 3A4/5 (CYP3A45), and drug efflux transporters such as p-glycoprotein (pgp)

play an important role in the oral bioavailability of many currently marketed drugs, including most of the narrow therapeutic index immunosuppressive drugs used in transplant patients, such as tacrolimus, sirolimus, and cyclosporine. This is a single center study to evaluate the intestinal (and hepatic) CYP3A4/5 and p-gp activity in recipients of isolated small bowel transplants, small bowel-pancreas transplants, and modified multivisceral transplants (stomach-small intestine-pancreas), early postoperatively and at four to nine months after transplant surgery, in comparison to that of normal healthy adults. We hypothesize that in the early post operative time period, intestinal CYP3A4/5 and p-gp activity will be lower due to the effects of ischemia-reperfusion injury and the inflammatory and immunologic insult to the transplanted graft, as compared to the control subjects. After four to nine months there will be no significant difference between the two groups as the graft would have recovered completely from the aforementioned injuries associated with the transplant surgery. Twenty-five patients who undergo small bowel, small bowel and pancreas, or modified multivisceral transplantation at the Thomas E. Starzl Transplantation Institute at the University of Pittsburgh Medical Center will participate in a screening session and two study sessions approximately four to nine months apart. Control subjects will undergo a screening session and only one pharmacokinetic study session. During pharmacokinetic study sessions, we will administer midazolam intravenously (IV) and orally to determine its bioavailability as a marker for CYP3A4/5 activity in the gut, and administer fexofenadine orally and determine the area under the plasma-concentration time curve as a measure of p-gp activity. Each subject will receive a dose of midazolam orally and one hour later a dose of fexofenadine orally, followed by an IV dose of midazolam six hours after the oral dose of fexofenadine. Blood samples will be taken for 20 hours after start of the study and plasma midazolam and fexofenadine levels will be measured by high performance liquid chromatography/mass spectrometry (LC/MS). In addition, blood concentrations of tacrolimus (an immunosuppressive drug that is a substrate for both CYP3A4/5 and p-gp, given to all small bowel transplant patients as standard of care) will also be measured in the same blood samples. We will also determine the concentrations of several cytokines and C-reactive protein (CRP) in plasma, and CYP3A and p-gp proteins in graft tissue from biopsies, whenever available during the first 21 days post-transplant and at a time point approximately four to nine months (a the time of the second study) after transplant in the transplant recipients. Cytokine and CRP levels will be measured in the healthy control subjects during each study period. We predict that plasma midazolam, fexofenadine, and tacrolimus levels will be significantly higher in the transplant patients at the earlier time point, but that levels will be significantly decreased to what is observed in normal healthy adults at about four to six months and that these changes will be associated with decreases in cytokine and CRP levels in blood and intestinal mucosa. We will also assess the contribution of CYP3A5/MDR1 polymorphisms on drug exposure in small bowel transplant recipients and normal healthy controls (a pilot study). Understanding the regulation of and changes in CYP3A4/5 and p-gp after small bowel transplantation will enable us to optimize drug therapy in this unique patient population.

1.   Objectives, Aims, Background and Significance

1.1 Objective: What is the overall purpose of this research study?

The primary objectives of this study are to determine the intestinal metabolic capacity as measured by CYP3A4/5 and intestinal efflux transport as measured by p-gp expression and activity in small bowel transplant recipients early and late after transplant surgery and to compare the intestinal CYP3A4/5 and first pass p-gp activity in small bowel transplant patients to that in normal healthy adults. We will also evaluate the cytokine profiles and C-reactive protein (CRP) expression in these patients during the early post operative period and at a later time point and correlate with the functional activity of CYP3A and P-gp. The secondary objective of this study (as a pilot study) is to assess the effect of CYP3A5/MDR1 polymorphisms on the oral availability of CYP3A4/5 and p-gp substrates in small bowel transplant patients. Our central hypothesis is that small intestinal transplant patients will have altered intestinal function during the early post operative period, but will have normal intestinal function when they are clinically stable, as measured by CYP3A and Pgp expression and activity.

1.2 Specific Aims: List the goals of the proposed study (e.g., describe the relevant hypotheses or the specific problems or issues that will be addressed by the study).

Question #1: What is the expression and functional activity of intestinal CYP3A4/5 and p-gp in the early post-transplant period?
Specific Aim #1: To determine the activity of intestinal CYP3A4/5 and p-gp in the early posttransplant period by measurement of the area under the curve (AUC—a measure of exposure to the drug) and the oral clearance of midazolam, a substrate for CYP3A4/5, oral fexofenadine, a substrate for p-gp. In the transplant subjects only, tacrolimus (a substrate for both CYP3A4/5 and p-gp given to all small bowel transplant recipients as standard of care) levels will also be measured using the same blood samples taken for the study. This aim will be accomplished by administration of a single dose of oral midazolam followed by a single dose of oral fexofenadine 60 minutes later, followed by a dose of intravenous midazolam, six hours later. Plasma levels of midazolam and fexofenadine and whole blood levels of tacrolimus (in the transplant patients) will be measured for 20 hours starting with the oral dose of midazolam. Various pharmacokinetic parameters will be determined for each subject and compared between groups. This study will allow for a better understanding of the dosing of CYP3A4/5 and p-gp substrates in the early post-operative period after small bowel transplantation.
Hypothesis #1: We hypothesize that intestinal transplantation leads to a transitory increase in expression of inflammatory and anti-inflammatory cytokines, which in turn leads to decreased expression and impairment of CYP3A4/5-mediated intestinal first-pass metabolism and p-gp-mediated intestinal efflux transport in the early postoperative period. This will then lead to a correspondingly increased AUC and bioavailability of orally administered CYP3A4/5 and p-gp substrates.

Question #2: What is the expression and functional activity of CYP3A4/5 and p-gp in the later post-transplant period?
Specific Aim #2: To determine the activity of intestinal CYP3A4/5 and p-gp in the later posttransplant period (approximately four to nine months post-surgery) by measurement of oral bioavailability of midazolam and AUC of oral fexofenadine. This aim will be accomplished by administration of a single dose of oral midazolam followed by a single dose of oral fexofenadine 60 minutes later, followed by a single dose of intravenous midazolam 6 hours later. Plasma concentrations of midazolam and fexofenadine and whole blood levels of tacrolimus (in the

transplant subjects only) will be measured for 20 hours starting with the dose of oral midazolam. Various pharmacokinetic parameters will be determined for each subject and compared between subjects and between groups. This will allow for a better understanding of dosing of CYP3A4/5 and p-gp substrates in the later post-operative period after small bowel transplantation.

Hypothesis #2: We hypothesize that the functional capacity (as measured by CYP3A4/5 and p-gp activity) of the intestine in clinically stable intestinal transplant patients (about four months after transplantation) will be no different than the functional capacity of the intestine in non-transplanted healthy adults.

Question #3: What is the time course of the concentration of inflammatory mediators such as cytokines and CRP after small bowel transplantation?
Specific Aim #3: To determine the expression pattern of various cytokines (including those associated with inflammation, ischemia-reperfusion injury, and inhibitory effects on CYP enzymes and p-gp) as well as CRP in the first 21 days after surgery and approximately four to nine months after surgery. This aim will be accomplished by measurement of serum cytokine and CRP levels, as well as graft cytokine levels (whenever graft tissue is available) in small bowel transplant patients during the first 21 days after surgery. This will allow for a better understanding of the expression of inflammatory and anti-inflammatory mediators immediately after small bowel transplantation. These mediators have been known to alter the expression of certain drug metabolizing enzymes and transporters.

Hypothesis #3: We hypothesize that graft ischemia, intestinal manipulation secondary to surgery, and ischemia-reperfusion injury will lead to increases in cytokine and CRP expression levels in blood and intestinal mucosa during the first three weeks after surgery. We hypothesize that these protein levels will decline over the course of time post-surgery. In addition, we hypothesize that these increased cytokine levels will result in decreased expression of CYP3A and p-gp proteins in small intestinal mucosal tissue.

Question #4: What are the effects of graft and native CYP3A4/5 and MDR1 variant genotypes on the bioavailability of orally administered CYP3A4/5 and p-gp substrates after small bowel transplantation?
Specific Aim #4: The purpose of this aim is to obtain pilot data on the effects of graft and native CYP3A4/5 and MDR1 variant genotypes on the bioavailability of CYP3A4/5 and p-gp substrates after small bowel transplantation. This aim will be accomplished in the transplant patients by genotyping of small bowel grafts using tissue samples taken from routine biopsies. In addition, we will use peripheral blood cells to determine native genotype in the small bowel transplant patients and healthy controls. We will then assess the relationship between genotype and pharmacokinetic parameters in the study subjects. This will provide pilot data towards a future study.

Hypothesis #4: Graft and native CYP3A4/5 and MDR1 variant genotypes are associated with alterations in the first pass metabolism of CYP3A4/5 and p-gp substrates, and will contribute to the variability in drug levels in small bowel transplant recipients and healthy controls.

1.3 Background: Briefly describe previous findings or observations that provide the background leading to this proposal.

Small bowel transplantation is an increasingly viable alternative to chronic intravenous nutrition in patients with intestinal failure. However, despite the importance of the small intestine in the absorption (uptake), metabolism and efflux of endogenous and exogenous materials, little is known about the time course of recovery of these functions in the graft, and whether the graft function recovers to what is normally observed in normal healthy subjects, in small bowel transplant patients. Earlier studies in our laboratory has shown that the AUC of tacrolimus after oral administration was higher during the early post operative period, compared to late postoperative period in dogs after small bowel transplantation. The AUC of tacrolimus was, however, similar between small bowel transplanted dogs and control non-transplanted dogs during the late post operative period (about 4 to 7 months) (Ishikawa et al. 2003). Other studies by our group have shown evidence of complete recovery of intestinal metabolic function in stable small bowel transplant patients a few months after transplantation. We have shown that the dose normalized trough concentration of tacrolimus (a substrate for CYP3A4/5 and p-gp) in clinically stable small bowel transplant patients (5 ng/ml/mg dose) is similar to that observed in other transplant (liver) patients (4.6 ng/ml/mg dose) at six months after transplant surgery (Jain et al. 1992; Jain et al. 1994). In clinically stable pediatric small bowel and liver transplant patients, the pharmacokinetic profile of tacrolimus was similar to that observed in pediatric liver transplant patients (Schubert et al. 2004). The blood concentration versus time profile of sirolimus (substrate for CYP3A4/5 and Pgp) in clinically stable small bowel transplant patients is similar to that observed in other transplant (liver) patients (Schubert et al. 2004). All of the above observations point to altered functional activity early post transplantation that completely recovers with time (in about 4-6 months) after transplantation.

Small bowel grafts are subjected to several types of injury leading to the release of inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFalpha), which may adversely affect the intestinal function in the early post-transplant period (Kadry et al. 2000; Tuler et al. 2002). Cytokines such as TNF-alpha and IL-6 have been shown to inhibit gene expression of various drug-metabolizing enzymes and drug transporters, including the key cytochrome P450 enzyme, CYP3A4, and p-glycoprotein, a key drug transporter. (Morgan et al. 1994; Carlson and Billings 1996; Pascussi et al. 2000; Bertilsson et al. 2001; Sukhai et al. 2001; Belliard et al. 2002; Belliard et al. 2004; Fernandez et al. 2004). These early post-operative changes are of concern in the setting of small bowel transplantation because the small intestine is the primary site of absorption of drugs. The enterocytes lining this organ contain CYP3A4/5 enzymes in their endoplasmic reticulum as well as p-gp transporters on their brush border, both of which contribute significantly to intestinal first-pass metabolism of orally administered medications. Many clinically used drugs, including the immunosuppressive medications tacrolimus, sirolimus, and cyclosporine, are given orally and are substrates for both CYP3A4/5 and p-gp. Earlier studies carried out in our lab have shown that intestinal metabolism plays an important role in the bioavailability of the CYP3A and p-gp substrate cyclosporine (Schwinghammer et al. 1991). Given the important role of CYP3A and pGP it is important to understand their activity in small bowel transplant patients.

In addition, genetic factors may also play a role in the pharmacokinetics of CYP3A4/5 and pgp substrates in small bowel transplant patients. Although inter-individual variability in CYP3A4 levels and CYP3A4-mediated metabolism has been noted in patients, of the 38 allelic variants of the CYP3A4 gene that have been described, only one has been found to cause alterations in metabolism (CYP3A4*20) and this variant is extremely rare (Westlind-Johnsson et al. 2006). In contrast, polymorphic differences in the CYP3A5 protein, an enzyme with substrate

specificity overlapping that of CYP3A4, are common in the U.S. population. It has been estimated that up to 90 percent of Caucasians express a nonfunctional version of this enzyme (CYP3A5*3) while up to 50 percent of African-Americans express a functional version of the enzyme (most commonly CYP3A5*1). CYP3A5 is the most predominant extrahepatic CYP3A isoform. Patients heterozygous and homozygous for the *1 allele have been shown in some studies to have decreased trough levels of tacrolimus, implying increased first-pass metabolism by CYP3A5 in the intestine (Lamba et al. 2002; Daly 2006). MDR1 (the gene encoding the p-gp protein) polymorphisms are also common and may cause alterations in the bioavailability of p-gp substrates after oral administration. Although study results have been conflicting, the C3435T and G2677T/A variants have been associated with decreased p-gp expression and function and increased bioavailability of p-gp substrates (Pauli-Magnus and Kroetz 2004; Yi et al. 2004). The genotype of the graft may therefore play a role in the pharmacokinetics of CYP3A4/5 and p-gp substrates in small bowel transplant patients.

1.4 Significance: Why is it important that this research be conducted? What gaps in existing information or knowledge is this research intended to fill?

A thorough understanding of the functional activity and time course of recovery of CYP3A4/5 and p-gp in small bowel transplant patients is important to optimize drug therapy in this population. Due to the increasing number of intestinal transplants performed for treatment of intestinal failure, the complicated biochemical changes that occur in both the recipient and the graft over time, the large number of drugs used in this patients, the importance of the small intestine in absorption and metabolism of drugs, a better understanding of the regulation of the metabolic and transport function of the transplanted gut in these patients is essential. This study will also evaluate the clinical utility of serum CRP and cytokine measurement and CYP3A and p-gp tissue protein measurement in the post-transplant period as ways of gauging the amount of systemic and graft inflammation in the recipient and its potential effect on drug disposition. Finally, with the growing awareness of the importance of genetic variants on drug metabolism, more information is needed on the effects of CYP3A4/5 and MDR1 variants on bioavailability of drugs in this unique patient population. Transplantation of a small bowel also potentially creates a situation where the liver and intestinal CYP3A4/5 and/or MDR1 polymorphisms may be different between native and transplanted organ. Given the importance of the small intestine to drug metabolism, it is crucial to investigate the pharmacokinetic consequences of such a situation.

2.   Research Design and Methods

2.1 Does this research study involve the use or evaluation of a drug, biological, or nutritional (e.g., herbal or dietary) supplement?

yes

2.1.1 Does this research study involve an evaluation of the safety and/or effectiveness of one or more marketed nutritional (e.g., herbal or dietary) supplements for the diagnosis,

prevention, mitigation or treatment of a specific disease or condition or symptoms characteristic of a specific disease or condition?

no

2.1.2 Does this research study involve the use or evaluation of one or more drugs or biologicals not currently approved by the FDA for general marketing?

no

2.1.3 Does this research involve an evaluation of the effectiveness and/or safety of one or more drugs or biologicals currently approved by the FDA for general marketing?

no

2.2 Will this research evaluate the safety and/or effectiveness of one or more devices?

no

2.3 Summarize the general classification (e.g., descriptive, experimental) and methodological design (e.g., observational, cross-sectional, longitudinal, randomized, open-label single-blind, double-blind, placebo-controlled, active treatment controlled, parallel arm, cross-over arm) of the proposed research study, as applicable.

This is an open-label pharmacokinetic study.

This is a single center pharmacokinetic study of oral and IV midazolam (a CYP3A4/5 substrate) and oral fexofenadine (a p-gp substrate) in patients who undergo isolated small bowel, small bowel-pancreas, or modified multivisceral transplantation for end stage intestinal failure at the Thomas E. Starzl Transplantation Institute at the University of Pittsburgh Medical Center, Pittsburgh, PA and in healthy controls. An appropriately signed informed consent will be obtained from each transplant patient once transplant candidacy is established and prior to any study-related procedures. No standard of care is being withheld by patient participation in this study. Similarly, an appropriately signed informed consent will be obtained from healthy control subjects before any study-related procedures are performed. Up to 50 subjects who have signed the informed consent and who continue to meet entry criteria will be enrolled and eligible for participation in the study sessions.

For transplant patients, the study consists of three phases: a screening visit and two study visits. For controls, the study consists of one screening visit and one study visit. Subject participation in the study will last for approximately 120-180 days from the day of transplantation (for transplant patients) or day of enrollment (healthy controls). For the transplant recipients, the first study session will be while they are still hospitalized after transplant surgery. However, for the second study session the transplant patients may be required to make a separate visit for study purposes alone, and the control subjects will make two separate visits to the study facility for study purposes alone. We will attempt to prescreen potential control subjects by

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telephone so that they are an appropriate match for the small bowel transplant patients (see recruitment section).

2.3.1 Does this research study involve a placebo-controlled arm?

no

2.4 Will any research subjects be withdrawn from known effective therapy for the purpose of participating in this research study?

no

2.5 Will screening procedures (i.e., procedures to determine research subject eligibility) be performed specifically for the purpose of this research study?

yes

2.5.1 List the screening procedures that will be performed for the purpose of this research study.

The investigators involved in the study are clinicians who are normally involved in the routine clinical care of the patients. These clinicians will be involved in screening the subjects for the study. Transplant patients (Screening will be done in transplant ICU after transplant):
Medical history (including medical conditions and drug allergies)
Volume of transplanted intestinal graft output
Total bilirubin
AST/ALT
gamma-glutamyl transferase
BUN/Cr
serum albumin
alkaline phosphatase
electrolyte panel and calcium, magnesium, phosphorus
CBC with differential
platelet count
ECG
hemoglobin/hematocrit
demographics (age, gender, height, weight, percent of ideal body weight)
indication for transplant
degree of hepatic steatosis/fibrosis from pre-transplant liver biopsy report
CMV status of donor and recipient
HLA mismatch
Control subjects (Screening will be done in Montefiore CTRC):
All of the above, except indication for transplant, pre-transplant liver biopsy report, CMV status, and HLA mismatch since these are N/A to controls.

2.5.2 What steps will be taken in the event that a clinically significant, unexpected disease or condition is identified during the conduct of the screening procedures?

If a clinically significant, unanticipated disease or condition is identified during the conduct of these screening procedures the subject will be excluded from the study and given a referral to a physician who specializes in the condition identified.

2.6 Provide a detailed description of all research activities (e.g., all drugs or devices; psychosocial interventions or measures) that will be performed for the purpose of this research study. This description of activities should be complete and of sufficient detail to permit an adequate assessment of associated risks. At a minimum this should include:
personnel performing the procedures
location of procedures
duration of procedures
timeline of study procedures

Study Visit 1 (Screening): Eligible small bowel, small bowel-pancreas, and modified multivisceral transplant recipients will be given the opportunity to sign informed consent for the study as soon as transplant candidacy is confirmed. Healthy control subjects will be recruited as the study subjects are recruited so that we are able to match based on demographics such as age, race, and gender. All patients who have signed the consent form and been enrolled in the study will undergo a preliminary screening lasting for approximately two hours. For the transplant subjects, the screening will take place in the transplant ICU after the transplant. The control subjects will have their screening visits at the Clinical and Translational Research Center (CTRC) at Montefiore hospital. The initial screening of subjects will include a medical history and the following clinical laboratory tests: serum bilirubin, alanineaminotrasferease, (ALT), aspartateaminotransferase (AST), serum creatinine (Scr), blood urea nitrogen (BUN), serum albumin, alkaline phosphatase, phosphorus, calcium, magnesium, an electrolyte panel, glucose, PT/PTT/INR, complete blood count with differential (CBC), platelets, as well as demographics (including age, gender, race, weight, and height). In the case of the transplant recipients, the indication for transplant and HLA mismatch will also be recorded as well as the cytomegalovirus (CMV) status of both the donor and the recipient. In addition, for the transplant subjects, gamma-glutamyltransferase will be measured in serum, output from the transplanted graft will be recorded, and the degree of hepatic steatosis and/or fibrosis from pre-transplant liver biopsy reports will be noted. If any subjects do not meet the inclusion criteria or meet any of the exclusion criteria based on the results of this screening session, they will be excluded from the study before any study procedures have been performed. If a clinically significant, unanticipated disease or condition is identified during the conduct of these screening procedures the subject will be excluded from the study and given a referral to physician who specializes in the condition identified.

