Department of Clinical Pharmacology
University of Helsinki
Finland

Effects of induction and inhibition of Cytochrome P-450 enzymes on the pharmacokinetics and pharmacodynamics of oral antidiabetic drugs

by

Mikko Niemi

ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of the University of Helsinki, for public examination in the small lecture hall 2 of Biomedicum Helsinki, Haartmaninkatu 8, on December 21st, 2001, at 12 noon.

Helsinki 2001

Supervisors: Professor Pertti Neuvonen, MD
Department of Clinical Pharmacology
University of Helsinki
Helsinki, Finland

Docent Kari Kivistö, MD
Department of Clinical Pharmacology
University of Helsinki
Helsinki, Finland

Reviewers: Docent Risto Huupponen, MD
Department of Pharmacology and Clinical Pharmacology
Institute of Biomedicine
University of Turku
Turku, Finland

Professor Olavi Tokola, MD
National Agency for Medicines
Helsinki, Finland

Opponent: Professor Pauli Ylitalo, MD
Department of Pharmacology, Clinical Pharmacology and Toxicology
University of Tampere
Tampere, Finland

ISBN 952-91-4219-6 (nid.)
ISBN 952-10-0260-3 (PDF)
Helsinki 2001
Yliopistopaino
This dissertation is available online at http://ethesis.helsinki.fi

To my family

CONTENTS

ABBREVIATIONS

ABC	ATP-binding cassette
ACE	angiotensin-converting enzyme
Ah	aryl hydrocarbon
ANOVA	analysis of variance
ATP	adenosine triphosphate
AUC(t-t_i)	area under the plasma drug concentration-time curve from t to t_i hours
CAR	constitutive androstane receptor
C_{max}	peak concentration
CV	coefficient of variation
CYP	cytochrome P-450
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HPLC	high-performance liquid chromatography
k_{el}	elimination-rate constant
KIR6.x	inwardly rectifying potassium channel
LC/MS/MS	liquid chromatography-tandem mass spectrometry
m/z	mass-to-charge ratio
NIDDM	non-insulin-dependent diabetes mellitus
NSAID	nonsteroidal anti-inflammatory drug
PPAR	peroxisome proliferator-activated receptor
PXR	pregnane X receptor
RXR	retinoid X receptor
SD	standard deviation
SEM	standard error of the mean
SUR	sulfonylurea receptor
t_{max}	time to peak concentration
$t_{½}$	elimination half-life
UGT	UDP-glucuronosyltransferase
UKPDS	UK Prospective Diabetes Study

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred to in the text by the Roman numerals I to VI.

I Niemi M, Kivistö KT, Backman JT, Neuvonen PJ. Effect of rifampicin on the pharmacokinetics and pharmacodynamics of glimepiride. *Br J Clin Pharmacol* 2000;50:591-5.

II Niemi M, Backman JT, Neuvonen M, Neuvonen PJ, Kivistö KT. Effects of rifampin on the pharmacokinetics and pharmacodynamics of glyburide and glipizide. *Clin Pharmacol Ther* 2001;69:400-6.

III Niemi M, Backman JT, Neuvonen M, Neuvonen PJ, Kivistö KT. Rifampin decreases the plasma concentrations and effects of repaglinide. *Clin Pharmacol Ther* 2000;68:495-500.

IV Niemi M, Backman JT, Neuvonen M, Laitila J, Neuvonen PJ, Kivistö KT. Effects of fluconazole and fluvoxamine on the pharmacokinetics and pharmacodynamics of glimepiride. *Clin Pharmacol Ther* 2001;69:194-200.

V Niemi M, Neuvonen PJ, Kivistö KT. Effect of gemfibrozil on the pharmacokinetics and pharmacodynamics of glimepiride. *Clin Pharmacol Ther* 2001;70:439-45.

VI Niemi M, Neuvonen PJ, Kivistö KT. The cytochrome P4503A4 inhibitor clarithromycin increases the plasma concentrations and effects of repaglinide. *Clin Pharmacol Ther* 2001;70:58-65.

ABSTRACT

The aims of this work were to investigate the effects of induction of drug-metabolizing enzymes, with rifampicin as the model inducer, on the pharmacokinetics and pharmacodynamics of the oral antidiabetic drugs, glibenclamide, glimepiride, glipizide, and repaglinide, and to investigate the effects of inhibition of CYP2C9 on the pharmacokinetics and pharmacodynamics of glimepiride, with fluconazole, fluvoxamine, and gemfibrozil as model inhibitors of CYP2C9. In addition, the effects of inhibition of CYP3A4 on the pharmacokinetics and pharmacodynamics of repaglinide were investigated, with clarithromycin as the model inhibitor.

A total of 9 to 12 healthy volunteers participated in each study, except in Study II, in which 20 volunteers participated, because it comprised two separate substudies. All the studies were randomized, placebo-controlled crossover studies with 2 to 3 phases in each. Pretreatment with the inducer or inhibitor was followed by a single dose of antidiabetic drug, whereafter blood samples were collected for the determination of plasma drug, blood glucose, and, in two of the six studies, serum insulin concentrations.

Rifampicin reduced the plasma concentrations of all of these oral antidiabetic drugs and also significantly reduced the blood glucose-lowering effects of glibenclamide and repaglinide. Rifampicin reduced the AUC of glipizide, glimepiride, glibenclamide, and repaglinide by 22% ($P < .05$), 34% ($P < .001$), 39% ($P < .001$), and 57% ($P < .001$), respectively. Fluconazole considerably elevated the plasma concentrations of glimepiride, whereas fluvoxamine and gemfibrozil caused only moderate increases in the plasma glimepiride concentrations. Fluconazole, fluvoxamine, and gemfibrozil elevated the AUC of glimepiride by 138% ($P < .0001$), 33% (n.s.), and 23% ($P < .005$), respectively. None of these drugs significantly affected the blood glucose-lowering effect of glimepiride in healthy volunteers. The low 250-mg twice-daily dose of clarithromycin raised the AUC and C_{max} of repaglinide by 40% ($P < .0001$) and 67% ($P < .005$), respectively. Clarithromycin raised the incremental AUC(0-3) of serum insulin and the maximum increase in the insulin concentration after repaglinide by 51% ($P < .05$) and 61% ($P < .01$), respectively.

ABSTRACT

The interactions of rifampicin with the sulfonylureas glibenclamide, glimepiride, and glipizide are probably mainly explained by induction of CYP2C9 by rifampicin. Induction of the P-glycoprotein by rifampicin probably also contributed to the interaction between rifampicin and glibenclamide. The susceptibilities of these antidiabetic drugs to interaction with rifampicin differ significantly, and glipizide seems to be least susceptible to a clinically significant interaction with rifampicin. Rifampicin had the greatest effect on the CYP3A4 substrate repaglinide, indicating that repaglinide is susceptible to clinically significant interactions resulting in loss of blood glucose control with rifampicin and other potent inducers of CYP3A4. CYP2C9 inhibitors can raise plasma concentrations of glimepiride, and the concomitant use of fluconazole or other potent inhibitors of CYP2C9 with glimepiride may markedly increase the risk of hypoglycemia. Clarithromycin raised the plasma concentrations and the effects of repaglinide probably by inhibiting its CYP3A4-mediated metabolism. The concomitant use of potent inhibitors of CYP3A4 with repaglinide may enhance its blood glucose-lowering effect and increase the risk of hypoglycemia.

INTRODUCTION

Cytochrome P-450 (CYP) enzymes play a central role in the biotransformation of a great number of drugs. Among the several CYP enzyme families, the first three, CYP1, CYP2, and CYP3, are involved in human drug metabolism (Wrighton & Stevens 1992). The primary organ for the biotransformation of drugs is the liver. However, there are significant quantities of CYP enzymes in several other organs as well. For example, the wall of the small intestine plays a major role in the presystemic elimination of many drugs, and intestinal drug metabolism can greatly reduce the oral bioavailability of drugs. CYP2C and CYP3A4 enzymes are significantly expressed in the small intestinal wall (Kivistö et al 1996, Zhang et al 1999).

The knowledge of harmful drug-drug interactions mediated by the CYP enzymes has grown considerably during the past 10 years. Drugs that inhibit CYP enzymes can greatly raise the plasma concentrations of certain other drugs metabolized by these enzymes and thereby enhance their pharmacological and toxicological effects. Induction of CYP enzymes can lower the plasma concentrations and effects of some substrates of CYP enzymes. On the other hand, if the substrate is a prodrug activated by CYP-mediated metabolism, inhibition of its metabolism can reduce its effects and induction can either enhance or reduce its effects and toxicity, depending on the effects of induction on the further metabolism or excretion of the active metabolite. Certain drugs, for instance the antimycotic fluconazole, competitively inhibit the metabolism of drugs metabolized by certain CYP enzymes (Lin & Lu 1998, Miners & Birkett 1998). Some other drugs, like many macrolide antibiotics, form inactive metabolic intermediate complexes with certain CYP enzymes and thereby inhibit their catalytic activities (Lindstrom et al 1993, Ohmori et al 1993). The antituberculosis drug rifampicin, as a potent inducer of several CYP enzymes and some other drug-metabolizing enzymes as well as the xenobiotic transporter P-glycoprotein, thereby reduces the plasma concentrations and effects of several drugs (Venkatesan 1992, Greiner et al 1999, Branch et al 2000). Rifampicin binds and activates the orphan nuclear receptor known as the pregnane X receptor (PXR), which leads to increased CYP3A4 and P-glycoprotein protein synthesis (Lehmann et al 1998, Geick et al 2001). Induction of CYP2C9 by rifampicin is probably caused by the same mechanism (Gerbal-Chaloin et al 2001).

The sulfonylureas used in the treatment of type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus; NIDDM) are eliminated primarily by metabolism, mainly by the CYP2C9 enzyme. The meglitinide analogue repaglinide is a new short-acting antidiabetic drug that acts mainly by the same mechanism as the sulfonylureas (i.e., by increasing insulin secretion from the pancreas). Repaglinide is metabolized to a significant extent by the CYP3A4 enzyme. However, few controlled studies examine the effects of inducers or inhibitors of CYP enzymes on the pharmacokinetics and pharmacodynamics of these antidiabetic drugs.

The purpose of this work was to investigate the effects of rifampicin on the pharmacokinetics and pharmacodynamics of oral antidiabetic drugs and to investigate the effects of inhibitors of CYP enzymes on the pharmacokinetics and pharmacodynamics of glimepiride and repaglinide.

REVIEW OF THE LITERATURE

1. Drug metabolism, CYP enzymes, and P-glycoprotein

1.1. Principles of drug metabolism

Most drugs are lipophilic, which facilitates their passing through biological membranes. Lipophilic compounds are not, however, readily excreted from the body and need to be biotransformed into more hydrophilic forms. This biotransformation is catalyzed in humans by a number of drug-metabolizing enzymes. Drug metabolism can be divided into functionalization (phase I) and conjugation (phase II) reactions. The functionalization reactions include dehydrogenation or hydrogenation, oxidation, hydrolysis, reduction, and monooxygenation (Meyer 1996). The metabolites produced by these reactions are polarized hydrophilic compounds and usually possess fewer pharmacologic and toxic properties than do the parent compounds. In other words, they are bioinactivated. On the other hand, some inactive prodrugs are activated by the functionalization reactions. In some cases, the metabolites possess toxic properties and cause, for instance, hepatotoxicity. The enzymes involved in phase II reactions transfer water-soluble chemicals, such as glutathione, glucuronide, or sulfate to other compounds. Many drugs undergo both phase I and phase II metabolism sequentially, but some can be excreted after either phase I or phase II reactions or even while still nonmetabolized (Krishna & Klotz 1994).

1.2. CYP enzymes

In phase I human drug metabolism, CYP enzymes play a major role. The CYP enzymes comprise a superfamily of heme-containing mono-oxygenases. In the presence of carbon monoxide, they have an absorption maximum at wavelength 450 nm and are therefore called P-450. The CYP superfamily is subdivided and classified on the basis of amino acid identity, phylogenetic criteria, and gene organization. The symbol CYP is followed by a number for families (in general, groups of proteins with more than 40% amino acid sequence identity), a letter for subfamilies (more than 55% identity), and a number for the individual enzyme (Nelson et al 1996).

Human beings have 17 known CYP gene families (http://drnelson.utmem.edu/human.p450s.html), of which only the first 3 seem to be important for drug metabolism (Wrighton & Stevens 1992). Apart from being involved in drug metabolism, the CYP enzymes also play an important role in cholesterol biosynthesis, vitamin D metabolism, bile acid metabolism, and biosynthesis of steroids and thromboxane A2 (Nelson 1999).

CYP enzymes can be induced and inhibited by various drugs and other xenobiotics (Pelkonen et al 1998), and also be affected by some diseases. Porphyrias are a group of diseases with genetic deficiencies in heme biosynthesis. Patients with certain forms of porphyria can have markedly decreased metabolism of certain drugs. Interestingly, in patients with variegate porphyria the prolonged $t_{½}$ of antipyrine (about 30 hours) can be shortened to 6 hours by a 3-day treatment with intravenous heme (Tokola et al 1988), and in these patients, even a single infusion of heme can reduce the $t_{½}$ of antipyrine to 13 hours (Mustajoki et al 1992).