Transplant recipients will also have serum cytokine (including TNF-alpha, IL-6, IL-1beta) and C-reactive protein (CRP) levels measured every other day from the day of transplant up until the second study session before post-transplant day 21. This range was chosen because we would like to do the study within a week of the patient being able to tolerate oral medications and enteral feedings, but we recognize that there is wide inter-individual variability in this.

Female subjects of childbearing age will undergo a urinary pregnancy testing at this phase prior to administration of any study medications.

Study Visits 2 and 3: For the transplant patients, the second study session will be between 2 and 4 days after the patient is able to tolerate enteral intake (usually within the first 2 weeks after surgery) and the third session will be approximately four to nine months after transplantation (120-240 days post-transplant). For the control subjects, the single study session will take place as soon as possible after enrollment and will be in the CTRC at UPMC Montefiore. In order to participate in the second and third study sessions, the transplant patients must be able to take medications orally. If greater than 30 days has elapsed since a control subject had labs done during the screening session, then the control will have the following labs drawn prior to the administration of study drugs: total bilirubin, AST/ALT, BUN/SCr, serum albumin, phosphorus, magnesium, alkaline phosphatase, and electrolyte panel. Transplant subjects will have these labs drawn daily or weekly as standard of care and those values will be used. During the pharmacokinetic study sessions, subjects will receive an oral dose of 5 mg midazolam, an oral dose of 60 mg fexofenadine 60 minutes later, and about 6 hours later an intravenous dose of 2 mg midazolam. Transplant subjects may receive the oral medications via jejunostomy tube at the earlier study session. Subjects will be monitored with an automated blood pressure cuff and pulse oximetry for two hours after each drug administration. The mean elimination half-life of midazolam in patients with normal hepatic and renal function is approximately 3 hours (range 1.8 to 6.4 hours). The semisimultaneous bioavailability approach will be used to characterize the pharmacokinetic parameters after midazolam administration. This method allows for administration of a second dose of midazolam while some of the first dose remains in the circulation, and the drug concentration-time profile is fitted to a mathematical model using nonlinear regression (Karlsson, 1989). The half life of fexofenadine is about 12 hours. However, the plasma concentration of fexofenadine is very low by 20 hrs after drug administration and correspondingly the AUC from 0-20 after fexofenadine administration will approximate the AUC from 0-infinity. Therefore in a total study time period of 20 hours we expect to capture a significant portion of the AUC for fexofenadine and adequately characterize the pharmacokinetic parameters of midazolam. Control subjects at all study sessions and transplant subjects at the later study session will be given a breakfast two hours after the dose of oral midazolam in the morning and will be given a lunch one hour after the dose of intravenous midazolam, followed by a dinner near the end of the session. Transplant subjects at the earlier study session will remain on their standard enteral feeding schedule. Any patients who experience emesis within four hours after oral administration of study drugs will be removed from the study, as full absorption of drug may not have taken place. Subjects will stay overnight after study sessions 2 and 3 either in their hospital bed (if inpatient transplant subjects) or the CTRC (if outpatient and/or control subjects). As an additional safety measure, all subjects will be required to remain in bed for six hours after each dose of midazolam, although they will be allowed bathroom privileges with assistance. The Observer's Assessment of Alertness/Sedation (OAA/S) scale will be used by the investigators to assess alertness and level of sedation. This assessment will be administered at baseline (before administration of study drug), and at 15, 30, 45, 60, and 120 minutes after each dose of midazolam.

Multiple blood samples (3 ml each) will be obtained in a purple top vacutainer from an indwelling catheter over a 12 hr time period. The sampling time points will include 0, 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 5, 7, 7.25, 7.5, 8, 10, 12, and 20 hours after the start of oral dosing for measurement of the concentration of drugs under study. One additional 3 ml blood sample will

be drawn to measure cytokine and CRP levels at time zero. Total blood volume removed during the study visits will be 51 mL, unless a control subject requires labs, in which case the total amount drawn will be 54 mL.

Urine samples will be taken at 1, 3, 5, 7, 9, and 12 hours in the transplant patients at the first pharmacokinetic study session and at 1, 3, 5, 7, 9, 12 and 20 hours at the second pharmacokinetic study session and also in controls, in order to measure levels of midazolam, 1'hydroxymidazolam, and 1'hydroxymidazolam glucuronide. Measurement of these compounds in the urine will allow for the characterization of formation clearance of the 1'hydroxymidazolam metabolite as well as other pharmacokinetic parameters such as renal clearance of unchanged drug (midazolam) as well as metabolite.

Blood samples will be centrifuged at 3000 rpm for 10 minutes immediately to obtain plasma and plasma and urine samples will be frozen at –70 degrees until analysis. Plasma and urine samples will be assayed for midazolam, 1'hydroxymidazolam, 1'hydroxymidazolam glucuronide, and fexofenadine concentrations by LC/MS in the PI's laboratory. Whole blood concentrations of tacrolimus will be measured by HPLC-MS.

Subjects will be asked not to consume grapefruit or grapefruit juice or cranberry juice (which contain known inhibitors of intestinal CYP3A4) or apple or orange juice (which may decrease fexofenadine concentrations) for 3 days before and during study visits 2 and 3. In addition, subjects will be asked not to consume caffeine-containing beverages for 72 hours prior to the study session, since caffeine is a weak CYP3A inhibitor. Subjects will be asked to abstain from consuming food and other beverages (except water) after midnight prior to the study day. Transplant patients will be allowed to take their routine medications at least two hours before administration of the study medications.

All transplant patients will receive tacrolimus for routine immunosuppression. Tacrolimus dose and blood levels that are routinely measured in the patients will be collected, as a measure of a combined measure of CYP3A and pgp activity.

Small intestinal mucosal tissue samples that are taken from the transplant recipients during two routine intestinal biopsies (one in the early post-transplant period and one approximately 4-9 months post-transplant around the time of the third study session) will be genotyped for the presence of the CYP3A5*1 and CYP3A5*3 alleles, as well as for the C3435T and G2677T/A variants of the MDR1 gene. Levels of cytokine and CRP mRNA expression in the tissue sample will also be measured whenever tissue samples from routine biopsies become available. Markers of ischemia and reperfusion injury may also be assessed. A peripheral blood sample from each subject (transplant patients and controls) will also be genotyped for the above polymorphisms. Finally, CYP3A and p-gp protein levels will be measured in routine biopsy samples by Western blot and immunohistochemical staining, using antibodies specific for CYP3A and p-gp. Two additional mucosal tissue samples (beyond the three typically taken during a routine biopsy in these patients) will be taken during each of the two biopsy sessions in order to have enough tissue for the PCR and Western blot procedures.

2.7 Will follow-up procedures be performed specifically for research purposes? Followup procedures may include phone calls, interviews, biomedical tests or other monitoring procedures.

yes

1) Transplant subjects at study session 2 will be in monitored hospital beds as standard of their post-operative care, and will be supervised by medical and nursing staff for the duration of the session.

2) Session 2 for control subjects and session 3 for transplant subjects will take place at the University of Pittsburgh Clinical and Translational Research Center (CTRC) at Montefiore hospital. The following precautions will be taken after drug administration:

a) Subjects will lie supine and have blood pressure, respiratory rate and heart rate measured every 15 minutes for two hours after study drug administration.

b) Subjects will wear a pulse oximeter continuously for two hours after study drug administration. Supplemental oxygen will be administered for oxygen saturations less than 94%.

c) Subjects will be required to remain in bed for six hours after each dose of midazolam, but will be allowed bathroom privileges with assistance.

d) Level of sedation will be assessed using the Observer's Assessment of Alertness/Sedation scale at baseline and 15, 30, 60, and 120 minutes after each dose of midazolam.

If this fails to increase the saturation or if the pulse ox is less than 90 percent, a physician will be called. In addition, a physician will be called if the blood pressure goes above 140/90 or below 90/60, depending on the patient's baseline blood pressure (the physician would of course be called for any dramatic increases or decreases).

e) Subjects will remain overnight in the CTRC to ensure that all study drugs are washed out of the system. In addition, CTRC nursing staff will assess level of sedation, blood pressure, and pulse ox before the subject is allowed to leave on the morning after the study session.

2.8 Does this research study involve the use of any questionnaires or survey instruments not listed in Appendix G of the IRB Reference Manual?

yes

2.9 If subjects are also patients, will any clinical procedures that are being used for their conventional medical care also be used for research purposes?

yes

If Yes, describe the clinical procedures (and, if applicable, their frequency) that will be used for research purposes:

1) In the potential transplant subjects, all labs used for screening purposes are drawn daily as part of routine clinical care--no extra labs will be drawn for screening in these subjects.

2) In the transplant subjects, all intestinal tissue biopsies are done as part of routine clinical care--no additional biopsies will be scheduled in these subjects for study purposes.

3) For control subjects, none of the procedures associated with any portion of the study are considered routine clinical care and thus all costs associated with their participation in the study will be paid for by the study and not billed to them or to their insurance (this is indicated in the fiscal review form).

2.10 Will blood samples be obtained as part of this research study?

yes

If Yes, address the frequency, volume per withdrawal, the total volume per visit, and the qualifications of the individual performing the procedure:

During the screening session, control subjects will have one 3 mL sample of blood collected to measure various biochemical parameters. Transplant patients will have blood drawn and labs done daily as part of routine clinical care so no additional blood draws will be required during screening for the transplant group.

During participation in each of the two pharmacokinetic study sessions, a total of 17 blood samples of 3 mL each will be collected for a total of 51 mL (approximately 3 tablespoonfuls). Research subjects will participate in no more than one screening session and two pharmacokinetic study sessions (one for controls) for a maximum total of 102 mL of blood collected at all study sessions combined. No additional blood will be drawn for genotyping since 1 mL of blood taken from study session 2 will be used for this purpose in both study groups.

2.11 What is the total duration of the subject's participation in this research study across all visits, including follow-up surveillance?

Approximately four to nine months

2.12 Does this research study involve any type of planned deception?

no

2.13 Does this research study involve the use of UPMC/Pitt protected health information that will be de-identified by an IRB approved "honest broker" system?

no

2.14 Will protected health information (PHI) from a UPMC/Pitt HIPAA covered entity be obtained for research purposes or will research data be placed in the UPMC/Pitt medical record?

no

2.14.1 Will protected health information from a non-UPMC/Pitt HIPAA covered entity be obtained for research purposes or will research data be placed in the non-UPMC/Pitt medical record?

no

2.15 Does this research study involve the long-term storage (banking) of biological specimens?

yes

2.15.1 Broadly describe the intended future use of the banked biological specimens:

1) Peripheral blood samples drawn from subjects in both the transplant and control groups will be genotyped for the presence of CYP3A5*1 and CYP3A5*3 alleles and the C3435T and G2677T/A variants of the MDR1 gene.
2) Intestinal tissue taken during biopsies performed as part of routine clinical care in the transplant subjects will also be genotyped for the above polymorphisms as well as analyzed for cytokine, CYP3A4/5, and p-gp levels.

2.15.2 Indicate the planned length of storage of the banked biological specimens:

indefinite

2.15.3 Will the biological specimens be de-identified prior to making these specimens available for use in this research study?

no

2.15.4 Will subjects (including family members, if applicable) be informed of their personal results from analyses performed on their biological specimens?

yes

2.15.4.2 Indicate when personal results will be disclosed.

Only upon the request of a research subject

2.15.4.3 Indicate how subjects will be informed of their personal results.

Information on the blood concentration time profile of tacrolimus and other drugs tested may be shown to the subjects. Since the implications of the initial results will not be clear no changes in routine management of the subjects will be made if any, until the completion of the study.

2.15.4.4 When will the results be disclosed?

At the conclusion of the research study

2.15.4.5 Describe potential risks associated with disclosure of personal results.

While the investigators will have procedures in place to ensure that all subject information is kept strictly confidential, there is the possibility that if genotype results became generally known, this information could have an impact on a subject's future insurability,

employability, reproductive plans, or could have a negative impact on family relationships, or could result in paternity suits or stigmatization.

2.15.4.5.1 Is germ-line genetic research being conducted on the banked biological specimens?

no

2.15.4.6 Is the laboratory performing the analyses on the biological specimens CLIA certified?

yes

2.15.4.7 Describe the procedures that will be employed to protect the confidentiality of subjects' private information associated with use of biological specimens:

All patient/subject data obtained over the course of this study will remain strictly confidential. Specimens collected will be coded and kept in the laboratory of the Principal Investigator. Only authorized personnel will have access to the specimens. All specimens will be labeled with a code and will not be identifiable by patient name, initials, social security number, or any other identifier that could be traced back to the patient. The list of codes linking specimens and identifiable patient information will be kept in a locked cabinet in the Principal Investigator's office. Only investigators in this study will have access to this information.

2.15.4.8 Will the banked biological specimens or data derived from them be provided with subject identifiers to any secondary investigators or external entities?

no

2.15.4.9 Will research subjects be remunerated in the event of the future commercial development of inventions or products based on the research use of their biological specimens?

no

2.16 Will research participants be asked to provide information about their family members or acquaintances?

no

2.17 What are the main outcome variables that will be evaluated in this study?

The functional activity of CYP3A and P-gp will be assessed from the concentration vs. time profile of midazolam, fexofenadine and tacrolimus. Pharmacokinetic data will be analyzed by noncompartmental methods. Plasma concentration versus time curves will be generated for each subject and the area under the plasma concentration versus time curve (AUC), which is the

primary outcome measure since it is a measure of drug exposure, will be calculated. Other pharmacokinetic parameters including disposition rate constant, disposition half life, mean residence time, clearance, and volume of distribution will be calculated after IV administration; and absorption rate constant, elimination rate constant, absorption half life, elimination half life, bioavailability, Cmax (maximum concentration), and Tmax (time of maximum concentration) will be calculated after oral administration of the study drugs. For tacrolimus, in addition, the ratio of the trough blood concentration vs. time will be evaluated over the entire study period.

2.18 Describe the statistical approaches that will be used to analyze the study data.

Various weight-normalized pharmacokinetic parameters will be compared between groups of patients at each time point (i.e. between transplant patients and controls) and within groups (between time points) by the use of a two-sample t test and two way ANOVA. A comparison of the systemic clearance and oral clearance of midazolam will provide information on the drug's intestinal clearance. Oral clearance and AUC of fexofenadine will be used as a measure of p-gp activity. AUC of tacrolimus will be used as a measure of combined CYP3A and Pgp activity. Trends in cytokine levels will be evaluated over time. Mean serum cytokine and CRP levels will be compared between the transplant patients and controls at both time points using the Wilcoxon rank-sum test, and correlated with various pharmacokinetic parameters. Descriptive plots of cytokine and CRP levels over time will also be created. Time course of the ratio of blood concentration to dose will be evaluated. In addition, in the statistical analysis of pharmacokinetic parameters within and between groups, subjects will be subdivided by presence or absence of CYP3A5 protein, and by CYP3A5 and MDR1 polymorphism and parameters compared by polymorphism. We will also use the genotype as a covariate in the population pharmacokinetic analysis (NONMEM software available in our department) of the data. Although these comparisons will not be likely to reach statistical significance due to the small sample size and unknown genetic makeup of the future groups, this information will nevertheless allow us to better understand the contribution of some of the more common CYP3A5 and MDR1 genotypes to intestinal first-pass metabolism in small bowel transplant recipients and healthy subjects and provide pilot data needed for future studies.

2.19 Will this research be conducted in a foreign country or at a site where the cultural background of the subject population differs substantially from that of Pittsburgh and its surrounding communities?

no

2.20 Will the Principal Investigator for this project be responsible for some or all research activities at other sites?

no

2.21 Will this research study be conducted within a nursing home located in Pennsylvania?

no

3. Human Subjects

3.1 What is the age range of the subject population?

18-65

3.2 What is their gender?

Both males and females

3.3 Will any racial or ethnic subgroups be explicitly excluded from participation?

no

3.4 For studies conducted in the U.S., do you expect that all subjects will be able to comprehend English?

yes

3.5 Participation of Children: Will children less than 18 years of age be studied?

no

If No, provide a justification for excluding children:

There will be no children included in the study. The large number of blood samples needed in this study for pharmacokinetic analysis would not be practical in pediatric patients.

3.6 Does this research study involve prisoners, or is it anticipated that the research study may involve prisoners?

no

3.7 Will pregnant women be knowingly and purposely be included in this research study?

no

3.8 Does this research study involve neonates?

no

3.9 Fetal Tissues: Does this research involve the use of fetal tissues or organs?

no

3.10 What is the total number of subjects to be studied at this site, including subjects to be screened for eligibility?

50

3.11 Identify each of the disease or condition specific subgroups (include healthy volunteers, if applicable) that will be studied.

Subgroup	Number to undergo research procedures	Number to undergo screening procedures
Small bowel transplant	**20**	**25**
Healthy controls	**20**	**25**

3.12 Provide a statistical justification for the total number of subjects to be enrolled into this research study at the multicenter sites or this site.

Sample size calculations for this study were performed to determine the appropriate sample size to ensure an 80 percent chance of finding a significant difference using a two-sided significance test with an alpha of 0.05. Data from the studies by Kirby et al. and Kharasch et al. were used as estimates of arithmetic AUC means and standard deviations for each drug in healthy subjects. The mean AUC values for small bowel transplant patients are not available in the literature but are predicted to be 25 percent higher than in controls, but standard deviations are predicted to be equal. Therefore, we estimate that 20 patients will be needed in each group to show a 25 percent difference in midazolam and fexofenadine AUCs between the transplant patients at two different time points and between transplant patients and controls. However, based on the survival, rejection incidence, and other possible complications we predict that some of the small bowel transplant patients in the study will not return for the second and/or third study sessions. Similarly, not all of the controls will be expected to complete the study. Therefore, a sample size of 25 in each group should provide an adequate number to account for dropouts.

3.13 Inclusion Criteria: List the specific criteria for inclusion of potential subjects.

Inclusion criteria (transplant patients and controls):

To be included in the study, a transplant patient must:
• Have signed appropriate informed consent
• Be between 18 and 65 years of age
• Have been accepted as an isolated small bowel, modified multivisceral, or small bowel-pancreas transplant candidate at the University of Pittsburgh Medical Center

• Be treated in accordance with the standard of care protocol(s) currently in effect for small bowel transplant recipients at the University of Pittsburgh Medical Center, including immunosuppression and other elements of pre- and post-operative care

• Be receiving oral or enteral medications at the time of the first study session

• Be within 30 percent of ideal body weight

To be included in the study, a control subject must:
• Have signed appropriate informed consent
• Be between 18 and 65 years of age
• Be within 30 percent of ideal body weight
• Have normal hepatic and renal function (as defined by total bilirubin, AST, ALT, BUN, and serum creatinine within normal limits.)

3.14 Exclusion Criteria: List the specific criteria for exclusion of potential subjects from participation.

Exclusion criteria (transplant patients and controls):
To be included in the study, a TRANSPLANT subject must NOT:
• Be a smoker
• Have a creatinine clearance less than 30 mL/minute as estimated by the Cockcroft-Gault equation
• Have a hemoglobin value less than 8.5 gm/dL
• Require supplemental oxygen
• Have a very high output (> 2500 mL per day) from their transplanted intestinal graft
• Be undergoing therapy with known CYP3A4/5 and/or p-gp inhibitors or inducers (i.e. cyclosporine, azole antifungals, rifampin, phenobarbital, St. John's Wort)
• Be undergoing multi-organ transplantation, except for small bowel and pancreas combined or modified multivisceral transplant
• Have had any previous organ transplant or cell infusion
• Be receiving any non-standard immunosuppression protocol or other non-standard treatment that could affect interpretation of the study results
• Have any known hypersensitivity to benzodiazepines or fexofenadine
• If female, be pregnant or nursing (as confirmed by pregnancy test at each visit)
Be under the age of 18 or over the age of 65:
-The large number of blood samples needed in this study for pharmacokinetic analysis would not be practical in pediatric patients (less than 18).
-In addition, patients over age 65 are generally not transplanted. Therefore, since controls are to be age-matched, no control subjects over 65 will be included.
To be included in the study, a control subject must NOT:
• Have had any previous organ transplant or cell infusion
• Have any medical condition known to affect gastrointestinal function or motility, such as diabetes mellitus, Crohn's disease, ulcerative colitis, or short gut syndrome
• Have liver disease (normal liver function as defined by AST/ALT, total bilirubin, albumin, and alkaline phosphatase all within normal limits, and no prior history of liver disease)
• Be a smoker

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• Have a creatinine clearance less than 50 mL/minute as estimated by the Cockcroft-Gault equation
• Have a hemoglobin value less than 8.5 gm/dL
• Have a history of bariatric surgery, such as gastric bypass
• Be undergoing therapy with known CYP3A4/5 and/or p-gp inhibitors or inducers (i.e. cyclosporine, azole antifungals, rifampin, phenobarbital, St. John's Wort)
• Be receiving any other investigational drug or be participating in any other investigational study
• Have any known hypersensitivity to benzodiazepines or fexofenadine
• If female, be pregnant or nursing (as confirmed by pregnancy test at each visit)

3.15 Will HIV serostatus be evaluated specifically for the purpose of participation in this research study?

no

4.   Recruitment and Informed Consent Procedures

4.1 Will potential research subjects be identified through the use of advertisements?

yes

4.2 Will potential research subjects be identified and recruited through the use of a "honest broker"?

no

4.3 Will you be accessing identifiable medical record information from your patient population for subsequent contact?

yes

4.3.1 Are you requesting a waiver of informed consent to access and record identifiable medical record information for recruitment purposes only?

No - The respective patients provided previously their written informed consent to permit access to their identifiable medical record information for future research study recruitment purposes. Prospective consent will be obtained under IRB #0307037 before transplant.

4.4 Will methods other than advertisements, an honest broker system/process, or the review of identifiable medical record information be used for the identification of potential research subjects?

no

Are you planning on using the CTSI Research Participant Registry as a recruitment tool?

yes

4.5 How will potential research subjects be initially contacted to ascertain their interest in study participation?

1) Transplant subjects: Interest in and eligibility for study participation will be ascertained by the transplant surgeon prior to transplant. This will be accomplished through the transplant research registry. IRB # 0307037

2) Control subjects: Control subjects will be recruited via advertisements posted around the University of Pittsburgh and UPMC campuses, the CTSI research participant registry, as well as in Audex messages and campus newspapers.

4.6 Are you requesting a waiver to document informed consent for any or all participants, for any or all procedures? (e.g., a verbal or computerized consent script will be used, but the subjects will not be required to sign a written informed consent document, such as with phone screening.

yes

4.6.1 Identify the specific research procedures and/or the specific subject populations for which you are requesting a waiver of the requirement to obtain a signed consent form.

Telephone screening interview for potential control subjects.

4.6.2 Indicate which of the following regulatory criteria is applicable to your request for a waiver of the requirement to obtain a signed consent form.

45 CFR 46.117(c)(2)

4.6.2.1 Address why the specific research procedures for which you are requesting a waiver of the requirement to obtain a signed consent form present no more than minimal risk of harm to the research subjects:

The pre-screening research procedures for potential control subjects involve the gathering of basic demographic and medical information that is necessary to obtain prior to scheduling a potential control for a screening session at the CTRC. No research interventions are performed during the telephone pre-screening, and all information obtained is kept strictly confidential. If subjects meet basic eligibility criteria, they are scheduled for a screening session, prior to which the PI goes through the consent form with them in person. If they then sign the consent form, screening procedures are performed. If subjects do not meet the basic eligibility criteria or do not wish to participate once the study is described to them, their information is shredded by the investigator unless they request otherwise (for example, some participants who are excluded

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based on weight may ask the investigator to keep their information in case they lose weight or the inclusion criteria change.)

4.6.2.2 Justify why the research listed in 4.6.1 involves no procedures for which written informed consent is normally required outside of the research context:

The phone screening involves only the gathering of information, which will be kept confidential and will be destroyed if the subject does not wish to participate.

4.6.3 Address the procedures that will be used and the information that will be provided (i.e., script) in obtaining and documenting the subjects' verbal informed consent for study participation:

The study is described to the potential subject and he/she is asked if interested in participating and if they have any questions. Potential control subjects are then asked prior to any pre-screening questions if it is acceptable to them that they be asked some questions about themselves, and assured that their answers will be kept confidential, and that their name and information will be kept in a locked cabinet at all times. They are also told that their information will be shredded if they are deemed ineligible to participate at this time unless they request otherwise (for example, some participants who are excluded based on weight may ask the investigator to keep their information in case they lose weight or the inclusion criteria change.) Verbal consent to phone screening is documented by the investigator.

4.7 Are you requesting a waiver of the requirement to obtain informed consent (from some or all potential subjects) for participation in this minimal risk. research study, or any minimal risk procedures associated with the conduct of this research study?

no

4.8 Are you requesting an exception to the requirement to obtain informed consent for research involving the evaluation of an 'emergency' procedure?

no

4.9 Consent form documents

See section C.2 and C.3.

4.10 Will all potential adult subjects be capable of providing direct consent for study participation?

Yes

4.11 At what point will you obtain the informed consent of potential research subjects or their authorized representative?

After performing certain of the screening procedures, but prior to performing any of the research interventions/interactions

4.11.1 Address why you feel that it is acceptable to defer obtaining written informed consent until after the screening procedures have been performed.

We will defer obtaining written informed consent (from control subjects) until after the telephone screening has been performed. We will obtain written consent when the potential research subject comes into the CTRC for the in-person screening session. This will be prior to any invasive screening procedures are done--i.e. prior to drawing labs, physical exam, or detailed medical history.

4.11.2 Taking into account the nature of the study and subject population, indicate how the research team will ensure that subjects have sufficient time to decide whether to participate in this study. In addition, describe the steps that will be taken to minimize the possibility of coercion or undue influence.

Potential transplant study subjects will be approached about possible study participation in the pre-transplant clinic or while inpatients being worked up for transplant. Potential subjects will not be approached immediately prior to transplant--i.e. on the day of surgery. One of the investigators will go through the consent form with the potential subject in the clinic or the hospital and stress that participation in the study is completely voluntary, that the choice of whether or not to participate in the study will have no effect on his/her relationship to the medical team or UPMC, and that he/she is free to withdraw from the study at any time after signing the consent form. The potential subject will then be free to take home the consent form and think about whether or not to participate in the study.

Control subjects will be recruited via advertisements so they will approach the investigators themselves. However, the same considerations will apply--they will be given time to think about whether or not to participate, and it will be stressed that participation is completely voluntary and they may withdraw from the study at any time for any reason.

4.12 Describe the process that you will employ to ensure the subjects are fully informed about this research study.

One of the investigators will go through the consent form with each potential study subject, describing each section of the form and each part of the study, including the purpose of the study, the procedures to be employed, and the potential risks and benefits of their participation in the study, as well as their rights as a research subject. The potential subject will be reassured that study participation is completely voluntary, that their decision whether or not to participate in the study will have no effect on their relationship with any of the investigators or with UPMC, and that even if they do decide to sign the consent form, they may still withdraw from the study at any time for any reason. No coercion will occur and the refusal of a potential subject to participate in the study will be final. In addition, the potential subject will be given ample time to ask questions about the study, and will be given the phone number of a study coordinator in case of further questions.

4.13 Are you requesting an exception to either IRB policy related to the informed consent process?

no

4.14 Will you inform research subjects about the outcome of this research study following its completion?

no

5.   Potential Risks and Benefits

5.1 Risks of Screening Procedures: Are there any potential risks (e.g., physical, psychological, social, etc.) associated with the screening procedures (i.e., procedures to determine research subject eligibility) that will be performed for the purpose of this research study?

Yes

5.1.1 Describe potential risks (physical, psychological, social, legal, economic or other) associated with screening procedures, research interventions/interactions, and follow-up/monitoring procedures performed specifically for this study:

Research Activity: Blood drawn for labs
Infrequent Risks: Pain Bleeding, bruising, fainting, and risk of infection

5.1.2 Describe the steps that will be taken to prevent or to minimize the severity of the potential risks:

All information collected will be kept confidential. All blood draws will be done by appropriate licensed healthcare personnel using sterile techniques and equipment. All data collected from subjects will be kept confidential under lock and key.

5.2 Risks of Experimental Interventions: Are there any potential risks (e.g., physical, psychological, social, etc.) associated with the experimental interventions that will be performed for the purpose of this research study?
yes

5.2.1 List Expected Incidence of Experimental Intervention Risk:

Research Activity	Common risks	Infrequent Risks	Other risks
Administration of oral fexofenadine	Fexofenadine (Allegra®) is usually well tolerated and has	Infrequent (Occurs in 1-10% of people): Viral infection (cold, flu):	

	a low incidence of side effects. However, possible side effects of fexofenadine (vs. placebo) from clinical trials for seasonal allergic rhinitis (hay fever) and chronic idiopathic urticaria (an allergic skin condition) include: Common (Occurs in 10-25% of people): headache 10.6% (vs. 7.5%) and 7.2% (vs. 6.6%)	2.5% (vs. 1.5%), Nausea: 1.6% (vs. 1.5%), Dysmenorrhea (difficult menstrual periods): 1.5% (vs. 0.3%), Drowsiness: 1.3% (vs. 0.9%) and 2.2% (vs. 0.0%), Dyspepsia (upset stomach): 1.3% (vs. 0.6%), Fatigue: 1.3% (vs. 0.9%), Upper Respiratory Tract Infection: 3.2% (vs. 3.1%) and 4.3% (vs. 1.7%), Back Pain: 2.8% (vs. 1.4%) and 2.2% (vs. 1.1%), Accidental Injury: 2.9% (vs. 1.3%), Coughing: 3.8% (vs. 1.3%), Fever: 2.4% (vs. 0.9%), Pain: 2.4% (vs. 0.4%), Otitis Media (ear infection): 2.4% (vs. 0.0%), Sinusitis (sinus infection): 2.2% (vs. 1.1%), Dizziness: 2.2% (vs. 0.0%) Rare (Occurs in less than 1% of people): none	
Administration of oral/intravenous midazolam	Decreased tidal volume and/or respiratory rate decrease (23.3% of patients after IV and 10.8% of patients after IM injection), apnea (15.4% of patients after IV administration), as well as variations in blood pressure and pulse rate (frequency not listed). In addition, the following adverse reactions were seen	Other adverse effects occurring after midazolam administration at an incidence of <1.0% in adults and children include: Respiratory: laryngospasm, bronchospasm, dyspnea, hyperventilation, wheezing, shallow respirations, airway obstruction, Cardiovascular: tachypnea, bigeminy, premature ventricular contractions, vasovagal episode, bradycardia, tachycardia,	

	after IV injection of midazolam: hiccoughs (3.9%), vomiting (2.6%), coughing (1.3%),"oversedation" (1.6%), headache (1.5%), drowsiness (1.2%). Local effects at the injection site include: tenderness (5.6%), pain during injection (5.0%), redness (2.6%), induration (1.7%), phlebitis (0.4%).	nodal rhythm, Gastrointestinal: acid taste, excessive salivation, retching, CNS/neuromuscular: retrograde amnesia, euphoria, hallucination, confusion, argumentativeness, nervousness, anxiety, grogginess, restlessness, emergence delirium or agitation, prolonged emergence from anesthesia, dreaming during emergence, sleep disturbance, insomnia, nightmares, athetoid movements, seizure-like activity, ataxia, dizziness, dysphoria, slurred speech, dysphonia, paresthesia, Special Senses: blurred vision, diplopia, nystagmus, pinpoint pupils, cyclic movements of eyelids, visual disturbance, difficulty focusing eyes, ears blocked, loss of balance, light-headedness, Integumentary: hive-like elevation at injection site, swelling or feeling of burning, warmth, or coldness at injection site, Hypersensitivity: allergic reactions including anaphylactoid reactions, hives, rash, pruritis, Miscellaneous: yawning, lethargy, chills, weakness, toothache, faint feeling hematoma.	
Genotyping			There are risks associated with

			genotyping. While the investigators will have procedures in place to ensure that all subject information is kept strictly confidential, there is the possibility that if genotype results became generally known, this information could have an impact on your future insurability, employability, reproductive plans, or could have a negative impact on family relationships, or could result in paternity suits or stigmatization.
Removal of two extra tissue samples during biopsy		Small risk of bleeding, infection, or intestinal perforation (exact incidence unknown, but rare). Since these subjects will be undergoing the biopsy as part of routine clinical care, we feel it is unlikely that the acquisition of two extra tissue samples beyond the usual three obtained as part of routine clinical care will pose significant extra risk to the subject.	

5.2.2 Describe the steps that will be taken to prevent or to minimize the severity of the potential risks associated with the experimental interventions:

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Inpatient subjects (transplant patients at study sessions 1 and 2) will be in monitored hospital beds and will be supervised by medical and nursing staff for the duration of the session. Outpatient study sessions will take place at the University of Pittsburgh Medical Center Clinical and Translational Research Center (CTRC). Study subjects will undergo pulse oximetry monitoring for the first two hours after oral midazolam administration while in the CTRC. Vital signs and pulse ox will be measured every two hours thereafter for the duration of blood sampling. In addition, experienced CTRC nursing staff will continuously monitor each subject during the study session to watch for adverse effects from study medications or any other medical complications. The Observer's Assessment of Alertness/Sedation (OAA/S) scale will be used by the investigators to assess alertness and level of sedation. This assessment will be administered at baseline (before administration of study drug), and at 15, 30, 45, 60, and 120 minutes after each dose of midazolam. Subjects will be required to remain in bed for six hours after midazolam administration (bathroom privileges with assistance). Upon recognition of possible adverse events, the patient's transplant physician will be notified immediately (in the case of transplant patients) and appropriate medical care given to the subject (all subjects).

5.3 Risks of Follow Up Procedures: Are there any potential risks (e.g., physical, psychological, social, etc.) associated with the follow up procedures (e.g., tests to measure the efficacy and/or safety of the experimental intervention(s); to include monitoring procedures and/or outcome measures) that will be performed for the purpose of this research study?

Yes

5.3.1 List Expected Incidence of Follow Up Procedure Risk:

Research Activity: Blood draws for labs and drug levels
Common Risks: Pain
Infrequent Risks: Bruising, bleeding, fainting, and risk of infection

5.3.2 Describe the steps that will be taken to prevent or to minimize the severity of the potential risks associated with the follow up procedures:

Patients will have a catheter inserted at the beginning of the study to facilitate blood draws. Blood draws will be performed by qualified CTRC nursing staff using sterile techniques.

5.4 Do any of the research procedures pose a physical or clinically significant psychological risk to women who are or may be pregnant or to a fetus?

yes

5.4.1 List the research procedures that pose a risk to pregnant women or fetuses:

Midazolam is classified as Pregnancy Category D by the FDA. An increase in birth defects has been seen in infants born to mothers who used benzodiazepine drugs (class of drugs that midazolam belongs to) during pregnancy. Fexofenadine is classified as Pregnancy Category

C by the FDA. This means that although animal studies have not shown the drug to cause birth defects, no adequate or well-controlled studies have been done in pregnant women to examine their effects on the fetus. In addition, midazolam is known to be excreted in breast milk. Therefore, being a part of this study while pregnant or breastfeeding may expose the unborn child or nursing infant to risks known and unknown. Therefore, pregnant and nursing women will not be included in this study.

5.4.2 Describe the steps that will be taken to rule out pregnancy prior to exposing women of child-bearing potential to the research procedures that pose a risk to pregnant women or fetuses:

A urine pregnancy test will be done on all women of childbearing potential during the screening visit and at each study session before administration of study drugs. It must be negative before they can enter this study or participate in any of the interventional study sessions.

5.4.3 Describe the measures to prevent pregnancy, and their required duration of use, that will be discussed with women of child-bearing potential during and following exposure to research procedures:

While enrolled in the study women of childbearing potential must agree to use appropriate methods of birth control for the duration of the study as well as for one week after the last dose of study drug. Medically acceptable birth control methods include: (1) surgical sterilization, (2) approved hormonal contraceptives (such as birth control pills or patches), (3) double-barrier methods (such as a condom and diaphragm together) used with a spermicide, (4) an intrauterine device (IUD), or (5) abstinence. Women will be advised not to take part in this study if they plan to become pregnant within one month after completing this study, are currently pregnant, or are currently breast feeding. They will be informed that they must notify their doctor and/or the investigators if they suspect they have become pregnant while participating in this study.

5.5 Do any of the research procedures pose a potential risk of causing genetic mutations that could lead to birth defects?

Unknown

5.5.1 List the research procedures that pose a potential risk of genetic mutations/birth defects:

Midazolam is classified as pregnancy category D by the FDA. An increase in birth defects has been seen in infants born to mothers who used benzodiazepine drugs during pregnancy. Fexofenadine is classified as pregnancy category C by the FDA, meaning that although animal studies have not shown the drug to cause birth defects, there are no adequate or well-controlled studies in pregnant women to examine its effects on the fetus.

5.5.2 Describe the measures to prevent pregnancy, and their required duration of use, in female subjects and female partners of male subjects during and following exposure to research procedures:

Pregnant and nursing women, or women who plan to become pregnant, are not included in the study. The potential risks of the study drugs during pregnancy and lactation are explained in the consent forms. Female subjects of childbearing potential must agree to use appropriate methods of birth control while enrolled in the study as well as for one week after the last dose of study drug. Acceptable methods of birth control include surgical sterilization, approved hormonal contraceptives, double barrier methods (such as condom and diaphragm) used with a spermicide, an intrauterine device, or abstinence. In addition, urine pregnancy tests are done at each study session before administration of study drugs.

5.6 Are there any alternative procedures or courses of treatment which may be of benefit to the subject if they choose not to participate in this study?

Not applicable; the experimental intervention does not involve a diagnostic/treatment procedure.

5.7 Describe the specific endpoints (e.g., adverse reactions/events, failure to demonstrate effectiveness, disease progression) or other circumstances (e.g., subject's failure to follow study procedures) that will result in discontinuing a subject's participation?

Any subject enrolled in the study (transplant patient or control) may withdraw from the study at any time if they elect to do so. No cold calling by the investigator or research staff will occur. No coercion will occur. Refusal by a subject to participate in the study will be final. Subjects will also be withdrawn from the study if they are unable to tolerate the study drugs.