The **CYP1A** subfamily comprises two members, CYP1A1 and CYP1A2; they share 68% amino acid sequence identity. CYP1A1 is expressed primarily in extrahepatic tissues, such as lungs, small intestine, and placenta, whereas CYP1A2 is expressed primarily in the liver (Wrighton & Stevens 1992), where it constitutes more than 10% of the total liver CYP content (Shimada et al 1994). CYP1A1 and CYP1A2 are inducible by xenobiotics, and this induction is mediated generally by the aryl hydrocarbon (Ah) receptor (Miners & McKinnon 2000). Cigarette smoke and charcoal grilled meat are typical inducers of CYP1A enzymes (Miners & McKinnon 2000). Human CYP1A induction shows marked heterogeneity, which may be caused by polymorphisms in the Ah receptor (Miners & McKinnon 2000). In addition, the CYP1A1 gene shows structural polymorphisms; furthermore the great variability in CYP1A2 activity is suggestive of genetic polymorphism (Miners & McKinnon 2000).

CYP1A enzymes are capable of activating procarcinogenic xenobiotics (Miners & McKinnon 2000). In addition, CYP1A2 is an important drug-metabolizing enzyme in the human liver (Table I). Substrates for CYP1A2 include caffeine, clozapine, theophylline, and R-warfarin (Miners & McKinnon 2000, Bertilsson et al 1994). Fluvoxamine and ciprofloxacin are known inhibitors of CYP1A2 (Rasmussen et al 1995, Fuhr et al 1992).

CYP2A6 comprises 1% to 4% of the total human liver CYP protein (Yun et al 1991, Shimada et al 1994) and is polymorphically expressed (Oscarson 2001). CYP2A6 protein is induced in cell cultures by rifampicin (Dalet-Beluche et al 1992), and results from an in vivo study indicate that also the antiepileptic drugs carbamazepine and phenytoin induce CYP2A6 (Sotaniemi et al 1995). CYP2A6 is the major coumarin 7-hydroxylase in human liver and is involved in the metabolism also of nicotine, halothane, valproic acid, and disulfiram (Pelkonen et al 2000, Oscarson 2001). Defective CYP2A6 alleles are associated with deficient nicotine metabolism (Nakajima et al 2001). According to some studies, these alleles tend to delay the age of starting regular smoking, reduce the number of cigarettes smoked, and increase the likelihood of quitting smoking (Pianezza et al 1998, Gu et al 2000). Interestingly, the CYP2A6 inhibitor methoxsalen (Kharasch et al 2000) inhibits nicotine first-pass metabolism and in a laboratory setting reduces smoking (Sellers et al 2000).

The **CYP2C** subfamily, the most complex mammalian subfamily, has 4 known human members: CYP2C8, CYP2C9, CYP2C18, and CYP2C19. CYP2C enzymes are primarily hepatic, but significant quantities of CYP2C protein exist in the small intestine, as well (Zhang et al 1999). CYP2C8, CYP2C9, and CYP2C19 are inducible in cell cultures by rifampicin, phenobarbital, and dexamethasone (Gerbal-Chaloin et al 2001), and in vivo data indicate that, in addition, carbamazepine and phenytoin induce CYP2C enzymes (Anderson 1998). CYP2C9 is the most abundant enzyme of the CYP2C subfamily in the liver and in one study accounted for more than 30% of the total human liver CYP content (Lasker et al 1998). CYP2C9 and CYP2C19 are polymorphically expressed, with approximately 3% of Caucasians and 20% of Asians being poor metabolizers of CYP2C19 substrates (Wedlund et al 1984, Nakamura et al 1985). CYP2C9 has at least 3 important allelic variants, Arg144/Ile359 (CYP2C9*1; wild-type), Cys144/Ile359 (CYP2C9*2), and Arg144/Leu359 (CYP2C9*3) (Miners & Birkett 1998). The CYP2C9*3 allele (in both heterozygous and homozygous individuals) is associated with decreased clearance of CYP2C9 substrates such as S-warfarin, celecoxib, phenytoin, and glipizide (Takahashi et al 1998, Tang et al 2001, Kidd et al 1999). CYP2C9 polymorphisms also affect warfarin and phenytoin dose requirements (Aithal et al 1999, van der Weide et al 2001) and risk of bleeding complications during warfarin therapy (Aithal et al 1999). Approximately 10% to 15% of Caucasians are heterozygous and 1% homozygous for the CYP2C9*3 allele (Yasar et al 1999).

Taxol (paclitaxel) is the prototypic substrate for CYP2C8 (Rettie et al 2000), and CYP2C8 plays an important role in the metabolism of cerivastatin, pioglitazone, and rosiglitazone (Mück 1998, Mudaliar & Henry 2001). CYP2C8 is also capable of metabolizing benzphetamine, retinoic acid, tolbutamide, benzo(a)pyrene, carbamazepine, and R-ibuprofen, although it is probably not the main enzyme responsible for the in vivo metabolism of these compounds (Rettie et al 2000). Many drug substrates for CYP2C9 have narrow therapeutic indexes (Miners & Birkett 1998), including S-warfarin, phenytoin, and the first-generation sulfonylurea tolbutamide (Table I). It seems that most of the sulfonylurea antidiabetic drugs are metabolized primarily by CYP2C9 (Relling et al 1990, Brian 2000, Kidd et al 1999, Langtry & Balfour 1998, Shon et al 2001). Other substrates for CYP2C9 include many nonsteroidal anti-inflammatory drugs (NSAIDs): ibuprofen, diclofenac, naproxen, piroxicam, and tenoxicam, as well as losartan (Miners & Birkett 1998). There seems to be no convincing evidence that CYP2C18 contributes to the in vivo metabolism of any drugs, although in vitro data show that it may contribute to the metabolism of diazepam, omeprazole and lansoprazole (Rettie et al 2000). S-mephenytoin is the prototypic substrate of CYP2C19 (Rettie et al 2000). Omeprazole and diazepam are also metabolized by CYP2C19, and the active cycloguanil metabolite of proguanil seems to be produced by CYP2C19 (Table I; Rettie et al 2000).