5.8 Will any individuals other than the investigators/research staff involved in the conduct of this research study and authorized representatives of the University Research Conduct and Compliance Office (RCCO) be permitted access to research data/documents (including medical record information) associated with the conduct of this research study?

no

5.9 Has or will a Federal Certificate of Confidentiality be obtained for this research study?

no

5.10 Question has been moved to 5.17

5.11 Question has been moved to 5.16

5.12 Does participation in this research study offer the potential for direct benefit to the research subjects?

There will be no direct benefits to the research subjects participating in this study. However, there may be indirect benefits to the transplant patients and to society as a whole from this research study because it will allow for a better understanding of the functional and metabolic changes occurring in the transplanted small bowel over time as well as the effects of certain genetic polymorphisms on these changes. This will enable clinicians who care for small bowel transplant patients to optimize drug dosing and potentially improve short- and long-term health outcomes in this population.

5.13 Describe the data and safety monitoring plan associated with this study. If the research study involves multiple sites, the plan must address both a local and central review process.

Study subjects will be closely monitored by the investigators and research personnel. The data and safety information obtained for each study subject will be reviewed by the principal investigator and the principal investigator's mentor in weekly meetings. The Starzl Transplantation Institute's Protocol Review Committee / DSMB committee will review the protocol and progress annually. This will evaluate the recruitment process, any breach of confidentiality and data collection. A summary report will be submitted annually to the IRB. We will comply with the IRB's policies for the reporting of serious and unexpected adverse events and breach of confidentiality as detailed in Chapter 3.0, section 3.3 of the IRB Reference Manual. If a serious life-threatening event occurs, the event will be reported immediately (i.e., within 24 hours) to the IRB. Unexpected reactions of moderate or greater severity will be reported to the IRB within 10 calendar days of the reaction. Minor events will be reported to the IRB at the time of annual review. All adverse events related to the research intervention will also be reported to the Food and Drug Administration (FDA) via the MedWatch adverse event reporting system in a timely fashion.

5.14 What precautions will be used to ensure subject privacy is protected? (e.g. the research intervention will be conducted in a private room; the collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected, drapes or other barriers will be used for subjects who are required to disrobe)

Study procedures are conducted in private rooms, either inpatient hospital rooms or private rooms in the CTRC. Investigators do not discuss study results or subjects in public areas or anywhere where anything may be overheard by non-study personnel. All biological samples from subjects are labeled without subject identifiers at all times.

5.15 What precautions will be used to maintain the confidentiality of identifiable information? (e.g., paper-based records will be kept in a secure location and only be accessible to personnel involved in the study, computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords, prior to access to any study-related information, personnel will be required to

sign statements agreeing to protect the security and confidentiality of identifiable information, whenever feasible, identifiers will be removed from study-related information, precautions are in place to ensure the data is secure by using passwords and encryption, because the research involves web-based surveys, audio and/or video recordings of subjects will be transcribed and then destroyed to eliminate audible identification of subjects)

All study records are and will be kept in a locked cabinet in the Principal Investigator's office. Only the PI (Jennifer Bonner) and another co-investigator (Darlene Koritsky) have access to the key to open this cabinet. Subjects are assigned numbers based on their order of recruitment and the names associated with subject numbers are kept in the aforementioned locked cabinet. All biological samples from subjects (blood/plasma/urine/biopsy tissue) are labeled without subject identifiers--therefore no laboratory personnel see subject identifiers at any time. Laboratory data generated from the above-referenced samples is analyzed without subject identifiers.

5.16 If the subject withdraws from the study, describe what, if anything, will happen to the subject's research data or biological specimens.

If a subject decides to withdraw from study participation, research data will be rendered anonymous.

5.17 Following the required data retention period, describe the procedures utilized to protect subject confidentiality. (e.g., destruction of research records; removal of identifiers; destruction of linkage code information; secured long-term retention)

At the end of the data retention period, all research records will be destroyed, including linkage code information.

6.   Costs and Payments

6.1 Will research subjects or their insurance providers be charged for any of the procedures (e.g., screening procedures, research procedures, follow-up procedures) performed for the purpose of this research study?

No

6.2 Will subjects be compensated in any way for their participation in this research study?

yes

6.2.1 Describe the amount of payment or other remuneration offered for complete participation in this research study.

For transplant subjects: $200 for completion of all 3 study sessions. For control subjects: $125 upon completion of both study sessions.

6.2.2 Describe the amount and term of payment or other remuneration that will be provided for partial completion of this research study.

$100 for completion of each study session during which drug is administered (i.e. study sessions 2 and 3)--applicable to transplant patients only.

7. Qualifications and Sources of Support

7.1 Summarize the qualifications and expertise of the principal investigator and listed co-investigators to perform the procedures outlined in this research study.

Kareem Abu-Elmagd, MD, PhD, FACS, is a transplant surgeon at the University of Pittsburgh Medical Center, professor of surgery at the University of Pittsburgh, and director of the Intestinal Rehabilitation and Transplant Center at UPMC.

Geoffrey Bond, MD, FRACS, is a transplant surgeon and assistant professor of surgery at the University of Pittsburgh. He has extensive clinical and research experience with small bowel and multivisceral transplant patients.

Jennifer Bonner, PharmD, is a clinical pharmacist working with the small bowel transplant team at UPMC and a graduate student in the Clinical Pharmaceutical Scientist PhD program at the University of Pittsburgh.

Guillherme Costa, MD, FACS, is a transplant surgeon and assistant professor of surgery at the University of Pittsburgh. He has extensive clinical and research experience with small bowel and multivisceral transplant patients.

Darlene Koritsky, RN, is a researcher at the Intestinal Rehabilitation and Transplant Center. She has several years' experience designing clinical research protocols in small bowel and multivisceral transplant patients.

Hossam Kandil, MD, PhD is a gastroenterologist and associate professor of medicine at the University of Pittsburgh. He has over 20 years' clinical and research experience working with transplant patients.

Stephen J. O'Keefe, MD, MSc, FRCP is the medical director of the Small Bowel Transplantation and Rehabilitation unit at UMPC and professor of medicine at the University of Pittsburgh. Dr. O'Keefe has extensive clinical and research experience with small bowel and multivisceral transplant patients.

Raman Venkataramanan, PhD, is a professor in the department of pharmaceutical sciences at the University of Pittsburgh School of Pharmacy. He has over 23 years of experience working with transplant patients, and has been the principal investigator on several pharmacokinetic studies in this patient population.

Sripal Mada Reddy, Ph.D. is a research assistant professor in the School of Pharmacy. He has several years of experience conducting pharmacokinetic studies in human subjects.

7.2 Indicate all sources of support for this research study.

Internal funding--this project will be supported by the Clinical Pharmacokinetics Laboratory, University of Pittsburgh (Director: Raman Venkataramanan); CTRC

7.3 Does any investigator involved in this study:
a) possess an equity interest in the publicly-traded entity that either sponsors this research or owns the technology being evaluated that exceeds 5% ownership interest or a current value of $10,000?
b) possess any equity interest in the non-publicly-traded entity that either sponsors this research or owns the technology being evaluated?
c) receive salary, consulting fees, honoraria, royalties or other payments from the entity that either sponsors this research or owns the technology being evaluated that is expected to exceed $10,000 in any twelve-month period?
d) have rights to the intellectual property (IP) being evaluated, as either the inventor of the IP for which a patent has been issued, or as the inventor of the IP that has been optioned or licensed to a company?
e) have a financial relationship with a Licensed Start-up Company (which is being monitored by the COI Committee) that has an option or license to utilize the technology being evaluated?
f) receive compensation of any amount when the value of the compensation would be affected by the outcome of the research, such as compensation that is explicitly greater for a favorable outcome than for an unfavorable outcome or compensation in the form of an equity interest in the entity that either sponsors this research or owns the technology being evaluated?
'Investigator' means any member of the study team who participates in the design, conduct, or reporting of this research, as well as his/her spouse, registered domestic partner, dependents, or other members of his/her household.
The PI is responsible for ensuring that s/he and all other relevant members of the study team review these questions.
If any of the above are true, select Yes below:

no

University of Pittsburgh   3459 Fifth Avenue

School of Medicine   Pittsburgh, PA 15213

Thomas E. Starzl Transplantation Institute   412-647-1458

CONSENT TO ACT AS PARTICIPANT IN A RESEARCH STUDY

(TRANSPLANT PATIENT)

TITLE: Short- and long-term evaluation of intestinal CYP3A4/5 and p-glycoprotein

function in small bowel transplant recipients

PRINCIPAL INVESTIGATOR:
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Why is this research being done?
The small intestine (also called the small bowel) is subjected to low temperature during transportation from the person who donates the small intestine (the donor) to the person who gets it (the recipient). When the small intestine is put inside the recipient it is warmed up to normal body temperature and the recipient's blood begins to flow into it. These changes, combined with the effects of surgery and healing, cause the release of substances in the body that may temporarily change the way the small intestine works soon after the surgery. However, with time the body recovers from the surgery and the new intestine usually begins to function normally. The main objective of this study is to look at the time course of this recovery process by comparing how three drugs are absorbed immediately after the transplant surgery and at a later time point after the transplant surgery. Another objective of this study is to examine the effects of certain genetic differences on oral drug absorption after small bowel transplantation. This information will then be used in the future to help health professionals such as your doctor determine the best dosing of drugs in small bowel transplant patients.

Who is being asked to take part in this research study?
You are being invited to take part in this research study because you are a small bowel transplant candidate and will receive a small bowel or small bowel and pancreas transplantation. Female and male small bowel, small bowel-pancreas, and modified multivisceral transplant patients, between the ages of 18 and 65 years of age are being asked to participate in this clinical study.

This study will take place at the University of Pittsburgh Medical Center, Pittsburgh, PA, and will include approximately 25 transplant subjects and 25 control subjects.

How will the study be done?
If you decide to participate in this study, you will undergo a total of three study sessions. The first is a screening visit that will occur at the University of Pittsburgh Medical Center (UPMC) during your transplant hospitalization (after your transplant) and will last approximately 2 hours. The second study session will take place within 21 days after your transplant surgery and will occur while you are still in the hospital. The third study session will require a separate visit to UPMC, unless you happen to be an inpatient at the hospital at that time for some other reason. The second and third study sessions will last approximately 24 hours each (including the overnight stay after the blood draws have been completed). At the second and third study sessions you will be administered a single dose of midazolam (an FDA-approved sedative) both orally and through a vein (blood vessel). You will also receive a single oral dose of fexofenadine (also FDA-approved and marketed under the brand name Allegra® for the treatment of allergy).

First study session - Screening
If you have signed the informed consent form before your transplant, you will be eligible to participate in the first study session. This session will occur after your transplant and will include vital signs measurement and clinical laboratory tests that are part of routine clinical care, including blood tests to evaluate liver and kidney function. In addition, blood levels of several inflammatory and anti-inflammatory substances that are secreted by the body normally and are secreted in higher amounts after transplant surgery will be measured. This first session will last approximately 2 hours and will take place in the transplant intensive care unit (TICU) of UPMC Montefiore hospital.

After surgery, medical information will be collected that is part of the routine care of small bowel transplant surgery which includes daily blood tests to evaluate your liver and kidney function, length of the small bowel transplant surgery, any signs or symptoms of injury to your transplanted organ, time admitted to the intensive care unit, time spent needing the ventilator (breathing machine) and information about the donor small intestine such as age, gender and weight. In addition, results of any liver biopsies done before your transplant will be recorded for study purposes. Blood levels of the substances associated with inflammation will be measured every other day from the first day after surgery until the day of the second study session within 21 days after your transplant. This will be done using blood left over from other routine lab work. No separate blood samples will be taken for this purpose.

Additional procedures
Small intestinal tissue samples taken during two routine biopsies of your transplanted organ (one in the first three weeks after your transplant and one approximately 4 to 9 months after your transplant) will be analyzed for levels of the same inflammatory substances. One of these tissue samples will also be tested for the presence and type of two specific genes. These two genes produce proteins that help the body metabolize (i.e. break down) and transfer drugs in the intestine. Finally, these same tissue samples will be analyzed for amounts of these two proteins. During each of the two routine biopsy sessions mentioned above, two separate tissue samples will be taken for research purposes, in addition to the three that are normally taken as part of routine clinical care. A blood sample (approximately 3 mL or 3/5 of a tablespoon) will also be

tested for the presence and type of the same genes.

Second and third study sessions

The second study session will occur in the transplant intensive care unit (TICU) or on Unit 12N or 11N of UPMC Montefiore hospital while you are still hospitalized, most likely within 21 days after your transplant. The third study session will probably require a separate visit to the CTRC approximately four to nine months after your transplant surgery. Before each of these sessions, you will be asked not to eat grapefruit or drink grapefruit juice, apple juice, cranberry juice, orange juice, or caffeine-containing drinks/foods for 3 days before this study session, because these foods may affect intestinal uptake of certain drugs. You will be asked not to drink water or consume any food after midnight on the day of the session except small amounts needed to take your routine medications. At each study session, you will receive a single dose of 5 milligrams (mg) of midazolam by mouth (or through a jejunostomy tube, if you have one at the time) with a small amount of water. Sixty minutes later you will receive a dose of 60 mg of fexofenadine by mouth with a small amount of water. Seven hours after the oral dose of midazolam you will be given a single dose of 2 mg of midazolam through a vein (intravenous). If you are eating a regular diet at the time of the study session, you will be given breakfast two hours after the oral dose of midazolam and lunch one hour after the intravenous dose of midazolam. Dinner will be provided in the early evening of each study session day. If you are receiving enteral feedings (feedings through a tube into your intestine) or some other dietary arrangements, they will proceed as directed by your physician.

Blood samples will be taken through a small tube that has been placed in your blood vessel (or from your central line, if you have one) a total of 16 times over a 20-hour period, starting immediately before the first dose of drug. In addition, a blood sample will be taken at the beginning of the session to measure levels of the inflammatory substances described above. The total amount of blood removed at each study session will be approximately 51 mL (approximately 3 tablespoonfuls plus one teaspoonful). For the entire study (both study sessions four to six months apart), approximately 102 mL or 7 tablespoonfuls of blood will be removed. These same blood samples will also be analyzed for level of tacrolimus (Prograf , an immunosuppressive medication you will be receiving after your transplant as part of your routine medical care.) Your dose of tacrolimus will not be changed for the purposes of this study, and no extra blood samples will be drawn to measure levels of it. In addition, your urine will be collected over a 12-hour period during the first study session and 20 hours at the second study session, for measurement of study drugs and their metabolites in your urine. You will undergo continuous blood pressure monitoring and pulse oximetry (a non-invasive test to measure the level of oxygen in your blood to make sure you are breathing adequately) for the first two hours after each drug administration. Experienced nursing staff will monitor your progress continually, and your level of sedation will be assessed periodically during the session. If you vomit within 4 hours of taking either of the oral drugs you will be removed from the study, since full absorption of the dose may not have taken place. You will be asked to remain overnight in the hospital unit or CTRC (wherever the session has taken place) after the second and third study sessions to ensure that both study drugs are completely gone from your body before you leave.

If you decide to participate in the study, your medical records will be reviewed for demographic information (age, gender, and race), lab results (such as liver and kidney function, done as part of

your routine pre- and post-transplant care), and medication information, during the transplant follow-up period.

What are the possible risks, side effects, and discomforts of this research study?
There may be certain risks associated with participation in this study. These may include side effects of midazolam and/or fexofenadine (which are described below), the risks associated with giving you midazolam, and risk associated with blood sampling for measuring drug levels. These include pain, bruising, bleeding, fainting, and risk of infection.

In addition, there are risks associated with genotyping. While the investigators will have procedures in place to ensure that all subject information is kept strictly confidential, there is the possibility that if genotype results became generally known, this information could have an impact on a subject's future insurability, employability, reproductive plans, or could have a negative impact on family relationships, or could result in paternity suits or stigmatization.

The doses of midazolam that you will receive (5 mg orally and 2 mg intravenously) are relatively low but may be sedating (may cause drowsiness) in some people. These doses of midazolam have been used safely for research purposes with minimal side effects in transplant patients. However, these are the most common side effects of midazolam seen at a wide range of doses:

The following adverse reactions to midazolam have been reported after intramuscular (IM) or intravenous (IV) injection:
Decreased tidal volume and/or respiratory rate decrease (decrease in breathing rate and volume of breath) (23.3% of patients after IV and 10.8% of patients after IM injection), apnea (stopping breathing while asleep) (15.4% of patients after IV administration), as well as variations in blood pressure and pulse rate (frequency not listed).

In addition, the following adverse reactions were seen after IV injection of midazolam: hiccoughs (hiccups) (3.9%), vomiting (2.6%), coughing (1.3%), "oversedation" (1.6%), headache (1.5%), drowsiness (1.2%). Local effects at the injection site include: tenderness (5.6%), pain during injection (5.0%), redness (2.6%), induration (indentation of the skin) (1.7%), phlebitis (inflammation of blood vessel through which drug was administered) (0.4%).

Other very rare adverse effects occurring after midazolam administration in less than 1.0% in adults and children include:
Respiratory: laryngospasm (tightening or spasm of the throat or airway), bronchospasm (spasm or tightening of the lungs), dyspnea (shortness of breath), hyperventilation, wheezing, shallow respirations, airway obstruction, tachypnea (rapid breathing).
Cardiovascular: bigeminy (pulse and heart rate abnormality), premature ventricular contractions (type of heart arrhythmia), vasovagal episode (fainting due to drop in blood pressure and heart rate), bradycardia (slow heart rate), tachycardia (rapid heart rate), nodal rhythm (type of heart arrhythmia).
Gastrointestinal: acid taste, excessive salivation, retching,
CNS/neuromuscular: retrograde amnesia (temporary loss of memory), euphoria (elevated mood), hallucination, confusion, argumentativeness, nervousness, anxiety, grogginess, restlessness, emergence delirium or agitation, prolonged emergence from anesthesia, dreaming during emergence, sleep disturbance, insomnia, nightmares, athetoid movements (involuntary writhing movements), seizure-like activity, ataxia (unsteady gait), dizziness, dysphoria (unpleasant feelings), slurred speech, dysphonia (difficulty speaking), paresthesia ("pins and needles"

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tingling sensation).

Special Senses: blurred vision, diplopia (double vision), nystagmus (involuntary rapid movement of eyeballs), pinpoint pupils, cyclic movements of eyelids, visual disturbance, difficulty focusing eyes, ears blocked, loss of balance, light-headedness,

Integumentary: hive-like elevation at injection site, swelling or feeling of burning, warmth, or coldness at injection site,

Hypersensitivity: allergic reactions including anaphylactoid reactions, hives, rash, pruritis (itching).

Miscellaneous: yawning, lethargy (low energy), chills, weakness, toothache, faint feeling, hematoma (blood clot within a tissue).

Fexofenadine (Allegra®) is usually well tolerated and has a low incidence of side effects. However, possible side effects of fexofenadine (vs. placebo) from clinical trials in patients with seasonal allergic rhinitis (hay fever) and chronic idiopathic urticaria (an allergic skin condition) include:

Common (Occurs in 10-25% of people): headache 10.6% (vs. 7.5%) and 7.2% (vs. 6.6%)

Infrequent (Occurs in 1-10% of people): Viral infection (cold, flu): 2.5% (vs. 1.5%), Nausea: 1.6% (vs. 1.5%), Dysmenorrhea (difficult menstrual periods): 1.5% (vs. 0.3%), Drowsiness: 1.3% (vs. 0.9%) and 2.2% (vs. 0.0%), Dyspepsia (upset stomach): 1.3% (vs. 0.6%), Fatigue: 1.3% (vs. 0.9%), Upper Respiratory Tract Infection: 3.2% (vs. 3.1%) and 4.3% (vs. 1.7%), Back Pain: 2.8% (vs. 1.4%) and 2.2% (vs. 1.1%), Accidental Injury: 2.9% (vs. 1.3%), Coughing: 3.8% (vs. 1.3%), Fever: 2.4% (vs. 0.9%), Pain: 2.4% (vs. 0.4%), Otitis Media (ear infection): 2.4% (vs. 0.0%), Sinusitis (sinus infection): 2.2% (vs. 1.1%), Dizziness: 2.2% (vs. 0.0%)

Rare (Occurs in less than 1% of people): none

In addition, complications of small intestinal biopsy are rare, but may include intestinal bleeding, infection, and perforation (puncture) of the intestine.