Currently no known specific inhibitors of CYP2C8 or CYP2C18 exist (Rettie et al 2000). Ketoconazole and diethyldithiocarbamate inhibited CYP2C8 at concentrations often thought specifically to inhibit CYP3A4 or CYP2E1, and some CYP3A substrates such as midazolam, quinine, terfenadine, and triazolam can also inhibit CYP2C8 in vitro (Ong et al 2000). Sulfaphenazole is a prototype inhibitor of CYP2C9 (Rettie et al 2000). The antiarrhythmic drug amiodarone and the azole antifungals fluconazole and miconazole are potent CYP2C9 inhibitors both in vitro and in vivo (Miners & Birkett 1998). Fluvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, can also inhibit CYP2C9 in vitro and in vivo (Miners & Birkett 1998), but only slightly affects the pharmacokinetics of the CYP2C9 substrates glibenclamide and tolbutamide in patients with NIDDM (Appel et al 1995). S-mephenytoin can competitively inhibit CYP2C19 in vitro only at high concentrations (Rettie et al 2000). The CYP2C19 substrate omeprazole seems capable of inhibiting CYP2C19 in vivo (Funck-Brentano et al 1997), and fluvoxamine inhibits CYP2C19, among other CYP enzymes (Rasmussen et al 1998).

CYP2D6 is the only functionally active isozyme of the CYP2D subfamily in humans, but its expression constitutes only about 2% to 5% of the total hepatic CYP content (Shimada et al 1994). It is, however, polymorphically expressed, and its expression varies more than 100-fold between the poor metabolizers and the most active extensive metabolizers – the ultrarapid CYP2D6 metabolizers (Zanger & Eichelbaum 2000). Approximately 7% of Caucasians are poor metabolizers of CYP2D6. In contrast to all other CYP enzymes involved in human drug metabolism, CYP2D6 seems not to be inducible (Zanger & Eichelbaum 2000).

Several widely used drugs are metabolized by CYP2D6 (Table I). These include antiarrhythmics, β-adrenergic receptor antagonists, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), neuroleptics, opiates, anticancer agents, and amphetamines (Zanger & Eichelbaum 2000). Quinidine, the SSRI fluoxetine, the antiarrhythmic flecainide, and the antimycotic terbinafine are known inhibitors of CYP2D6 (Zanger & Eichelbaum 2000, Abdel-Rahman et al 1999).

CYP3A is the most important drug-metabolizing CYP subfamily in man. It has been estimated that CYP3A enzymes are involved in the metabolism of more than 50% of all clinically used drugs (Wrighton & Thummel 2000). The most prominently expressed CYP enzyme in the human liver may be CYP3A4. In one study, CYP3A4 accounted for almost 30% of the total CYP in the liver (Shimada et al 1994), and is the dominant CYP enzyme in the small intestinal mucosa (Kivistö et al 1996, Zhang et al 1999), with its greatest activity in the proximal small intestine (Zhang et al 1999). Hepatic and intestinal CYP3A4 can be induced by several widely used drugs, such as rifampicin, dexamethasone, carbamazepine, phenytoin, and St John's wort (*Hypericum Perforatum*) (Wrighton & Thummel 2000, Backman et al 1996a, Backman et al 1996b, Dürr et al 2000). CYP3A4 catalyzes the metabolism of a wide variety of commonly prescribed drugs, such as the psychotropic drugs buspirone, alprazolam, midazolam, and triazolam, the HMG-CoA reductase inhibitors atorvastatin, lovastatin, and simvastatin, the calcium-channel blockers felodipine, nifedipine, and verapamil, and the gastroprokinetic cisapride (Dresser et al 2000; Table I). The CYP3A4 enzyme has two substrate binding sites and is allosterically regulated (Ueng et al 1997, Korzekwa et al 1998, Shou et al 1999). The drug interactions caused by inhibition of CYP3A4 exhibit substrate dependency

(Wang et al 2000), and one should be careful in extrapolating drug-drug interactions studied for one CYP3A4 substrate to another substrate.

CYP3A5 is the second functionally active member of the CYP3A subfamily. It has an amino acid identity of 88% with CYP3A4 (Schuetz et al 1989). CYP3A5 is present at readily detectable levels in only about 30% of human livers (Wrighton & Thummel 2000), but it is the most abundant CYP3A isozyme in the human kidney, where it may be important in the hydroxylation of endogenous molecules. It also exists in other extrahepatic tissues, such as the gastrointestinal tract, lung, and pancreas (Wrighton & Thummel 2000). CYP3A5 is inducible in hepatocyte cultures by rifampicin and phenobarbital (Wrighton & Thummel 2000) and in one human lung adenocarcinoma cell line by glucocorticoids and phenobarbital (Hukkanen et al 2000). The substrate specificity of CYP3A5 seems to be in general similar to that of CYP3A4 (Wrighton & Thummel 2000). CYP3A7 is found mainly in fetal tissues and comprises about 50% of the total CYP expressed in the human fetal liver. It is not present in significant quantities in adult liver, but can be found in appreciable quantities in the endometrium and placenta (Wrighton & Thummel 2000). CYP3A7 has an amino acid identity of 88% with CYP3A4, and the metabolic capabilities of CYP3A7 and CYP3A4 seem to be similar (Wrighton & Thummel 2000).

Apart from being involved in the metabolism of a great number of drugs, CYP3A enzymes can also be inhibited by a considerable number of drugs. Known inhibitors of CYP3A include the macrolide antibiotics erythromycin and clarithromycin, the calcium-channel blockers diltiazem, mibefradil, and verapamil, the azole antimycotics ketoconazole and itraconazole, the HIV protease inhibitors ritonavir, indinavir, and saquinavir, the novel antidepressant nefazodone, and grapefruit juice (Dresser et al 2000, Wrighton & Thummel 2000).

1.3. P-glycoprotein

The impact of drug transporters on pharmacokinetics has been widely recognized in the past few years. Several transporters with different functions have been characterized in various organs. P-glycoprotein, a product of the multiple drug resistance 1 (MDR1) gene, is one of the most studied drug transporters and belongs to the superfamily of adenosine triphosphate (ATP)-binding cassette (ABC) proteins (Sharom 1997). It was originally recognized as a cause of the multidrug resistance in cancer chemotherapy. Like the CYP enzymes, P-glycoprotein is also able to interact with a large number of structurally distinct drugs and xenobiotics (Silverman 2000).

P-glycoprotein is a transmembrane efflux protein that actively transports drugs, other xenobiotics, and cellular metabolites out of the cells. P-glycoprotein is expressed in the epithelial cells on the luminal surfaces of many organs with an excretory or barrier function: the liver, kidneys, and small intestine, and the endothelial cells of the blood-brain and blood-testes barriers. Therefore, P-glycoprotein can affect drug disposition, for instance by inhibiting drug absorption from the gastrointestinal tract and by facilitating drug excretion into the bile and urine (Silverman 2000).

The known drug substrates for P-glycoprotein include a number of anticancer agents and other drugs, such as digoxin (Silverman 2000; Table I). Of the antidiabetic drugs studied, glibenclamide is a substrate for the P-glycoprotein (Goldstein et al 1999), and verapamil, an inhibitor of P-glycoprotein, elevates the plasma concentrations of glibenclamide (Semple et al 1986). It is not yet known whether glipizide, glimepiride, or repaglinide is a substrate for the P-glycoprotein.

1.4. Induction and inhibition

1.4.1. Mechanisms of induction of CYP enzymes

The induction of CYP enzymes can be caused by at least 5 different mechanisms. Ethanol selectively induces CYP2E1 primarily by stabilizing the enzyme protein (Fuhr 2000). Other types of induction of CYP enzymes seem to be mediated by intracellular receptors, namely the Ah receptor, the constitutive

androstane receptor (CAR), the PXR, and the peroxisome proliferator-activated receptor (PPAR) (Fuhr 2000). The Ah receptor is a transcription factor that belongs to the basic-helix-loop-helix-PAS (bHLH-PAS) family, whereas CAR, PXR, and PPAR are orphan nuclear receptors (Waxman 1999).

Polycyclic aromatic hydrocarbons, found for instance in tobacco smoke and charcoal grilled meat, bind to the Ah receptor. The inducer-Ah receptor complex, together with the Ah receptor nuclear translocator (Arnt), binds to a deoxyribonucleic acid (DNA) response element and increases protein synthesis. The most important enzyme induced through this mechanism is CYP1A2. Other enzymes induced by this mechanism include CYP1A1 and some phase II enzymes such as glutathione S-transferases (GSTs) and UDP-glucuronosyltransferases (UGTs) (Fuhr 2000). A clear dose dependency is evident between smoking and caffeine clearance (an indicator of CYP1A2 activity) with a 1.22-fold increase in caffeine clearance in subjects who smoke 1 to 5 cigarettes per day and a 1.72-fold increase in subjects who smoke more than 20 per day (Tantcheva-Poór et al 1999). Smoking also raises the systemic elimination of other CYP1A2 substrates, such as theophylline, tacrine, and clozapine (Fuhr 2000).

The mechanism of the induction of protein synthesis by the nuclear receptors CAR, PXR, and PPAR is essentially similar. An inducer binds to CAR, PXR, or PPAR, and the inducer-receptor complex forms a heterodimer with the retinoid X receptor (RXR). This heterodimer binds to a DNA response element and enhances DNA transcription and eventually protein synthesis (Waxman 1999).

Phenobarbital binds to CAR and affects the expression of approximately 50 genes. Of the CYP enzymes, phenobarbital seems to have the greatest effect on CYP2B6, but clearly also induces CYP1A2, CYP2C8, CYP2C9, and CYP3A4 and also some UGTs (Fuhr 2000). Phenobarbital also induces CYP2C19, although to a smaller extent than CYP2C8 or CYP2C9 (Gerbal-Chaloin et al 2001).

PXR is activated by a number of compounds that are known to induce CYP3A4, such as rifampicin, phenobarbital, dexamethasone, and St John’s wort (Lehmann et al 1998, Moore et al 2000). Recent studies suggest that PXR is also involved in the induction of CYP2C8 and CYP2C9 (Gerbal-Chaloin et al 2001). Interestingly, apart from activating the PXR receptor, dexamethasone can induce

CAR, PXR, and RXR protein synthesis through its effects on the glucocorticoid receptor and thus can potentiate the inducing effects of CAR and PXR activators (Pascussi et al 2000a, Pascussi et al 2000b).

A new group of antidiabetic drugs, the thiazolidinediones or glitazones, are known to bind to the PPARγ, whereas the fibrate drugs bind to the PPARα. PPARα activates the transcription of genes that encode for proteins involved in lipoprotein and fatty acid metabolism, and PPARγ controls adipocyte differentiation and adipogenesis (Fuhr 2000). PPARα is involved in the induction of CYP4A enzymes (Waxman 1999).

1.4.2. Mechanisms of inhibition of CYP enzymes

The mechanisms of CYP inhibition can be roughly divided into 2 groups: reversible inhibition and irreversible inhibition, with the former being probably the more common mechanism (Lin & Lu 1998).

Reversible inhibition can be divided, on a kinetic basis, into competitive, noncompetitive, and uncompetitive inhibition. In competitive inhibition, the inhibitor competes with the substrate for the same binding site within a CYP enzyme. In noncompetitive inhibition, the inhibitor binds to the same enzyme as does the substrate, but the binding site differs. In uncompetitive inhibition, the inhibitor binds only to an enzyme that forms a complex with the substrate (Ito et al 1998). Potent reversible inhibitors of CYP enzymes include, for example, itraconazole (CYP3A4), fluoxetine (CYP2D6), miconazole (CYP2C9), and ciprofloxacin (CYP1A2) (von Moltke et al 1996, Stevens & Wrighton 1993, O'Reilly et al 1992, Fuhr et al 1992).

Irreversible inhibition also includes what can be considered quasi-irreversible inhibition. In quasi-irreversible inhibition, the inhibitor undergoes metabolic activation by the CYP enzymes to form inhibitory intermediate metabolites. These metabolites form stable inactive complexes with the prosthetic heme of CYP. In vitro, the metabolic activity of the inactive CYP can be reversed during incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site, or by irradiation, or by oxidation to the ferric state by the addition of potassium ferricyanide. In vivo, these complexes are, however, so stable that the CYP enzymes involved are unavailable for drug

metabolism, and synthesis of new enzymes is required to overcome the inhibition; hence the name quasi-irreversible inhibition (Lin & Lu 1998). In irreversible inhibition, certain drugs with functional groups are oxidized by the CYP enzymes to form reactive intermediate metabolites that covalently bind and irreversibly inactivate CYP. Because metabolic activation is needed, the inhibitors are often called mechanism-based inactivators or suicide substrates (Lin & Lu 1998). The metabolic intermediates of the macrolide antibiotics erythromycin and clarithromycin form complexes with the iron of the heme of CYP3A4 and thus inactivate it. Erythromycin and clarithromycin are potent inhibitors of CYP3A4 (Lindstrom et al 1993, Gascon & Dayer 1991, Ohmori et al 1993). In addition, furafylline (CYP1A2), gestodene (CYP3A4), and grapefruit juice (CYP3A4) are known irreversible inhibitors of CYP enzymes (Kunze & Trager 1993, Guengerich 1990, Lown et al 1997).