Pregnancy

Midazolam is classified as Pregnancy Category D by the FDA. An increase in birth defects has been seen in infants born to mothers who used benzodiazepine drugs (class of drugs that midazolam belongs to) during pregnancy. Fexofenadine is classified as Pregnancy Category C by the FDA. This means that although animal studies have not shown the drug to cause birth defects, no adequate or well-controlled studies have been done in pregnant women to examine their effects on the fetus. In addition, midazolam is known to be excreted in breast milk. Therefore, being a part of this study while pregnant or breastfeeding may expose the unborn child or nursing infant to risks known and unknown. Therefore, pregnant and nursing women will not be included in this study. If you are a woman of childbearing potential, a urine pregnancy test will be done at each study session before administration of study drugs. It must be negative before you can enter this study. While enrolled in the study you must agree to use appropriate methods of birth control as well as for one week after the last dose of study drug. Medically acceptable birth control methods include: (1) surgical sterilization, (2) approved hormonal contraceptives (such as birth control pills or patches), (3) double barrier methods (such as a condom and diaphragm) used with a spermicide, (4) an intrauterine device (IUD), or (5) abstinence.

You should not take part in this study if you plan to become pregnant within one month after completing this study, are currently pregnant, or you are currently breast feeding.

You must notify your doctor if you suspect you have become pregnant while participating in this study.

What are possible benefits from taking part in this study?
There are no direct benefits to you from taking part in this study. However, the information learned from this study will be used in the future to help health professionals such as your doctor to determine the best dosing of drugs in small bowel transplant recipients after surgery. Therefore, your participation may help others in the future by what the doctors learn from your involvement in this study.

What treatment or procedures are available if I decide not to take part in this research study?
If you decide not to take part in this research study, you will undergo normal procedures associated with the small bowel transplantation surgery. No routine treatment will be withheld.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?
You have been informed previously that the personal results of this research study will be provided to you upon your request. However, you will be promptly notified if any other information about this research study develops during the course of the study which may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?
If you choose to participate in the study, study drugs, nursing care, and meals during study sessions will be provided free of charge. Measurement of midazolam and fexofenadine blood levels, measurement of inflammatory substances, and genetic testing will also be performed free of cost to you. All costs and tests done to treat you before and after your small bowel transplant should be covered by your medical insurance. These are tests that would normally be performed in patients undergoing small bowel transplant surgery. No compensation will be provided by the makers of Versed® (midazolam) or the makers of Allegra® (fexofenadine). This includes no financial support for lost wages, disability, pain or discomfort.

Will I be paid if I take part in this research study?
You will not be paid for taking part in the initial (screening) study session prior to transplant surgery. However, you will be paid $100.00 for each of the second and third study visits, up to a maximum of $200.00. Your biological sample or genetic material may lead, in the future, to new inventions or products. If the research investigators are able to develop new products from the use of your biological sample or genetic material, there are currently no plans to share with you any money or other rewards that may result from the development of the new product.

Who will pay if I am injured as a result of taking part in this study?
If you believe that the research procedures have resulted in an injury to you, immediately contact

the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Who will know about my participation in this research study?
Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. Only the investigators listed on the first page of this consent form will have access to information linking your case number to your name, which will be stored in a locked cabinet in the Principal Investigator's office. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Will this research study involve the use or disclosure of my identifiable medical information?
This research study will involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g., physician office) records. The information that will be recorded will be limited to information concerning demographics (age, gender, and race) and concurrent conditions and medications you are receiving.
Your personal research results from this study will not be put in your medical record and you will not be identified in any publication of this research study. In addition, your written authorization will be obtained prior to providing personal research results to relatives, personal physicians, insurance companies, or any other third party.

Who will have access to identifiable information related to my participation in this research study?
In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

Authorized representatives of UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study participation; (2) addressing correct payment for tests

and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

Authorized representatives of the U.S. Food and Drug Administration may review and/or obtain identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of monitoring the accuracy of the research data. While the U.S. Food and Drug Administration understands the importance of maintaining the confidentiality of your identifiable research and medical information, the University of Pittsburgh and UPMC cannot guarantee the confidentiality of this information after it has been obtained by the U.S. Food and Drug Administration.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?
If you agree to participate in the research project, use of your biological sample and genetic material will be under the control of the principal investigator of this research project. All the blood and tissue samples collected from you will be labeled using an identification number and without your name. They will be stored and in the laboratory of the researchers until all the data is obtained from these samples. The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of 5 years and for as long (indefinite) as it may take to complete this research study. The blood and tissue samples collected in this study will be kept for an indefinite time period until a complete report of the study has been published. The sample with out the identification may be utilized in future studies by the investigators. These samples will not be shared with any secondary investigators not listed on the current research study.

May I have access to my medical information that results from my participation in this research study?
In accordance with UPMC Notices of Privacy Practices document that you have been given, you are permitted access to information (including information resulting from your participation in this research study) contained within your medical records filed with your health care provider. You will be given the results of your genetic testing related to this study if you request it from the investigators. If you request this information from the investigators, you will be given it at the end of the research study in a private meeting with one or more of the investigators and the information will be kept confidential. If you wish, the investigators will refer you to medical or genetic counseling at this time.

Is my participation in this research study voluntary?
Your participation in this research study is completely voluntary. You do not have to take part in this research study and, should you change your mind, you can withdraw from the study at any

time. Your current and future care at a UPMC facility and any other benefits for which you qualify will be the same whether you participate in this study or not.

Your doctor may be an investigator in this research study, and as an investigator, is interested both in your medical care and in the conduct of this research. Before entering this study or at any time during the research, you may discuss your care with another doctor who is in no way associated with this research project. You are not under any obligation to participate in any research study offered by your doctor.

May I withdraw, at a future date, my consent for participation in this research study?
You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research or medical information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above. Samples collected before your withdrawal from the study will be destroyed, if you so request.
To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

If you decide to withdraw your consent for participation in this research study it will have no effect on your current or future relationship with the University of Pittsburgh. If you decide to withdraw your consent for participation in this research study it will have no affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to take part in this research study, can I be removed from the study without my consent?
It is possible that you may be removed from the research study by the researchers if you do not follow all the instructions. You may be removed from the study if you experience unexpected side effects and in the opinion of the investigators that it is in your best interest. The study may also be stopped by the investigators or the sponsor if it felt that it is in the best interest of the subjects.

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VOLUNTARY CONSENT
All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-

2668).

I give my permission to use my biological sample or genetic material, with personal identifiers, in other research projects involving the study of small bowel transplantation.

Yes _____ No _____

I give my permission to be recontacted to obtain my consent if there is a desire to use my biological sample or genetic material, with personal identifiers, in other research projects involving the study of different diseases or conditions (i.e. diseases or conditions other than those specified in the Description section of this consent form).

Yes _____ No _____

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____
Participant's Signature   Date

CERTIFICATION of INFORMED CONSENT
I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise."

_____ _____
Printed Name of Person Obtaining Consent Role in Research Study

_____ _____
Signature of Person Obtaining Consent   Date

C.3   CONSENT FORM FOR CONTROL SUBJECTS

University of Pittsburgh   3459 Fifth Avenue

School of Medicine   Pittsburgh, PA 15213

Thomas E. Starzl Transplantation Institute   412-647-1458

CONSENT TO ACT AS PARTICIPANT IN A RESEARCH STUDY (CONTROL

SUBJECT)

TITLE: Short- and long-term evaluation of intestinal CYP3A4/5 and p-glycoprotein

function in small bowel transplant recipients

PRINCIPAL INVESTIGATOR:
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Co-Investigators:
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Why is this research being done?
The small intestine (also called the small bowel) is subjected to low temperature during transportation from the person who donates the small intestine (the donor) to the person who gets it (the recipient). When the small intestine is put inside the transplant recipient it is warmed up to normal body temperature and the recipient's blood begins to flow into it. These changes, combined with the effects of surgery and healing, cause the release of substances in the body that may temporarily change the way the small intestine absorbs oral medications. However, with time the body recovers from the surgery and the new intestine usually begins to function normally. The main objective of this study is to look at the time course of this recovery process by comparing how two drugs are absorbed immediately after the transplant surgery and at a later time after the transplant surgery. Another objective of this study is to examine the effects of certain genetic differences on oral drug absorption after small bowel transplantation. This information will then be used in the future to help health professionals such as your doctor determine the best dosing of drugs in small bowel transplant patients.

Who is being asked to take part in this research study?
You are being invited to take part in this research study to act as a control subject. In addition to small bowel transplant subjects, healthy men and women between the ages of 18 and 65 years of age are being asked to participate in this clinical study so that we can compare small bowel

transplant patients and healthy people of the same age, gender, and race. This study will take place at the University of Pittsburgh Medical Center, Pittsburgh, PA, and will include approximately 25 transplant subjects and 25 control subjects.

How will the study be done?
If you decide to participate in this study, you will undergo a total of two study sessions, each of which will require a separate visit to the University of Pittsburgh Medical Center (UPMC). The first study session is a screening visit that will take approximately 2 hours. The second study session involves drug administration and will last approximately 24 hours. At the second session you will be administered a single dose of midazolam (an FDA-approved sedative) once by mouth and once through a vein (blood vessel). You will also receive fexofenadine (also FDA-approved and marketed under the brand name Allegra® for treatment of allergy) one time by mouth.

First study session - Screening
If you have signed the informed consent form you will be eligible to participate in the first study session, a screening session. At this session, the investigators will decide if you are eligible to participate in the additional study session. This screening session will include a medical history, vital signs measurement, and clinical laboratory tests, including blood tests to evaluate liver and kidney function. An electrocardiogram (ECG) will be performed to check for problems with your heart rhythm. This first session will last approximately 2 hours and will take place in the Clinical and Translational Research Center (CTRC) at the University of Pittsburgh Medical Center (UPMC).

Second study session
The second study session will occur within approximately one month after the first (screening) study session. All study sessions will take place at the CTRC of UPMC. Before the second study session, you will be asked not to eat grapefruit or drink grapefruit juice, apple juice, cranberry juice, orange juice, or caffeine-containing drinks/foods for 3 days before the study session, because these foods may affect how certain drugs are taken up by the body. You will be asked not to drink liquids or eat any food after midnight on the day of the study, except small amounts of water as required to take medications. At the study session, you will receive a single dose of 5 milligrams (mg) of midazolam by mouth with a small amount of water. Sixty minutes later you will receive a dose of 60 mg of fexofenadine by mouth with a small amount of water. Seven hours after the oral dose of midazolam you will be given a single dose of 2 mg midazolam intravenously (into a blood vessel). You will be given breakfast two hours after the oral dose of midazolam, lunch one hour after the intravenous dose of midazolam, and dinner in the early evening of the study session day. Blood samples will be taken through a small tube inserted into a blood vessel in your forearm over a 20-hour period, starting immediately before the first dose of drug, for a total of 16 times. In addition, a 3 mL sample of blood will be taken to measure amounts of substances that are secreted by the body normally and are secreted in higher amounts after transplant surgery, before any drug is given. That blood sample will also be tested for the presence and type of two specific genetic markers. These two genes produce proteins that help the body metabolize (i.e. break down) and transport drugs that are taken by mouth. The total amount of blood removed at the second study session will be approximately 51 mL (approximately 3 tablespoonfuls plus one teaspoonful). If it has been greater than 30 days since your screening session, you will also have an additional 3 mL of blood drawn at this session for

labs to assess liver and kidney function. In addition, your urine will be collected over a 20-hour period during the study session, for measurement of study drugs and their metabolites in your urine. You will undergo blood pressure monitoring and pulse oximetry (a non-invasive test to measure the level of oxygen in your blood to make sure you are breathing adequately) continuously for the first two hours after each drug administration.

Experienced nursing staff will monitor your progress continually and your level of sedation will be assessed periodically during the session. If you vomit within 4 hours of taking either of the oral drugs you will be removed from the study, since full absorption of the dose may not have taken place. You will be asked to remain overnight in the CTRC in order to complete the blood and urine sampling and to make sure that both study drugs are gone from your body before you leave.

What are the possible risks, side effects, and discomforts of this research study?
There may be certain risks associated with participation in this study. These may include side effects of midazolam and/or fexofenadine (which are described below), the risks associated with giving you the intravenous midazolam, and risk associated with blood sampling for measuring drug levels. These include pain, bruising, bleeding, fainting, and risk of infection.

In addition, there are risks associated with genotyping. While the investigators will have procedures in place to ensure that all subject information is kept strictly confidential, there is the possibility that if genotype results became generally known, this information could have an impact on your future insurability, employability, reproductive plans, or could have a negative impact on family relationships, or could result in paternity suits or stigmatization.

The doses of midazolam that you will receive (5 mg orally and 2 mg intravenously) are relatively low but may be sedating (may cause drowsiness) in some people since midazolam is a sedative medication. These doses of midazolam have been used safely for research purposes with minimal side effects. However, these are the most common side effects of midazolam seen at a wide range of doses:

The following adverse reactions to midazolam have been reported after intramuscular (IM) or intravenous (IV) injection:

Decreased tidal volume and/or respiratory rate decrease (decrease in breathing rate and volume of breath) (23.3% of patients after IV and 10.8% of patients after IM injection), apnea (stopping breathing while asleep) (15.4% of patients after IV administration), as well as variations in blood pressure and pulse rate (frequency not listed).

In addition, the following adverse reactions were seen after IV injection of midazolam: hiccoughs (hiccups) (3.9%), vomiting (2.6%), coughing (1.3%), "oversedation" (1.6%), headache (1.5%), drowsiness (1.2%). Local effects at the injection site include: tenderness (5.6%), pain during injection (5.0%), redness (2.6%), induration (indentation of the skin) (1.7%), phlebitis (inflammation of blood vessel through which drug was administered) (0.4%).

Other very rare adverse effects occurring after midazolam administration in less than 1.0% in adults and children include:

Respiratory: laryngospasm (tightening or spasm of the throat or airway), bronchospasm (spasm or tightening of the lungs), dyspnea (shortness of breath), hyperventilation, wheezing, shallow respirations, airway obstruction, tachypnea (rapid breathing).

Cardiovascular: bigeminy (pulse and heart rate abnormality), premature ventricular contractions (type of heart arrhythmia), vasovagal episode (fainting due to drop in blood pressure and heart rate), bradycardia (slow heart rate), tachycardia (rapid heart rate), nodal rhythm (type of heart arrhythmia).

Gastrointestinal: acid taste, excessive salivation, retching,

CNS/neuromuscular: retrograde amnesia (temporary loss of memory), euphoria (elevated mood), hallucination, confusion, argumentativeness, nervousness, anxiety, grogginess, restlessness, emergence delirium or agitation, prolonged emergence from anesthesia, dreaming during emergence, sleep disturbance, insomnia, nightmares, athetoid movements (involuntary writhing movements), seizure-like activity, ataxia (unsteady gait), dizziness, dysphoria (unpleasant feelings), slurred speech, dysphonia (difficulty speaking), paresthesia ("pins and needles" tingling sensation).

Special Senses: blurred vision, diplopia (double vision), nystagmus (involuntary rapid movement of eyeballs), pinpoint pupils, cyclic movements of eyelids, visual disturbance, difficulty focusing eyes, ears blocked, loss of balance, light-headedness,

Integumentary: hive-like elevation at injection site, swelling or feeling of burning, warmth, or coldness at injection site,

Hypersensitivity: allergic reactions including anaphylactoid reactions, hives, rash, pruritus (itching).

Miscellaneous: yawning, lethargy (low energy), chills, weakness, toothache, faint feeling, hematoma (blood clot within a tissue).

Fexofenadine (Allegra®) is usually well tolerated and has a low incidence of side effects. However, possible side effects of fexofenadine (vs. placebo) from clinical trials for seasonal allergic rhinitis (hay fever) and chronic idiopathic urticaria (an allergic skin condition) include:
Common (Occurs in 10-25% of people): headache 10.6% (vs. 7.5%) and 7.2% (vs. 6.6%)
Infrequent (Occurs in 1-10% of people): Viral infection (cold, flu): 2.5% (vs. 1.5%), Nausea: 1.6% (vs. 1.5%), Dysmenorrhea (difficult menstrual periods): 1.5% (vs. 0.3%), Drowsiness: 1.3% (vs. 0.9%) and 2.2% (vs. 0.0%), Dyspepsia (upset stomach): 1.3% (vs. 0.6%), Fatigue: 1.3% (vs. 0.9%), Upper Respiratory Tract Infection: 3.2% (vs. 3.1%) and 4.3% (vs. 1.7%), Back Pain: 2.8% (vs. 1.4%) and 2.2% (vs. 1.1%), Accidental Injury: 2.9% (vs. 1.3%), Coughing: 3.8% (vs. 1.3%), Fever: 2.4% (vs. 0.9%), Pain: 2.4% (vs. 0.4%), Otitis Media (ear infection): 2.4% (vs. 0.0%), Sinusitis (sinus infection): 2.2% (vs. 1.1%), Dizziness: 2.2% (vs. 0.0%)
Rare (Occurs in less than 1% of people): none

Pregnancy
Midazolam is classified as Pregnancy Category D by the FDA. An increase in birth defects has been seen in infants born to mothers who used benzodiazepine drugs (class of drugs that midazolam belongs to) during pregnancy. Fexofenadine is classified as Pregnancy Category C by the FDA. This means that although animal studies have not shown the drug to cause birth defects, no adequate or well-controlled studies have been done in pregnant women to examine their effects on the fetus. In addition, midazolam is known to be excreted in breast milk. Therefore, being a part of this study while pregnant or breastfeeding may expose the unborn

child or nursing infant to risks known and unknown. Therefore, pregnant and nursing women will not be included in this study. If you are a woman of childbearing potential, a urine pregnancy test will be done during the screening visit and at each study session before administration of study drugs. It must be negative before you can enter this study. While enrolled in the study you must agree to use appropriate methods of birth control for the duration of the study as well as for one week after the last dose of study drug. Medically acceptable birth control methods include: (1) surgical sterilization, (2) approved hormonal contraceptives (such as birth control pills or patches), (3) double-barrier methods (such as a condom and diaphragm together) used with a spermicide, (4) an intrauterine device (IUD), or (5) abstinence.

You should not take part in this study if you plan to become pregnant within one month after completing this study, are currently pregnant, or you are currently breast feeding. You must notify your doctor if you suspect you have become pregnant while participating in this study.

What are possible benefits from taking part in this study?
There are no direct benefits to you from taking part in this study. However, the information learned from this study will be used in the future to help health professionals such as your doctor to determine the best dosing of drugs in small bowel transplant recipients after surgery. Therefore, your participation may help others in the future by what the doctors learn from your involvement in this study.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?
You have been informed previously that the personal results of this research study will be provided to you upon your request. However, you will be promptly notified if any other information about this research study develops during the course of the study which may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?
If you choose to participate in the study, study drugs, nursing care, and meals during the second study session will be provided free of charge. Measurement of midazolam and fexofenadine blood levels, clinical laboratory testing, and genetic testing will also be performed free of cost to you (your insurance will not be billed either). No compensation will be provided by makers of Versed® (midazolam) or makers of Allegra® (fexofenadine). This includes no financial support for lost wages, disability, pain or discomfort.

Will I be paid if I take part in this research study?
You will not be paid for taking part in the initial (screening) study session. However, you will be paid $125.00 upon completion of the second study session. Your biological sample or genetic material may lead, in the future, to new inventions or products. If the research investigators are able to develop new products from the use of your biological sample or genetic material, there are currently no plans to share with you any money or other rewards that may result from the development of the new product.

Who will pay if I am injured as a result of taking part in this study?

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Who will know about my participation in this research study?
Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. Only the investigators listed on the first page of this consent form will have access to the information linking your case number to your name, which will be stored in a locked cabinet in the Principal Investigator's office. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Will this research study involve the use or disclosure of my identifiable medical information?
This research study will involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g., physician office) records. The information that will be recorded will be limited to information concerning demographics (age, gender, and race) and concurrent conditions and medications you are receiving.
Your personal research results from this study will not be put in your medical record and you will not be identified in any publication of this research study. In addition, your written authorization will be obtained prior to providing personal research results to relatives, personal physicians, insurance companies, or any other third party.

Who will have access to identifiable information related to my participation in this research study?
In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

Authorized representatives of UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic

procedures) associated with research study participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

Authorized representatives of the U.S. Food and Drug Administration may review and/or obtain identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of monitoring the accuracy of the research data. While the U.S. Food and Drug Administration understands the importance of maintaining the confidentiality of your identifiable research and medical information, the University of Pittsburgh and UPMC cannot guarantee the confidentiality of this information after it has been obtained by the U.S. Food and Drug Administration.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?
If you agree to participate in the research project, use of your biological sample and genetic material will be under the control of the principal investigator of the research project. All the blood samples collected from you will be labeled using an identification number and with out your name. They will be stored and in the laboratory of the researchers until all the data is obtained from these samples. The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of 5 years and for as long (indefinite) as it may take to complete this research study.
The blood samples collected in this study will be kept for an indefinite time period until a complete report of the study has been published. The sample with out the identification may be utilized in future studies by the investigators. These samples will not be shared with any secondary investigators not listed on the current research study.

May I have access to my medical information that results from my participation in this research study?
In accordance with UPMC Notices of Privacy Practices document that you have been given, you are permitted access to information (including information resulting from your participation in this research study) contained within your medical records filed with your health care provider. You will be given the results of your genetic testing related to this study if you request it from the investigators. If you request this information from the investigators, you will be given it at the end of the research study in a private meeting with one or more of the investigators, and the information will be kept confidential. If you wish, the investigators will refer you to medical or genetic counseling at this time.

Is my participation in this research study voluntary?
Your participation in this research study is completely voluntary. You do not have to take part in

this research study and, should you change your mind, you can withdraw from the study at any time. Your current and future care at a UPMC facility and any other benefits for which you qualify will be the same whether you participate in this study or not.

Your doctor may be an investigator in this research study, and as an investigator, is interested both in your medical care and in the conduct of this research. Before entering this study or at any time during the research, you may discuss your care with another doctor who is in no way associated with this research project. You are not under any obligation to participate in any research study offered by your doctor.

May I withdraw, at a future date, my consent for participation in this research study?
You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research or medical information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above. Samples collected before your withdrawal from the study will be destroyed, if you so request.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

If you decide to withdraw your consent for participation in this research study it will have no effect on your current or future relationship with the University of Pittsburgh. If you decide to withdraw your consent for participation in this research study it will have no affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to take part in this research study, can I be removed from the study without my consent?
It is possible that you may be removed from the research study by the researchers if you do not follow all the instructions. You may be removed from the study if you experience unexpected side effects and in the opinion of the investigators that it is in your best interest. The study may also be stopped by the investigators or the sponsor if it felt that it is in the best interest of the patients.

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VOLUNTARY CONSENT
All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

I give my permission to use my biological samples or genetic material, with personal identifiers, in other research projects involving the study of small bowel transplantation.

Yes _____ No _____

I give my permission to be recontacted to obtain my consent if there is a desire to use my biological samples or genetic material, with personal identifiers, in other research projects involving the study of different diseases or conditions (i.e. diseases or conditions other than those specified in the Description section of this consent form).

Yes _____ No _____

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____
Participant's Signature   Date

CERTIFICATION of INFORMED CONSENT
I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise."

_____ _____
Printed Name of Person Obtaining Consent   Role in Research Study

_____ _____
Signature of Person Obtaining Consent   Date

C.4   ADDENDUM TO CONSENT FORM

University of Pittsburgh   3459 Fifth Avenue

School of Medicine   Pittsburgh, PA 15213

Thomas E. Starzl Transplantation Institute   412-647-1458

ADDENDUM

CONSENT TO ACT AS PARTICIPANT IN A RESEARCH STUDY

TITLE: Short- and long-term evaluation of intestinal CYP3A4/5 and p-glycoprotein

function in small bowel transplant recipients

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NEW INFORMATION:
You are currently a participant in a research study to evaluate the effects of small bowel
transplantation on oral drug absorption. This study includes a total of two pharmacokinetic study
sessions where study drugs are given and blood and urine samples are collected.

The original consent form you signed reads that we will take a total of sixteen 3-mL blood
samples from you at each study session, for a total of 48 mL of blood (approximately three
tablespoonfuls) at each session.

Review of early study results has shown that taking one additional blood sample at each study
session will improve estimation of peak concentrations of fexofenadine and tacrolimus.
Therefore, we request your permission to take seventeen blood samples during each study
session, for a total of 51 mL of blood (approximately three tablespoonfuls plus one teaspoonful)
at each session.

The risks associated with blood sampling for measuring drug levels include pain, bruising,
bleeding, fainting, and risk of infection.

There are no direct benefits to you from taking part in this study. However, the information
learned from this study will be used in the future to help health professionals such as your doctor
to determine the best dosing of drugs in small bowel transplant recipients after surgery.
Therefore, your participation may help others in the future by what the doctors learn from your
involvement in this study.

There is no change in the monetary compensation for study participation.

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

RIGHT TO WITHDRAW
You understand that can withdraw from this research study at any time. Your other care and benefits will be the same whether you participate in this research study or not. You also understand that you may be removed from this research study by the investigators in the event of a significant risk to your health.

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VOLUNTARY CONSENT

All of the above has been explained to me and all of my questions have been answered. I understand that, if not already done, I may request that my questions be answered by a physician involved in the research study. I also understand that any future questions I have about this research will be answered by the investigator(s) listed on the first page of this addendum to the consent document at the telephone number(s) listed. Any questions I have about my rights as a research subject will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668). By signing this form, I agree to continue to participate in this research study.

_____ _____
Patient/Subject Signature   **Date**

INVESTIGATOR'S CERTIFICATION
I certify that I have explained this new information and its significance to the above individual and that any questions about this information have been answered.

_____ _____
Investigator's Signature   **Date**

BIBLIOGRAPHY

Abbott (2010) Norvir prescribing information, Abbott Laboratories, North Chicago, IL.

Abete JF, Martin-Davila P, Moreno S, Quijano Y, de Vicente E and Pou L (2004) Pharmacokinetics of oral valganciclovir and intravenous ganciclovir administered to prevent cytomegalovirus disease in an adult patient receiving small-intestine transplantation. *Antimicrob Agents Chemother* **48**(7): 2782-2783.

Abu-Elmagd K, Todo S, Tzakis A, Furukawa H, Bonet H, Mohamed H, Nour B, Reyes J, Green M, Manez R, Pasculle W, Fung J and Starzl T (1994) Intestinal transplantation and bacterial overgrowth in humans. *Transplant Proc* **26**(3): 1684-1685.

Abu-Elmagd KM, Costa G, Bond GJ, Soltys K, Sindhi R, Wu T, Koritsky DA, Schuster B, Martin L, Cruz RJ, Murase N, Zeevi A, Irish W, Ayyash MO, Matarese L, Humar A and Mazariegos G (2009) Five hundred intestinal and multivisceral transplantations at a single center: major advances with new challenges. *Ann Surg* **250**(4): 567-581.

Aitken AE and Morgan ET (2007) Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. *Drug Metab Dispos* **35**(9): 1687-1693.

Amarri S, Masetti M, Benatti F, Callegari M, Morelli L, Villa E, Balli F, Jovine E and Pinna A (2002) Small intestine microflora after intestinal/multivisceral transplantation: preliminary results. *Transplant Proc* **34**: 953-954.

Amidon GL, Lennernas H, Shah VP and Crison JR (1995) A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res* **12**(3): 413-420.

Amundsen R, Åsberg A, Ohm IK and Christensen H (2011) Cyclosporine A and tacrolimus mediated inhibition of cytochrome P450 3A4 and 3A5 *in vitro*. *Drug Metab Dispos* **doi:10.1124/dmd.111.043018**.

Anapolsky A, Teng S, Dixit S and Piquette-Miller M (2006) The role of pregnane X receptor in 2-acetylaminofluorene-mediated induction of drug transport and -metabolizing enzymes in mice. *Drug Metab Dispos* **34**(3): 405-409.

Anglicheau D, Flamant M, Schlageter MH, Martinez F, Cassinat B, Beaune P, Legendre C and Thervet E (2003) Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. *Nephrology Dialysis Transplantation* **18**: 2409-2414.

APP (2003) Midazolam: prescribing information, American Pharmaceutical Partners, Inc., Schaumburg, IL.

Assenat E, Gerbal-Chaloin S, Larrey D, Saric J, Fabre J-M, Maurel P, Vilarem M-J and Pascussi JM (2004) Interleukin 1beta inhibits CAR-induced expression of hepatic genes involved in drug and bilirubin clearance. *Hepatology* **40**(4): 951-960.

Astellas (September 2011) Prograf prescribing information, Astellas Pharma US, Inc., Deerfield, IL.

Balimane P, Tamai I, Guo A, Nakanishi T, Kitada H, Leibach F, Tsuji A and Sinko P (1998) Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. *Biochem Biophys Res Commun* **250**(2): 246-251.

Barrett KE (2006) *Gastrointestinal physiology*. Lange Medical Books/McGraw-Hill, New York.

Beaumont K (2003) The importance of gut wall metabolism in determining drug bioavailability, in *Drug bioavailability: estimation of solubility, permeability, absorption and bioavailability* (van de Waterbeemd H, Lennernas H and Artursson P eds) pp 311-328, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Becquemont L, Glaeser H, Drescher S, Hitzl M, Simon N, Murdter TE, Heinkele G, Hofmann U, Schaefer C, Burk O, Verstuyft C, Eichelbaum M and Fromm MF (2006) Effects of ursodeoxycholic acid on p-glycoprotein and cytochrome P450 3A4-dependent pharmacokinetics in humans. *Clin Pharmacol Ther* **79**: 449-460.

Behrns KE, Sarr MG, Hanson RB and Zinsmeister AR (1996) Jejunoileal transplantation: effects on characteristics of canine jejunal motor activity *in vivo*. *Dig Dis Sci* **41**(5): 884-893.

Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C and Feingold KR (2002) Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response. *Biochem Biophys Res Commun* **293**: 145-149.

Belliard A-M, Lacour B, Farinotti R and Leroy C (2004) Effect of tumor necrosis factor-α and interferon-γ on intestinal p-glycoprotein expression, activity, and localization in Caco-2 cells. *J Pharm Sci* **93**: 1524-1536.

Belliard A-M, Tardivel S, Farinotti R, Lacour B and Leroy C (2002) Effect of hr-IL2 treatment on intestinal p-glycoprotein expression and activity in Caco-2 cells. *J Pharm Pharmacol* **54**: 1103-1109.

Benet LZ (2009) Predicting drug disposition via application of a biopharmaceutics drug disposition classification system. *Basic Clin Pharmacol Toxicol* **106**: 162-167.

Benet LZ, Amidon GL, Barends DM, Lennernas H, Polli JE, Shah VP, Stavchansky SA and Yu LX (2007) The use of BDDCS in classifying the permeability of marketed drugs. *Pharm Res* **52**(3): 483-488.

Benet LZ, Broccatelli F and Oprea TI (2011) BDDCS applied to over 900 drugs. *AAPS J* **13**(4): 519-547.

Berggren S, Gall C, Wollnitz N, Ekelund M, Karlbom U, Hoogstraate J, Schrenk D and Lennernas H (2006) Gene and protein expression of p-glycoprotein, MRP1, MRP2, and CYP3A4 in the small and large human intestine. *Mol Pharm* **4**(2): 252-257.

Berney T, Kato T, Nishida S, Tector AJ, Mittal NK, Madariaga J, Nery JR, Cantwell GP, Ruiz P and Tzakis AG (2002) Portal versus systemic drainage of small bowel allografts: comparative assessment of survival, function, rejection, and bacterial translocation. *J Am Coll Surg* **195**: 804-813.

Bertilsson G, Heidrich J, Svensson K, Åsman M, Jendeberg L, Sydow-Bäckman M, Ohlsson R, Postlind H, Blomquist P and Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for *CYP3A* induction. *Proc Natl Acad Sci U S A* **95**: 12208-12213.

Bland JM and Altman DG (1995) Calculating correlation coefficients with repeated observations: Part 1 - correlation within subjects. *BMJ* **310**: 446.

Blumberg RS and Stenson WF (2003) The immune system and gastrointestinal inflammation, in *Textbook of gastroenterology* (Yamada T, Alpers DH, Kaplowitz N, Laine L, Owyang C and Powell DW eds), Lippincott Williams & Wilkins, Philadelphia.

Buchman AL, Scolapio J and Fryer J (2003) AGA technical review on short bowel syndrome and intestinal transplantation. *Gastroenterology* **124**: 1111-1134.

Campbell WL, Abu-Elmagd K, Federle MP, Thaete FL, Furukawa H, Tzakis AG and Todo S (1993) Contrast examination of the small bowel in patients with small-bowel transplants: findings in 16 patients. *Am J Roentgenol* **161**: 969-974.

Canaparo R, Finnstrom N, Serpe L, Nordmark A, Muntoni E, Eandi M, Rane A and Zara GP (2007) Expression of CYP3A isoforms and p-glycoprotein in human stomach, jejunum and ileum. *Clin Exp Pharmacol Physiol* **34**: 1138-1144.

Cavicchi M, Beau P, Crenn P, Degott C and Messing B (2000) Prevalence of liver disease and contributing factors in patients receiving home parenteral nutrition for permanent intestinal failure. *Ann Intern Med* **132**: 525-532.

Cervera C, Fillela X, Linares L, Pineda M, Esteva C, Anton A, Marcos M, Cofan F, Navasa M, Perez-Villa F, Pumarola T and Moreno A (2007) Th1/Th2 cytokine release pattern during in vivo cytomegalovirus disease in solid organ transplantation. *Transplant Proc* **39**: 2233-2235.

Chalasani N, Gorski C, Patel NH, Hall SD and Galinsky RE (2001) Hepatic and intestinal cytochrome P450 3A activity in cirrhosis: effects of transjugular intrahepatic portosystemic shunts. *Hepatology* **34**: 1103-1108.

Chaobal HN and Kharasch ED (2005) Single-point sampling for assessment of constitutive, induced, and inhibited cytochrome P450 3A activity with alfentanil or midazolam. *Clin Pharmacol Ther* **78**: 529-539.

Chen C (2007) Some pharmacokinetic aspects of the lipophilic terfenadine and zwitterionic fexofenadine in humans. *Drugs in R&D* **8**(5): 301-314.

Choudhuri S and Klaasen CD (2006) Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. *Int J Toxicol* **25**: 231-259.

Choudry S, Bonner J, Capitano B, Abu-Elmagd K, Bond G, Costa G, Koritsky D, Martin D and Venkataramanan R (2007) Pharmacokinetics of orally administered voriconazole in small bowel and multivisceral transplant patients, in *IXth International Small Bowel Transplant Symposium*, Los Angeles, CA.

Christians U, Schmitz V and Haschke M (2005) Functional interactions between p-glycoprotein and CYP3A in drug metabolism. *Expert Opin Drug Metab Toxicol* **1**(4): 641-654.

Cruz Jr RJ, Costa G, Bond G, Soltys K, Stein WC, Wu G, Martin L, Koritsky D, McMichael J, Sindhi R, Mazariegos G and Abu-Elmagd KM (2010) Modified "liver-sparing" multivisceral transplant with preserved native spleen, pancreas, and duodenum: technique and long-term outcome. *J Gastrointest Surg* **14**: 1709-1721.

Cruz Jr RJ, Costa G, Bond GJ, Soltys K, Rubin E, Humar A and Abu-Elmagd KM (2011) Modified multivisceral transplantation with spleen-preserving pancreaticoduodenectomy for patients with familial adenomatous polyposis "Gardner's syndrome". *Transplantation* **91**(12): 1417-1423.

Cvetkovic M, Leake B, Fromm MF, Wilkinson GR and Kim RB (1999) OATP and p-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos* **27**(8): 866-871.

Dahan A and Amidon GL (2009) Segmental dependent transport of low permeability compounds along the small intestine due to p-glycoprotein: the role of efflux transport in the oral absorption of BCS class III drugs. *Mol Pharm* **6**(1): 19-28.

Daniel V, Naujokat C, Sadeghi M, Wiesel M, Hergesell O and Opelz G (2005) Association of circulating interleukin (IL)-12- and IL-10-producing dendritic cells with time posttransplant, dose of immunosuppression, and plasma cytokines in renal-transplant recipients. *Transplantation* **79**(11): 1498-1506.

Dansirikul C, Staatz CE, Duffull SB, Taylor PJ, Lynch SV and Tett SE (2004) Sampling times for monitoring tacrolimus in stable adult liver transplant recipients. *Ther Drug Monit* **26**(6): 593-599.

Davis S, Hardy J and Fara J (1986) Transit of pharmaceutical dosage forms through the small intestine. *Gut* **27**: 886-892.

de Jonge H, de Loor H, Verbeke K, Vanrenterghem Y and Kuypers D (2011) *In vivo* CYP3A activity is significantly lower in cyclosporine-treated as compared with tacrolimus-treated renal allograft recipients. *Clin Pharmacol Ther* **90**(3): 414-422.

Dowling TC, Briglia AE, Fink JC, Hanes DS, Light PD, Stackiewicz L, Karyekar CS, Eddington ND, Weir MR and Henrich WL (2003) Characterization of hepatic cytochrome P4503A activity in patients with end-stage renal disease. *Clin Pharmacol Ther* **73**: 427-434.

Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ and Kim RB (2002) Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* **71**: 11-20.

Eap CB, Buclin T, Hustert E, Bleiber G, Golay KP, Aubert A-C, Baumann P, Telenti A and Kerb R (2004) Pharmacokinetics of midazolam in CYP3A4- and CYP3A5-genotyped subjects. *Eur J Clin Pharmacol* **60**: 231-236.

El-Sankary W, Plant NJ, Gibson GG and Moore DJ (2000) Regulation of the CYP3A4 gene by hydrocortisone and xenobiotics: role of the glucocorticoid and pregnane X receptors. *Drug Metab Dispos* **28**(5): 493-496.

FDA (2000) Guidance for industry: waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system, Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, MD.

Finel M, Gardner-Stephen DA, Bratton S, Mackenzie PI and Radominska-Pandya A (2005) Human UDP-glucuronosyltransferase 1A5: identification, expression, and activity. *J Pharmacol Exp Ther* **315**(3): 1143-1149.

Fishbein TM (2009) Intestinal transplantation. *N Engl J Med* **361**(10): 998-1008.

Fisher CD, Lickteig AJ, Augustine LM, Ranger-Moore J, Jackson JP, Ferguson SS and Cherrington NJ (2009) Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease. *Drug Metab Disp* **37**(10): 2087-2094.

Foster DR, Gonzales JP, Amidon GL and Welage LS (2009) Intestinal dipeptide absorption is preserved during thermal injury and cytokine treatment. *JPEN J Parenter Enteral Nutr* **33**: 520-528.

Frye RF, Schneider VM, Frye CS and Feldman AM (2002) Plasma levels of TNF-α and IL-6 are inversely related to cytochrome P450-dependent drug metabolism in patients with congestive heart failure. *J Card Fail* **8**(5): 315-319.

Fujishiro J, Pech T, Finger T, Praktinjo M, Stoffels B, Standop J, Abu-Elmagd K, Tuerler A, Hirner A, Kalff J and Schaefer N (2010) Influence of immunosuppression on alloresponse, inflammation and contractile function of graft after intestinal transplantation. *Am J Transplant* **10**: 1545-1555.