1.4.3. Induction and inhibition of P-glycoprotein

Several studies provide convincing evidence that P-glycoprotein is inducible by some of the same drugs that are known to induce drug-metabolizing enzymes (Schuetz et al 1996, Greiner et al 1999, Westphal et al 2000, Dürr et al 2000, Hamman et al 2001). Recently, P-glycoprotein induction by rifampicin was shown to be mediated by the orphan nuclear receptor PXR (Geick et al 2001), which is involved in CYP3A4 induction. A number of PXR activators induce either CYP3A4 or P-glycoprotein or both (Lehmann et al 1998, Moore et al 2000, Geick et al 2001). PXR messenger ribonucleic acid (mRNA) is present in marked quantities in the liver and the small intestine but to a much lesser extent, if at all, in the kidneys (Kliewer et al 1998). Consequently, rifampicin seems to induce P-glycoprotein mainly in the small intestine and the liver (Greiner et al 1999, Westphal et al 2000, Hamman et al 2001).

Several widely used drugs inhibit the P-glycoprotein (Table I) and may therefore cause drug interactions with P-glycoprotein substrates (Silverman 2000). Inhibition of the intestinal P-glycoprotein may increase the systemic availability of P-glycoprotein substrates, whereas inhibition of the P-glycoprotein in the kidneys may reduce the renal excretion of P-glycoprotein substrates. For example, itraconazole raises plasma digoxin concentrations by reducing its renal clearance (Jalava et al 1997). On the other hand, inhibition of the P-glycoprotein at the blood-brain barrier may lead to an increase in the permeation of drugs

through the blood-brain barrier and to an increase in their effects on the central nervous system, as is seen with the antidiarrheal opioid loperamide after quinidine administration (Sadeque et al 2000). Apart from its role as a substrate for the P-glycoprotein, glibenclamide is also an inhibitor of the P-glycoprotein (Goldstein et al 1999). Furthermore, recent studies suggest that glibenclamide may be a general inhibitor of the ABC transporters (Payen et al 2001).

Table I. Examples of substrates, inhibitors, and inducers of CYP enzymes and P-glycoprotein. Adapted from Bertz & Granneman 1997 (1), Pelkonen et al 1998 (2), and Silverman 2000 (3) (other references in text).

SUBSTRATES					
CYP1A2	**CYP2C9***	**CYP2C19***	**CYP2D6***	**CYP3A4**	**P-glycoprotein**
Caffeine[1] Clozapine[1] Imipramine[1] Tacrine[1] Theophylline[1]	Celecoxib Diclofenac[1] Glibenclamide[1] Glimepiride[1] Glipizide[1] Ibuprofen[1] Losartan[1] Naproxen[1] Phenytoin[1] Piroxicam[1] S-warfarin[1] Tolbutamide[1] Tenoxicam	Amitriptyline[1] Diazepam[1] Imipramine[1] Lansoprazole[1] Omeprazole[1] S-mephenytoin[2]	Amitriptyline[1] Codeine[1] Debrisoquine[2] Flecainide[1] Fluoxetine[1] Fluvoxamine[1] Haloperidol[1] Imipramine[1] Metoprolol[1] Nortriptyline[1] Ondansetron[1] Oxycodone[1] Perphenazine[1] Propafenone[1] Risperidone[1] Thioridazine[1] Tramadol[1] Venlafaxine[1]	Alprazolam[1] Amiodarone[1] Carbamazepine[1] Cisapride[1] Ciclosporin[1] Diltiazem[1] Felodipine[1] HIV-protease inhibitors[1] Loratadine[1] Lovastatin[1] Midazolam[1] Nefazodone[1] Nifedipine[1] Repaglinide Simvastatin[1] Tacrolimus[1] Triazolam[1] Verapamil[1]	Celiprolol[3] Digoxin[3] Diltiazem[3] Erythromycin[3] Glibenclamide Indinavir[3] Ivermectin[3] Loperamide[3] Morphine[3] Nifedipine[3] Nelfinavir[3] Progesterone[3] Rifampicin[3] Saquinavir[3]
INHIBITORS					
Ciprofloxacin[1] Fluvoxamine[1] Furafylline[2]	Amiodarone Fluconazole[1] Miconazole Sulphaphenazole[2]	Fluconazole[1] Fluvoxamine Omeprazole[1]	Flecainide Fluoxetine Terbinafine Quinidine[1]	Clarithromycin[1] Erythromycin[1] Grapefruit juice HIV-protease inhibitors Itraconazole[1] Verapamil	Amiodarone[3] Ciclosporin[3] Glibenclamide Ketoconazole[3] Quinidine[3] Verapamil[3]
INDUCERS					
Charcoal grilled meat Cigarette smoke[2] Omeprazole[2]	Carbamazepine Dexamethasone Phenobarbital[2] Phenytoin Rifampicin[2]	Carbamazepine Dexamethasone Phenobarbital[2] Phenytoin Rifampicin[2]	not inducible	Carbamazepine[2] Dexamethasone[2] Phenobarbital[2] Phenytoin Rifampicin[2] St John's wort	Rifampicin St John's wort

* Enzyme exhibiting genetic polymorphism.

2. Oral antidiabetic drugs

2.1. General aspects

The first attempts to treat human diabetes by orally active drugs were made between 1925 and 1930 with synthalines and their derivatives. However, because of their toxicity, these compounds were never used in clinical practice. The hypoglycemic activity of some of the antibacterial sulfonamides was discovered in the 1940's, with carbutamide and tolbutamide the first sulfonamide derivative antidiabetic drugs in clinical use. The second-generation sulfonylurea, glibenclamide, has been in clinical use since 1969 and is per milligram 500 times as active as tolbutamide (Loubatières 1969). The oral antidiabetic drugs form the basis of the modern pharmacological treatment of NIDDM.

There are currently 5 groups of oral antidiabetic drugs in clinical use: the sulfonylureas, meglitinide analogues, biguanides, thiazolidinediones, and α-glucosidase inhibitors. In addition, also the dietary fiber guar gum can be used to treat hyperglycemia in patients with diabetes (Nuttall 1993). On the basis of their primary mechanism of action, the oral antidiabetic drugs can be divided into those that act by enhancing insulin secretion from the pancreas and those that act through extrapancreatic effects. The former include the sulfonylureas; the meglitinide analogues repaglinide and nateglinide are new short-acting insulin secretagogues (Landgraf 2000). Nateglinide differs from repaglinide in that it is even shorter-acting (Kalbag et al 2001) and is eliminated by metabolism by both CYP2C9 and CYP3A4 (Dunn & Faulds 2000). The effects of inducers or inhibitors of CYP enzymes on the pharmacokinetics and pharmacodynamics of nateglinide have not yet been studied.