Furness JB, Nguyen TV, Nurgali K and Shimizu Y (2009) The enteric nervous system and its extrinsic connections, in *Textbook of Gastroenterology* (Yamada T ed) pp 15-39, Wiley-Blackwell, Chichester, West Sussex, UK.

Furukawa H, Brown M, Abu-Elmagd K, Hutson W, Campbell W, Reyes J, Tzakis A, Todo S and Starzl T (1994) Abnormal gastric emptying after intestinal transplantation. *Transplant Proc* **26**(3): 1634.

Furuta S, Kamada E, Suzuki T, Sugimoto T, Kawabata Y, Shinozaki Y and Sano H (2001) Inhibition of drug metabolism in human liver microsomes by nizatidine, cimetidine and omeprazole. *Xenobiotica* **31**(1): 1-10.

Giacomini KM, Huang S-M, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren K, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ and Zhang L (2010) Membrane transporters in drug development. *Nat Rev Drug Discov* **9**: 215-236.

Glaeser H, Bailey D, Dresser G, Gregor J, Schwarz U, McGrath J, Jolicoeur E, Lee W, Leake B, Tirona R and Kim R (2007) Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin Pharmacol Ther* **81**(3): 362-370.

Granero GE, Ramachandran C and Amidon GL (2003) Gastrointestinal dissolution and absorption of drugs, in *Drug bioavailability: estimation of solubility, permeability, absorption, and bioavailability* (van de Waterbeemd H, Lennernas H and Artursson P eds), WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Gregory PA, Lewinsky RH, Gardner-Stephen DA and Mackenzie PI (2004) Regulation of UDP-glucuronosyltransferases in the gastrointestinal tract. *Toxicol Appl Pharmacol* **199**: 354-363.

Grootjans J, Lenaerts K, Derikx JP, Matthijsen RA, de Bruine AP, van Bijnen AA, van Dam RM, Dejong CH and Buurman WA (2010) Human intestinal ischemia-reperfusion-induced inflammation characterized. *Am J Pathol* **176**(5): 2283-2291.

Gunness P, Aleska K and Koren G (2011) Acyclovir is a substrate for the human breast cancer resistance protein (BCRP/ABCG2): implications for renal tubular transport and acyclovir-induced nephrotoxicity. *Can J Physiol Pharmacol* **89**(9): 675-680.

Hagenbuch B and Gui C (2008) Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica* **38**(7-8): 778-801.

Hartman AJ, Lough DM, Barupal DK, Fiehn O, Fishbein T, Zasloff M and Eisen JA (2009) Human gut microbiome adopts an alternative state following small bowel transplantation. *Proc Natl Acad Sci U S A* **106**(40): 17187-17192.

Haslam I, Wright J, O'Reilly D, Sherlock D, Coleman T and Simmons N (2011) Intestinal ciprofloxacin efflux: the role of breast cancer resistance protein BCRP (ABCG2). *Drug Metab Dispos*.

Hasler WL (2009a) Motility of the small intestine and colon, in *Textbook of Gastroenterology* (Yamada T ed) pp 231-263, Wiley-Blackwell, Chichester, West Sussex, UK.

Hasler WL (2009b) The physiology of gastric motility and gastric emptying, in *Textbook of Gastroenterology* (Yamada T ed) pp 207-230, Wiley-Blackwell, Chichester, West Sussex, UK.

He P, Court MH, Greenblatt DJ and von Moltke LL (2006) Factors influencing midazolam hydroxylation activity in human liver microsomes. *Drug Metab Dispos* **34**(7): 1198-1207.

Healy J, Borley NR and Wigley C (2005) Jejunum and ileum, in *Gray's anatomy: the anatomical basis of clinical practice* (Standring S, Ellis H, Healy J, Johnson D and Williams A eds) pp 1167-1172, Elsevier, Edinburgh.

Imanaga J, Kotegawa T, Imai H, Tsutsumi K, Yoshizato T, Ohyama T, Shirasaka Y, Tamai I, Tateishi T and Ohashi K (2011) The effects of the *SLO2B1* c.1457C>T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenet Genomics* **21**: 84-93.

Ishikawa T, Iwanami K, Okuda T, Zhu Y, Fukuda A, Zhang S, Ou J, Nalesnik MA, Venkataramanan R and Murase N (2003) Morphology and function of canine small intestinal autografts: with particular interest in the influence of ex vivo graft irradiation. *J Gastrointest Surg* **7**: 662-671.

Iwanami K, Ishikawa T, Nalesnik MA, Okuda T, Neto JS, Zhu Y, Turler A, Moore BA, Bauer AJ, Venkataramanan R and Murase N (2003) Long-term function and morphology of intestinal autografts and allografts in outbred dogs. *Am J Transplant* **3**: 1083-1090.

Jain A, Venkataramanan R, Lever J, Warty V, Abu-Elmagd K, Furukawa H, Reyes J, Nour B, Asrian A, Tzakis A, Todo S, Fung J and Starzl T (1994) FK 506 in small bowel transplant recipients: pharmacokinetics and dosing. *Transplant Proc* **26**(3): 1609-1610.

Jain A, Venkataramanan R, Todo S, Abu-Elmagd K, Fung J, Warty V, Tzakis A, Reyes J, Alessiani M and Starzl T (1992) Intravenous, oral pharmacokinetics, and oral dosing of FK 506 in small bowel transplant patients. *Transplant Proc* **24**(3): 1181-1182.

Jaisue S, Gerber J and Davey A (2010) Pharmacokinetics of fexofenadine following LPS administration to rats. *Xenobiotica* **40**(11): 743-750.

Kadry Z, Roux-Lombard P, Dayer J-M, Lieberman J, Marks W, Dafoe D, Cretin N, Cighetti G, Buhler L, Charbonnet P and Morel P (2000) Impact of intestinal ischemia-reperfusion on cytokine profile and enterocyte viability in human syngeneic intestinal transplantation. *Transplant Proc* **32**: 1292-1293.

Kahn E and Daum F (2010) Anatomy, histology, embryology, and developmental anomalies of the small and large intestine, in *Sleisenger and Fordtran's gastrointestinal and liver disease* (Feldman M, Friedman LS and Brandt LJ eds) pp 1615-1642, Saunders Elsevier, Philadelphia, PA.

Kalliokoski A and Niemi M (2009) Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* **158**: 693-705.

Kapelyukh Y, Paine MJ, Marechal J-D, Sutcliffe MJ, Wolf CR and Roberts GC (2008) Multiple substrate binding by cytochrome P450 3A4: estimation of the number of bound substrate molecules. *Drug Metab Dispos* **36**: 2136-2144.

Kaplan B, Lown K, Craig R, Abecassis M, Kaufman D, Leventhal J, Stuart F, Meier-Kriesche H-U and Fryer J (1999) Low bioavailability of cyclosporine microemulsion and tacrolimus in a small bowel transplant recipient: possible relationship to intestinal p-glycoprotein activity. *Transplantation* **67**(2): 333-335.

Kasparek M, Fatima J, Iqbal C, Duenes J and Sarr M (2008) Effect of chronic, extrinsic denervation on functional NANC innervation with vasoactive intestinal polypeptide and substance P in longitudinal muscle of rat jejunum. *Neurogastroenterol Motil* **20**: 243-252.

Kato R, Yamashita S, Moriguchi J, Nakagawa M, Tsukura Y, Uchida K, Amano F, Hirotani Y, Ijiri Y and Tanaka K (2008) Changes of midazolam pharmacokinetics in Wistar rats treated with lipopolysaccharide: relationship between total CYP and CYP3A2. *Innate Immunity* **14**(5): 291-297.

Katsakiori PF, Papapetrou EP, Goumenos DS, Nikiforidis GC and Flordellis CS (2010) Investigation of clinical interaction between omeprazole and tacrolimus in CYP3A5 non-expressors, renal transplant recipients. *Ther Clin Risk Manag* **6**: 265-269.

Katzenmaier S, Markert C and Mikus G (2010) Proposal of a new limited sampling strategy to predict CYP3A activity using a partial AUC of midazolam. *Eur J Clin Pharmacol* **66**(11): 1137-1141.

Kaufman SS, Lyden ER, Brown CR, Iverson AK, Davis CK, Sudan DL, Fox IJ, Horslen SP, Shaw BW and Langnas A (2000) Disaccharidase activities and fat assimilation in pediatric patients after intestinal transplantation. *Transplantation* **69**(3): 362-365.

Khan KK, He YQ, Domanski TL and Halpert JR (2002) Midazolam oxidation by cytochrome P450 3A4 and active-site mutants: an evaluation of multiple binding sites and of the metabolic pathway that leads to enzyme inactivation. *Mol Pharmacol* **61**(3): 495-506.

Kharasch E, Walker A, Isoherranen N, Hoffer C, Sheffels P, Thummel K, Whittington D and Ensign D (2007) Influence of CYP3A5 genotype on the pharmacokinetics and pharmacodynamics of the cytochrome P4503A probes alfentanil and midazolam. *Clin Pharmacol Ther* **82**(4): 410-426.

Kharasch ED, Walker A, Hoffer C and Sheffels P (2005) Evaluation of first-pass cytochrome P4503A (CYP3A) and p-glycoprotein activities using alfentanil and fexofenadine in combination. *J Clin Pharmacol* **45**: 79-88.

Kiang TK, Ensom MH and Chang TK (2005) UDP-glucuronosyltransferases and clinical drug-drug interactions. *Pharmacol Ther* **106**: 97-132.

Kirby B, Kharasch ED, Thummel KE, Narang VS, Hoffer CJ and Unadkat JD (2006) Simultaneous measurement of in vivo p-glycoprotein and cytochrome P450 3A activities. *J Clin Pharmacol* **46**: 1313-1319.

Kirwan CJ, Lee T, Holt DW, Grounds RM, MacPhee IA and Phillips BJ (2009) Using midazolam to monitor changes in hepatic drug metabolism in critically ill patients. *Intensive Care Med* **35**(7): 1271-1275.

Kita Y, Iwaki Y, Demetris AJ and Starzl TE (1994) Evaluation of sequential serum interleukin-6 levels in liver allograft recipients. *Transplantation* **57**(7): 1037-1041.

Kita Y, Iwaki Y, Noguchi K, Griffith B, Tzakis A, Todo S, Fung J and Starzl T (1996a) Daily serum interleukin-6 monitoring in multiple organ transplantation with or without liver allografts. *Transplant Proc* **28**(3): 1229-1234.

Kita Y, Iwaki Y, Noguchi K, Tzakis A, Todo S, Fung J and Starzl T (1996b) Daily serum inflammatory cytokine (tumor necrosis factor-α, interleukin-6) monitoring in liver transplantation focusing on allograft rejection: a five-case report. *Transplant Proc* **28**(3): 1237-1240.

Klaasen CD and Aleksunes LM (2010) Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* **62**(1): 1-96.

Klieber S, Hugla S, Ngo R, Arabeyre-Fabre C, Meunier V, Sadoun F, Fedeli O, Rival M, Bourrie M, Guillou F, Maurel P and Fabre G (2008) Contribution of the N-glucuronidation pathway to the overall in vitro metabolic clearance of midazolam in humans. *Drug Metab Dispos* **36**(5): 851-862.

Kocoshis SA, Reyes J, Todo S and Starzl TE (1997) Small intestinal transplantation for irreversible intestinal failure in children. *Dig Dis Sci* **42**(10): 1997-2008.

Konishi H, Sumi M, Shibata N, Takada K, Minouchi T and Yamaji A (2004) Decrease in oral bioavailability of ciclosporin by intravenous pulse of methylprednisolone succinate in rats. *J Pharm Pharmacol* **56**: 1259-1266.

Krishnamurthy P and Schuetz J (2006) Role of ABCG2/BCRP in biology and medicine. *Annu Rev Pharmacol Toxicol* **46**: 381-410.

Kubala L, Číž M, Vondráček J, Čížová H, Černý J, Němec P, Studenik P, Dušková M and Lojeck A (2001) Peri- and post-operative course of cytokines and the metabolic activity of neutrophils in human liver transplantation. *Cytokine* **16**(3): 97-101.

Kuebler JF, Toth B, Rue III LW, Bland KI and Chaudry IH (2003) Differential alterations in intestinal permeability after trauma-hemorrhage. *J Surg Res* **112**: 198-204.

Kurosawa S, Uchida S, Ito Y and Yamada S (2009) Effect of ursodeoxycholic acid on the pharmacokinetics of midazolam and CYP3A in the liver and intestine of rats. *Xenobiotica* **39**(2): 162-170.

Lampen A, Christians U, Guengerich FP, Watkins PB, Kolars JC, Bader A, Gonschior A-K, Dralle H, Hackbarth I and Sewing K-F (1995) Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variability. *Drug Metab Dispos* **23**(12): 1315-1324.

Lappin G, Shishikura Y, Jochemsen R, Weaver RJ, Gesson C, Houston B, Oosterhuis B, Bjerrum OJ, Rowland M and Garner C (2010) Pharmacokinetics of fexofenadine: evaluation of a microdose and assessment of absolute oral bioavailability. *Eur J Pharm Sci* **40**: 125-141.

Lauzurica R, Pastor M, Bayes B, Malumbres S, Homs M, Llopis M, Bonet J and Romero R (2007) Subclinical inflammation in renal transplant recipients: impact of cyclosporine microemulsion versus tacrolimus. *Transplant Proc* **39**: 2170-2172.

Le Vee M, Gripon P, Stieger B and Fardel O (2008) Down-regulation of organic anion transporter expression in human hepatocytes exposed to the proinflammatory cytokine interleukin 1beta. *Drug Metab Dispos* **36**(2): 217-222.

Lee J-I, Chaves-Gnecco D, Amico JA, Kroboth PD, Wilson JW and Frye RF (2002) Application of semisimultaneous midazolam administration for hepatic and intestinal cytochrome P450 3A phenotyping. *Clin Pharmacol Ther* **60**: 14-24.

Lee LS, Bertino Jr JS and Nafziger AN (2006) Limited sampling models for oral midazolam: midazolam plasma concentrations, not the ratio of 1-hydroxymidazolam to midazolam plasma concentrations, accurately predicts AUC as a biomarker of CYP3A activity. *J Clin Pharmacol* **46**: 229-234.

Lehmann J, McKee DD, Watson MA, Willson TM, Moore JT and Kliewer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate *CYP3A4* gene expression and cause drug interactions. *J Clin Invest* **102**(5): 1016-1023.

Lennernas H (1998) Human intestinal permeability. *J Pharm Sci* **87**(4): 403-410.

Lennernas H and Abrahamsson B (2005) The use of biopharmaceutic classification of drugs in drug discovery and development: current status and future extension. *J Pharm Pharmacol* **57**: 273-285.

Li X-Q, Andersson TB, Ahlstrom M and Weidof L (2004) Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos* **32**(8): 821-827.

Link B, Haschke M, Grignaschi N, Bodmer M, Aschmann YZ, Wenk M and Krahenbuhl S (2008) Pharmacokinetics of intravenous and oral midazolam in plasma and saliva in humans: usefulness of saliva as matrix for CYP3A phenotyping. *Br J Clin Pharmacol* **66**(4): 473-484.

Lipinski CA, Lombardo F, Dominy BW and Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* **46**: 3-26.

Lown KS, Bailey DG, Fontana RJ, Janardan S, Adair CH, Fortlage LA, Brown MB, Guo W and Watkins PB (1997) Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* **99**(10): 2545-2553.

Luke DR and Foulds G (1997) Disposition of oral azithromycin in humans. *Clin Pharmacol Ther* **61**: 641-648.

Ma JD, Tsunoda SM, Bertino Jr JS, Trivedi M, Beale K and Nafziger AN (2010) Evaluation of *in vivo* p-glycoprotein phenotyping probes. *Clin Pharmacokinet* **49**(4): 223-237.

MacLean C, Moenning U, Reichel A and Fricker G (2010) Regional absorption of fexofenadine in rat intestine. *Eur J Pharm Sci* **41**: 670-674.

Masuda S, Uemoto S, Goto M, Fujimoto Y, Tanaka K and Inui K-I (2004) Tacrolimus therapy according to mucosal MDR1 levels in small-bowel transplant recipients. *Clin Pharmacol Ther* **75**: 352-361.

Matarese LE, Dvorchik I, Costa G, Bond GJ, Koritsky DA, Ferraris RP, Touger-Decker R, O'Sullivan-Maillet JK and Abu-Elmagd KM (2009) Pyridoxal-5'-phosphate deficiencey after intestinal and multivisceral transplantation. *Am J Clin Nutr* **89**: 204-209.

Mathur A, Baz M, Staples ED, Bonnell M, Speckman JM, Hess PJ, Klodell CT, Knauf DG, Moldawer LL and Beaver TM (2006) Cytokine profile after lung transplantation: correlation with allograft injury. *Ann Thorac Surg* **81**: 1844-1850.

Matsushima S, Maeda K, Hayashi H, Debori Y, Schinkel AH, Schuetz JD, Kusuhara H and Sugiyama Y (2008) Involvement of multiple efflux transporters in hepatic disposition of fexofenadine. *Mol Pharmacol* **73**(5): 1474-1483.

McConnell EL, Fadda HM and Basit AW (2008) Gut instincts: explorations in intestinal physiology and drug delivery. *Int J Pharm* **364**: 213-226.

Meier D, Cagnola H, Ramisch D, Rumbo C, Chirdo F, Docena G, Gondolesi G and Rumbo M (2010) Analysis of immune cells draining from the abdominal cavity as a novel tool to study intestinal transplant immunobiology. *Clin Exp Immunol* **162**: 138-145.

Meier Y, Eloranta JJ, Darimont J, Ismair MG, Hiller C, Fried M, Kullak-Ublick GA and Vavricka SR (2007) Regional distribution of solute carrier mRNA expression along the human intestinal tract. *Drug Metab Dispos* **35**(4): 590-594.

Miao J, Jin Y, Marunde R, Kim S, Quinney S, Radovich M, Li L and Hall S (2009) Association of genotypes of the CYP3A cluster with midazolam disposition *in vivo*. *Pharmacogenomics J* **9**(5): 319-326.

Miura M, Inoue K, Kagaya H, Satoh S, Tada H, Sagae Y, Habuchi T and Suzuki T (2007a) Influence of rabeprazole and lansoprazole on the pharmacokinetics of tacrolimus in relation to CYP2C19, CYP3A5 and MDR1 polymorphisms in renal transplant recipients. *Biopharm Drug Dispos* **28**: 167-175.

Miura M, Uno T, Tateishi T and Suzuki T (2007b) Pharmacokinetics of fexofenadine enantiomers in healthy subjects. *Chirality* **19**: 223-227.

Morini S, Elias G, Brown M, Subbotin V, Rastellini C and Cicalese L (2010) Chronic morpho-functional damage as a consequence of transient ischemia/reperfusion injury of the small bowel. *Histol Histopathol* **25**: 277-286.

Mouly SJ, Matheny C, Paine MF, Smith G, Lamba J, Lamba V, Pusek SN, Schuetz EG, Stewart PW and Watkins PB (2005) Variation in oral clearance of saquinavir is predicted by *CYP3A5*1* genotype but not by enterocyte content of cytochrome P450 3A5. *Clin Pharmacol Ther* **78**: 605-618.

Mousa H, Bueno J, Griffiths J, Kocoshis S, Todo S, Reyes J and Di Lorenzo C (1998) Intestinal motility after small bowel transplantation. *Transplant Proc* **30**: 2535-2536.

Nagpal K, Minocha VR, Agrawal V and Kapur S (2006) Evaluation of intestinal mucosal permeability function in patients with acute pancreatitis. *Am J Surg* **192**: 24-28.

Nakada K, Ikoma A, Suzuki T, Reynolds J, Campbell W, Todo S and Starzl T (1994) Long-term effect of jejunoileal autotransplantation on motility and transit time in canine small bowel. *Transplant Proc* **26**(3): 1635-1636.