Biguanides are an effective treatment in hyperglycemia, acting mainly by promoting glucose utilization and reducing hepatic glucose production (Dunn & Peters 1995). The only biguanide drug currently in clinical use in Finland is metformin. It is not metabolized to a significant extent and is excreted primarily unaltered in the urine (Dunn & Peters 1995). The thiazolidinediones rosiglitazone and pioglitazone enhance the effects of insulin on cellular glucose and lipid metabolism. Rosiglitazone and pioglitazone are both eliminated by extensive metabolism in the liver, mainly by the CYP2C8 enzyme (Mudaliar & Henry 2001). CYP2C9 catalyzes a minor pathway in the metabolism of

rosiglitazone, and secondary metabolic enzymes for pioglitazone include CYP3A4, CYP2C9, and CYP1A1/2 (Mudaliar & Henry 2001, Gillies & Dunn 2000). The effects of inducers or inhibitors of CYP enzymes on the pharmacokinetics and pharmacodynamics of rosiglitazone and pioglitazone have not been studied (Balfour & Plosker 1999, Gillies & Dunn 2000). The α-glucosidase inhibitors slow down carbohydrate absorption from the gut (Balfour & McTavish 1993). The first of the α-glucosidase inhibitors in clinical use was acarbose. It is eliminated through cleavage by the intestinal digestive enzymes and through biotransformation by the intestinal bacteria. Only trace amounts of unaltered acarbose are absorbed from the gastrointestinal tract into the systemic circulation (Balfour & McTavish 1993). Miglitol is a newer α-glucosidase inhibitor, and in contrast to acarbose, it is almost completely absorbed from the gastrointestinal tract after low doses, but its absorption is saturable (Scott & Spencer 2000). Miglitol is not metabolized and is excreted very rapidly unaltered (Scott & Spencer 2000).

2.2. Antidiabetic drugs studied

2.2.1. Pharmacodynamics

The oral antidiabetic drugs glimepiride, glibenclamide, glipizide, and repaglinide all act primarily by stimulating glucose-induced insulin release from pancreatic β-cells (Creutzfeldt 1969, Wensing 1989, Langtry & Balfour 1998, Guay 1998). The sulfonylureas and repaglinide block ATP-sensitive potassium channels in the β-cells (Sturgess et al 1985), which leads to membrane depolarization, influx of calcium into the cells, and eventually, to release of insulin. The ATP-sensitive potassium channel is a heteromultimeric protein comprising 2 subunits: the sulfonylurea receptor (SUR) and the inwardly rectifying potassium channel (KIR6.x) subunits, in 4:4 stoichiometry (Aguilar-Bryan et al 1998). The KIR6.x has 2 and the SUR has 3 subtypes (KIR6.1, KIR6.2, SUR1, SUR2A, SUR2B). The combination of KIR6.2 and SUR1 forms the pancreatic ATP-sensitive potassium channel, whereas the combination of KIR6.2 and either of the 2 SUR2 subtypes forms the ATP-sensitive potassium channels expressed in the heart (SUR2A) and smooth muscle (SUR2B) (Aguilar-Bryan et al 1998). The first-generation sulfonylurea tolbutamide has been proposed to bind relatively selectively to SUR1 (Gribble et al 1998), whereas glibenclamide, glimepiride, and repaglinide seem to bind both SUR1 and SUR2A with approximately

similar affinities (Gribble et al 1998, Song & Ashcroft 2001, Dabrowski et al 2001). However, glimepiride and repaglinide bind to different sites on SUR than does glibenclamide (Kramer et al 1994, Fuhlendorff et al 1998), and recent evidence suggests that glimepiride has fewer effects on the cardiovascular ATP-sensitive potassium channel and thereby possibly fewer cardiovascular adverse effects than glibenclamide has (Mocanu et al 2001).

The sulfonylureas and repaglinide may, in part, act also via extrapancreatic effects, and some evidence suggests that they may enhance glycogen synthesis and inhibit glycogenolysis and gluconeogenesis in the liver; they may also improve peripheral glucose uptake by the muscles (Wensing 1989, Langtry & Balfour 1998, Guay 1998).

2.2.2. Glimepiride

Glimepiride is the newest of the sulfonylurea drugs for the treatment of NIDDM. The daily dose of glimepiride is usually between 1 mg and 4 mg once daily. The maximum recommended dose is 6 mg daily.

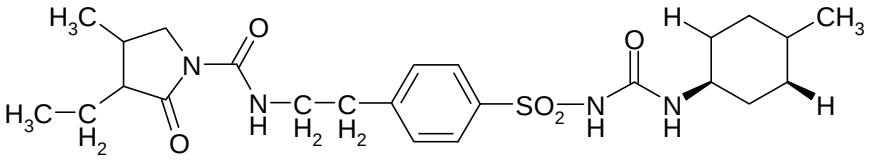

Figure 1. Chemical structure of glimepiride.

Pharmacokinetics. The pharmacokinetic characteristics of glimepiride have been summarized by Langtry and Balfour (1998). After oral administration, glimepiride is completely absorbed and has an oral bioavailability of nearly 100%. The peak plasma concentrations of about 100 ng/ml after a 1 mg dose of glimepiride are reached within 1 to 3 hours. Glimepiride is highly (more than 99%) bound to plasma proteins, and its volume of distribution is about 9 liters. Glimepiride is metabolized in the liver, mainly by CYP2C9, to an active hydroxy (M1) metabolite (Badian et al 1996), which is further dehydrogenated by the cytosolic alcohol and aldehyde dehydrogenase enzymes to an inactive carboxy (M2) metabolite. Approximately 50% of the total glimepiride dose is excreted in the urine as M1 and M2 metabolites; its $t_{½}$ is about 2 hours. The

pharmacokinetics of glimepiride are not significantly affected by age. In renal failure, its clearance may be slightly increased (Rosenkranz et al 1996).

Adverse effects. As with all sulfonylureas, the most common adverse effect of glimepiride is hypoglycemia (Langtry & Balfour 1998). However, the risk of hypoglycemia seems to be somewhat smaller with glimepiride than with either glibenclamide or glipizide (Langtry & Balfour 1998). Other common adverse effects include dizziness, headache, or asthenia, or gastrointestinal reactions such as nausea (Langtry & Balfour 1998). Allergic reactions and hepatic, renal, and hematological adverse effects are rare.

Pharmacokinetic interactions. Little is known about the pharmacokinetic interactions of glimepiride. It has no significant effect on the pharmacokinetics of warfarin (Langtry & Balfour 1998). Acetylsalicylic acid slightly reduced the C_{max} of glimepiride and reduced its AUC by 34% (Amaryl prescribing information 2000). The concomitant administration of propranolol with glimepiride has raised plasma glimepiride concentrations by approximately 20% and prolonged its $t_{½}$ by about 15% (Langtry & Balfour 1998). Cimetidine and ranitidine seem to have no major effects on glimepiride pharmacokinetics (Langtry & Balfour 1998).