Narang VS, Fraga C, Kumar N, Shen J, Throm S, Stewart CF and Waters CM (2008) Dexamethasone increases expression and activity of multidrug resistance transporters at the rat blood-brain barrier. *Am J Physiol Cell Physiol* **295**: C440-C450.

Noguchi K, Yoshida Y, Yagihashi A, Kita Y, Takenaka T, Terasawa K, Hayashi S, Konno A, Kobayashi M, Nakamura K, Reyes J, Abu-Elmagd K, Demetris A, Tzakis A, Todo S, Iwaki Y and Starzl T (1992) Serum levels of interleukin-6, tumor necrosis factor-alpha, and interleukin-2 in rejecting human small bowel allografts. *Transplant Proc* **24**(3): 1152.

Novartis (2009) Neoral prescribing information, Novartis Pharmaceuticals Corporation, East Hanover, NJ.

O'Keefe SJ, Buchman AL, Fishbein TM, Jeejeebhoy K, Jeppesen PB and Schaffer J (2006) Short bowel syndrome and intestinal failure: consensus definitions and overview. *Clin Gastroenterol Hepatol* **4**: 6-10.

O'Keefe SJ, Burnes JU and Thompson RL (1994) Recurrent sepsis in home parenteral nutrition patients: an analysis of risk factors. *JPEN J Parenter Enteral Nutr* **18**(3): 256-263.

O'Keefe SJ, Emerling M, Koritsky D, Martin D, Stamos J, Kandil H, Matarese L, Bond G and Abu-Elmagd K (2007) Nutrition and quality of life following small intestinal transplantation. *Am J Gastroenterol* **102**: 1093-1100.

Ogihara T, Kano T, Wagatsuma T, Wada S, Yabuuchi H, Enomoto S, Morimoto K, Shirasaka Y, Kobayashi S and Tamai I (2009) Oseltamivir (tamiflu) is a substrate of peptide transporter 1. *Drug Metab Dispos* **37**(8): 1676-1681.

Ogle CK, Mao J-X, Wu J-Z, Ogle JD and Alexander JW (1994) The 1994 Lindberg Award. The production of tumor necrosis factor, interleukin-1, interleukin-6, and prostaglandin E_2 by isolated enterocytes and gut macrophages: effect of lipopolysaccharide and thermal injury. *J Burn Care Rehabil* **15**: 470-477.

Ogura J, Kobayashi M, Itagaki S, Hirano T and Iseki K (2008) Alteration of Mrp2 and P-gp expression, including expression in remote organs, after intestinal ischemia-reperfusion. *Life Sci* **82**: 1242-1248.

Paine MF, Hart HL, Ludington SS, Haining RL, Rettie AE and Zeldin DC (2006) The human intestinal cytochrome P450 "pie". *Drug Metab Dispos* **34**(5): 880-886.

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD and Thummel KE (1997) Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* **283**(3): 1552-1562.

Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, Barr DM, Gillies BS and Thummel KE (1996) First-pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther* **60**: 14-24.

Pascussi JM, Gerbal-Chaloin S, Fabre J-M, Maurel P and Vilarem M-J (2000) Dexamethasone enhances constitutive androstane receptor expression in human hepatocytes: consequences on cytochrome P450 regulation. *Mol Pharmacol* **58**(6): 1441-1450.

Pentikainen P, Valisalmi L, Himberg J and Crevoisier C (1989) Pharmacokinetics of midazolam following intravenous and oral administration in patients with chronic liver disease and in healthy subjects. *J Clin Pharmacol* **29**: 272-277.

Peters WH, Roelofs HM, Nagengast FM and van Tongeren JH (1989) Human intestinal glutathione S-transferases. *Biochem J* **257**: 471-476.

Petri N, Tannergren C, Rungstad D and Lennernas H (2004) Transport characteristics of fexofenadine in the Caco-2 cell model. *Pharm Res* **21**(8): 1398-1404.

Pichard L, Fabre I, Daujat M, Domergue J, Joyeux H and Maurel P (1992) Effect of corticosteroids on the expression of cytochromes P450 and on cyclosporin A oxidase activity in primary cultures of human hepatocytes. *Mol Pharmacol* **41**: 1047-1055.

Porter CJ and Charman WN (2001) Intestinal lymphatic drug transport: an update. *Advanced Drug Delivery Reviews* **50**(1-2): 61-80.

Pratt SL, Ogle CK, Mao J-X, Zhao W, Lovell G and Horseman ND (2000) Interleukin-6 signal transduction in human intestinal epithelial cells. *Shock* **13**(6): 435-440.

Reynolds DP, Lanevskij K, Japertas P, Didziapetris R and Petrauskas A (2009) Ionization-specific analysis of human intestinal absorption. *J Pharm Sci* **98**(11): 4039-4054.

Rivory L, Slaviero K and Clarke S (2002) Hepatic cytochrome P450 3A drug metabolism is reduced in cancer patients who have an acute-phase response. *Br J Cancer* **87**: 277-280.

Robbins DK, Castles MA, Pack DJ, Bhargava VO and Weir SJ (1998) Dose proportionality and comparison of single and multiple dose pharmacokinetics of fexofenadine (MDL 16455) and its enantiomers in healthy male volunteers. *Biopharm Drug Dispos* **19**: 455-463.

Roche (2003) Fortovase prescribing information, Roche Laboratories Inc., Nutley, NJ.

Rogers JF, Nafziger AN, Kashuba AD, Streetman DS, Rocci Jr. ML, Choo EF, Wilkinson GR and Bertino Jr JS (2002) Single plasma concentrations of 1'hydroxymidazolam or the ratio of 1'hydroxymidazolam:midazolam do not predict midazolam clearance in healthy subjects. *J Clin Pharmacol* **42**: 1079-1082.

Rosner B (2006) *Fundamentals of biostatistics*. Thomson Brooks/Cole, Belmont, CA.

Rovera G, Strohm S, Bueno J, Kocoshis S, Abu-Elmagd K, Todu S and Reyes J (1998) Nutritional monitoring of pediatric intestinal transplant recipients. *Transplant Proc* **30**(6): 2519-2520.

Rubin DC (2009) Small intestine: anatomy and structural anomalies, in *Textbook of Gastroenterology* (Yamada T ed) pp 1085-1107, Wiley-Blackwell, Chichester, West Sussex, UK.

Russell T, Stoltz M and Weir S (1998) Pharmacokinetics, pharmacodynamics, and tolerance of single- and multiple-dose fexofenadine hydrochloride in healthy male volunteers. *Clin Pharmacol Ther* **64**: 612-621.

Sadeghi M, Daniel V, Weimer R, Wiesel M, Hergesell O and Opelz G (2003a) Differential early posttransplant cytokine responses in living and cadaver donor renal allografts. *Transplantation* **75**(8): 1351-1355.

Sadeghi M, Daniel V, Weimer R, Wiesel M, Hergesell O and Opelz G (2003b) Pre-transplant Th1 and post-transplant Th2 cytokine patterns are associated with early acute rejection in renal transplant recipients. *Clin Transplant* **17**: 151-157.

sanofi-aventis (2007) Allegra [prescribing information], sanofi-aventis, Bridgewater, NJ.

Schmid T, Korozsi G, Oberhuber G, Klima G and Margreiter R (1990) Lymphatic regeneration after small-bowel transplantation. *Transplant Proc* **22**(6): 2446.

Scholten EM, Cremers SC, Schoemaker RC, Rowshani AT, van Kan EJ, den Hartigh J, Paul LC and de Fijter JW (2005) AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. *Kidney Int* **67**: 2440-2447.

Schubert M, Venkataramanan R, Holt DW, Shaw LM, McGhee W, Reyes J, Webber S and Sindhi R (2004) Pharmacokinetics of sirolimus and tacrolimus in pediatric transplant patients. *Am J Transplant* **4**: 767-773.

Schwarz NT, Kalff JC, Turler A, Speidel N, Grandis JR, Billiar TR and Bauer AJ (2004) Selective jejunal manipulation causes postoperative pan-enteric inflammation and dysmotility. *Gastroenterology* **126**: 159-169.

Scully P, McKernan DP, Keohane J, Groeger D, Shanahan F, Dinan TG and Quigley EM (2010) Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity. *Am J Gastroenterol* **105**: 2235-2243.

Shelly MP, Dixon J and Park G (1989) The pharmacokinetics of midazolam following orthotopic liver transplantation. *Br J Clin Pharmacol* **27**: 629-633.

Shimizu M, Fuse K, Okudaira K, Nishigaki R, Maeda K, Kusuhara H and Sugiyama Y (2005) Contribution of OATP (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans. *Drug Metab Dispos* **33**(10): 1477-1481.

Shimomura M, Masuda S, Saito H, Sakamoto S, Uemoto S, Tanaka K and Inui K-I (2002) Roles of the jejunum and ileum in the first-pass effect as absorptive barriers for orally administered tacrolimus. *J Surg Res* **103**: 215-222.

Shord SS, Chan L-N, Camp JR, Vasquez EM, Jeong H-Y, Molokie RE, Baum CL and Xie H (2010) Effects of oral clotrimazole troches on the pharmacokinetics of oral and intravenous midazolam. *Br J Clin Pharmacol* **69**(2): 160-166.

Shugarts S and Benet LZ (2009) The role of transporters in the pharmacokinetics of orally administered drugs. *Pharm Res* **26**(9): 2039-2054.

Soars M, Grime K and Riley R (2006) Comparative analysis of substrate and inhibitor interactions with CYP3A4 and CYP3A5. *Xenobiotica* **36**(4): 287-299.

Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B and Basit AW (2008) The gastrointestinal microbiota as a site for the biotransformation of drugs. *Int J Pharm* **363**: 1-25.

Spindler-Vesel A, Wraber B, Vovk I and Kompan L (2006) Intestinal permeability and cytokine inflammatory response in multiply injured patients. *J Interferon Cytokine Res* **26**: 771-776.

Staatz CE and Tett SE (2004) Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* **43**(10): 623-653.

Standring S, Borley NR, Collins P, Crossman AR, Gatzoulis MA, Healy JC, Johnson D, Mahadevan V, Newell RL and Wigley C (eds) (2008) *Gray's anatomy: the anatomical basis of clinical practice*. Elsevier, London, UK.

Sugawara M, Huang W, Fei Y, Leibach F, Ganapathy V and Ganapathy M (2000) Transport of valganciclovir, a ganciclovir prodrug, via peptide transporters PEPT1 and PEPT2. *J Pharm Sci* **89**(6): 781-789.

Sukhai M, Yong A, Pak A and Piquette-Miller M (2001) Decreased expression of p-glycoprotein in interleukin-1β and interleukin-6 treated rat hepatocytes. *Inflamm Res* **50**: 362-370.

Sutton SC (2009) Role of physiological intestinal water in drug absorption. *AAPS J* **11**(2): 277-285.

Swift B, Tian X and Brouwer KL (2009) Integration of preclinical and clinical data with pharmacokinetic modeling and simulation to evaluate fexofenadine as a probe for hepatobiliary transport function. *Pharm Res* **26**(8): 1942-1951.

Tahara H, Kusuhara H, Fuse E and Sugiyama Y (2005) P-glycoprotein plays a major role in the efflux of fexofenadine in the small intestine and blood-brain barrier, but only a limited role it its biliary excretion. *Drug Metab Dispos* **33**(7): 963-968.

Takagi T, Ramachandran C, Bermejo M, Yamashita S, Yu LX and Amidon GL (2006) A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol Pharm* **3**(6): 631-643.

Takano M, Yumoto R and Murakami T (2006) Expression and function of efflux drug transporters in the intestine. *Pharmacol Ther* **109**: 137-161.

Tamura S, Tokunaga Y, Ibuki R, Amidon GL, Sezaki H and Shinji Y (2003) The site-specific transport and metabolism of tacrolimus in rat small intestine. *J Pharmacol Exp Ther* **306**(1): 310-316.

Tanaka T, Van Klompenberg LH and Sarr MG (2001) Selective role of vagal and nonvagal innervation in initiation and coordination of gastric and small bowel patterns of interdigestive and postprandial motility. *J Gastrointest Surg* **5**: 418-433.

Tannergren C, Knutson T, Knutson L and Lennernas H (2002) The effect of ketoconazole on the *in vivo* intestinal permeability of fexofenadine using a regional perfusion technique. *Br J Clin Pharmacol* **55**: 182-190.

Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Bacchi CE, Marsh CL, McVicar JP, Barr DM, Perkins JD and Carithers Jr RL (1994a) Use of midazolam as a human cytochrome P450 3A probe: II. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation. *J Pharmacol Exp Ther* **271**(1): 557-566.

Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Hartwell PS, Raisys VA, Marsh CL, McVicar JP, Barr DM, Perkins JD and Carithers Jr RL (1994b) Use of midazolam as a human cytochrome P450 3A probe: I. *In vitro-in vivo* correlations in liver transplant patients. *J Pharmacol Exp Ther* **271**(1): 549-556.

Thörn M, Finnström N, Lundgren S and Lööf L (2005) Cytochromes P450 and *MDR1* mRNA expression along the human gastrointestinal tract. *Br J Clin Pharmacol* **60**(1): 54-60.

Tixier E, Lalanne F, Just I, Galmiche J-P and Neunlist M (2005) Human mucosa/submucosa interactions during intestinal inflammation: involvement of the enteric nervous system in interleukin-8 secretion. *Cell Microbiol* **7**(12): 1798-1810.

Tomalik-Scharte D, Doroshyenko O, Kirchheiner J, Jetter A, Lazar A, Klaasen T, Frank D, Wyen C, Fätkenheuer G and Fuhr U (2008) No role for the CYP3A5*3 polymorphism in intestinal and hepatic metabolism of midazolam. *Eur J Clin Pharmacol* **64**: 1033-1035.

Tomita M, Takizawa Y, Kishimoto H and Hayashi M (2008) Assessment of ileal p-glycoprotein dysfunction induced by ischemia/reperfusion using in vivo animal model. *Drug Metab Pharmacokinet* **23**(5): 356-363.

Tuler A, Abu-Elmagd K, Kalff JC, Bond GJ, Brunagel G, Schraut WH, Moore BA and Bauer AJ (2002) Molecular inflammatory events within the human intestinal muscularis during small bowel transplantation. *Transplant Proc* **34**: 921.

Ufer M, Dilger K, Leschhorn L, Daufresne L, Mosyagin I, Rosenstiel P, Haesler R, Kuelbacher T, Nikolaus S, Schreiber S and Cascorbi I (2008) Influence of CYP3A4, CYP3A5, and ABCB1 genotype and expression on budesonide pharmacokinetics: a possible role of intestinal CYP3A4 expression. *Clin Pharmacol Ther* **84**(1): 43-46.

Ujie K, Oda M, Kabayashi M and Saitoh H (2008) Relative contribution of absorptive and secretory transporters to the intestinal absorption of fexofenadine in rats. *Int J Pharm* **DOI: 10.1016/j.ijpharm.2008.05.004**.

Üner A, Weinberg A, Nautrup CP, Kassianoff I, Lüdemann W, Schier F, Claus P and von Rautenfeld DB (2001) Spontaneous reanastomosis between lymphatic vessels following syngeneic transplantation of the small intestine in the rat. *Surg Radiol Anat* **23**: 383-387.

Uno S, Kawase A, Tsuji A, Tanino T and Iwaki M (2007) Decreased intestinal CYP3A and p-glycoprotein activities in rats with adjuvant arthritis. *Drug Metab Pharmacokinet* **22**(4): 313-321.

Venkataramanan R, Jain A, Cadoff E, Warty V, Iwasaki K, Nagase K, Krajack A, Imventarza O, Todo S, Fung J and Starzl T (1990) Pharmacokinetics of FK 506: preclinical and clinical studies. *Transplant Proc* **22**(1 Suppl 1): 52-56.

Venkataramanan R, Jain A, Warty V, Abu-Elmagd K, Alessiani M, Lever J, Krajak A, Flowers J, Mehta S, Zuckerman S, Fung J, Todo S and Starzl T (1991a) Pharmacokinetics of FK 506 in transplant patients. *Transplant Proc* **23**(6): 2736-2740.

Venkataramanan R, Jain A, Warty V, Abu-Elmagd K, Furakawa H, Imventarza O, Fung J, Todo S and Starzl T (1991b) Pharmacokinetics of FK 506 following oral administration: a comparison of FK 506 and cyclosporine. *Transplant Proc* **23**(1): 931-933.

Villikka K, Varis T, Backman J, Neuvonen P and Kivisto K (2001) Effect of methylprednisolone on CYP3A4-mediated drug metabolism in vivo. *Eur J Clin Pharmacol* **57**(6-7): 457-460.

Vollmar B and Menger MD (2011) Intestinal ischemia/reperfusion: microcirculatory pathology and functional consequences. *Langenbecks Arch Surg* **396**: 13-29.

Wang J, Dong Y, Sun J-Z, Taylor R, Guo C, Alegre M-L, Williams I and Newell K (2006) Donor lymphoid organs are a major site of alloreactive T-cell priming following intestinal transplantation. *Am J Transplant* **6**: 2563-2571.

Washington N, Washington C and Wilson CG (2003a) Cell membranes, epithelial barriers, and drug absorption, in *Physiological pharmaceutics: barriers to drug absorption* pp 1-18, Taylor & Francis, New York, NY.

Washington N, Washington C and Wilson CG (2003b) Drug absorption from the small intestine, in *Physiological pharmaceutics: barriers to drug absorption* pp 109-141, Taylor & Francis, New York, NY.

Watanabe T, Hoshino K, Tanabe M, Kawachi S, Wakabayashi G, Shimazu M, Morikawa Y and Kitajima M (2008) Correlation of motility and neuronal integrity with a focus on the grade of intestinal allograft rejection. *Am J Transplant* **8**: 529-536.

Watkins RE, Wisely GB, Moore LB, Collins JL, Lambert MH, Williams SP, Willson TM, Kliewer SA and Redinbo MR (2001) The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science* **292**: 2329-2333.

Welcker K, Martin A, Kolle P, Siebeck M and Gross M (2004) Increased intestinal permeability in patients with inflammatory bowel disease. *Eur J Med Res* **9**(10): 456-460.

Wen X-H, Kong H-Y, Zhu S-M, Xu J-H, Huang S-Q and Chen Q-L (2004) Plasma levels of tumor necrotic factor-alpha and interleukin-6, -8 during orthotopic liver transplantation and their relations to postoperative pulmonary complications. *Hepatobiliary Pancreat Dis Int* **3**: 38-41.

Weseman RA (2007) Review of incidence and management of chylous ascites after small bowel transplantation. *Nutr Clin Pract* **22**(5): 482-484.

Wilkinson GR (2005) Drug metabolism and variability among patients in drug response. *N Engl J Med* **352**: 2211-2221.

Winkelaar GB, Smith LJ, Martin GR and Sigalet DL (1997) Fat absorption after small intestinal transplantation in the rat. *Transplantation* **64**(4): 566-571.

Wright J, Haslam I, Coleman T and Simmons N (2011) Breast cancer resistance protein BCRP (ABCG2)-mediated transepithelial nitrofurantoin secretion and its regulation in human intestinal epithelial (Caco-2) layers. *Eur J Pharmacol* **672**(1-3): 70-76.

Xie R, Tan LH, Polasek EC, Hong C, Teillol-Foo M, Gordi T, Sharma A, Nickens DJ, Arakawa T, Knuth DW and Antal EJ (2005) CYP3A and p-glycoprotein activity induction with St. John's Wort in healthy volunteers from 6 ethnic populations. *J Clin Pharmacol* **45**: 352-356.

Yang J, Hao C, Yang D, Shi D, Song X, Luan X, Hu G and Yan B (2010) Pregnane X receptor is required for interleukin-6-mediated down-regulation of cytochrome P450 3A4 in human hepatocytes. *Toxicol Lett* **197**: 219-226.