2.2.3. Glibenclamide

Glibenclamide is one of the most widely used second-generation sulfonylureas and has been used in Europe since 1969. Its dosage is between 1.75 mg once daily and three 3.5 mg tablets daily (maximum recommended single dose 7 mg).

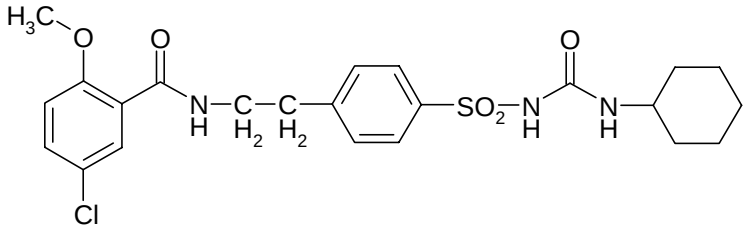

Figure 2. Chemical structure of glibenclamide.

Pharmacokinetics. Glibenclamide is well absorbed after oral administration; as a micronized formulation, it has an oral bioavailability that approaches 100% (Rydberg et al 1995). The peak plasma glibenclamide concentrations of about 100 ng/ml (after oral administration of 1.75 mg) are reached in 1 to 2 hours (Peart et al 1989). Glibenclamide is approximately 99% bound to plasma protein and has a low volume of distribution (9 to 10 liters) (Pearson 1985). It is extensively metabolized in the liver and has two hydroxylated metabolites: 4-trans-hydroxy-glibenclamide and 3-cis-hydroxy-glibenclamide (Pearson 1985). According to in vitro studies, these metabolites are produced primarily by CYP2C9 (Brian 2000). The hydroxylated metabolites of glibenclamide show hypoglycemic activity (Rydberg et al 1994, Rydberg et al 1997). However, this does not significantly contribute to its overall hypoglycemic activity, because the metabolites are rapidly excreted in urine and bile (Pearson et al 1986). The $t_{½}$ of glibenclamide is between 2 and 3 hours (Peart et al 1989, Coppack et al 1990). The free fraction of glibenclamide is increased, and its $t_{½}$ is prolonged in the elderly (Schwinghammer et al 1991).

Adverse effects. The most common serious adverse effect of glibenclamide is hypoglycemia (Asplund et al 1983). Usually it is mild but may, in some cases, be severe, prolonged, and potentially life-threatening. Glibenclamide may, though rarely, also cause hematological adverse effects such as leukopenia, thrombocytopenia, pancytopenia, agranulocytosis, aplastic anemia, or hemolytic anemia. Other adverse effects associated with glibenclamide therapy are gastrointestinal adverse effects such as nausea, abdominal discomfort, or anorexia, and allergic skin reactions such as pruritus, erythema, urticaria, morbilliform or maculopapular rash, or photosensitivity (Diabeta prescribing information 2000).

Pharmacokinetic interactions. Glibenclamide is metabolized primarily by CYP2C9. However, few controlled studies have investigated the effects on the pharmacokinetics of glibenclamide of known inducers or inhibitors of CYP2C9. In one study, fluconazole, a known inhibitor of CYP2C9, at 100 mg daily for 7 days boosted the AUC of glibenclamide by 44% and the maximum serum levels by 19% (Stockley 1999a). In that study, the blood glucose levels were not affected statistically significantly, but more subjects experienced symptomatic hypoglycemia associated with fluconazole. At least one death caused by hypoglycemia has been reported during concomitant use of fluconazole and glibenclamide (Diflucan prescribing information 1998). Miconazole is another potent inhibitor of CYP2C9. It is most often used topically, but case reports have described patients treated with unnamed sulfonylureas who developed severe hypoglycemia after taking systemic miconazole (Girardin et al 1992). A single oral dose of 120 mg verapamil increased the AUC of glibenclamide by about 26% (Semple et al 1986). It is possible that this was caused by inhibition of the P-glycoprotein, because verapamil is an inhibitor and glibenclamide is a substrate of the P-glycoprotein (Goldstein et al 1999).

One case report suggests that rifampicin may reduce the effects of glibenclamide (Self et al 1989). Surekha and coworkers studied the effects of rifampicin (450 or 600 mg daily) on blood glucose concentrations in 29 NIDDM patients treated with glibenclamide, and found that both fasting and post-prandial blood glucose levels were significantly worsened by rifampicin (Surekha et al 1997). Modifications of glibenclamide doses were needed in 15 patients.

A recent case report described an interaction between the quinolone antibiotic ciprofloxacin 250 mg twice daily and glibenclamide 5 mg daily in an elderly woman with NIDDM; this resulted in profound hypoglycemia (Roberge et al 2000). A study in patients with NIDDM showed that treatment with 1 g ciprofloxacin daily for one week caused a statistically non-significant increase of 25 to 36% in the AUC of glibenclamide (Stockley 1999b). One patient treated with glibenclamide developed severe hypoglycemia after starting treatment with gemfibrozil 1200 mg daily (Ahmad 1991), but the effects of gemfibrozil on the pharmacokinetics of glibenclamide have not undergone study. Ibuprofen increases the free fraction of glibenclamide, but has no effect on its pharmacokinetic parameters (Kubacka et al 1996). One patient treated with glibenclamide 2.5 mg daily lost consciousness because of hypoglycemia after a 150 mg dose of ibuprofen (Hirohito et al 2001). In a retrospective study on

glibenclamide-associated hypoglycemia, the concomitant use of trimethoprim-sulfamethoxazole was associated with increased risk of hypoglycemia (Asplund et al 1983). However, trimethoprim-sulfamethoxazole does not seem to affect the pharmacokinetics of glibenclamide (Sjöberg et al 1987). One patient had severe hypoglycemia associated with concomitant use of ranitidine and glibenclamide (Lee et al 1987). However, in one study, ranitidine had no effect on the pharmacokinetics of glibenclamide, whereas cimetidine boosted its AUC by about 37% (Kubacka et al 1987). Erythromycin seems to elevate slightly the rate of absorption of glibenclamide and enhance slightly its blood glucose-lowering effect (Fleishaker & Phillips 1991). Magnesium hydroxide may greatly enhance the absorption and hypoglycemic activity of non-micronized glibenclamide, whereas it has only a slight effect on the currently used micronized glibenclamide (Neuvonen & Kivistö 1991).

2.2.4. Glipizide

Glipizide is another second-generation sulfonylurea indicated for the treatment of NIDDM. The dosage of glipizide is between 2.5 mg and 15 mg once daily. It is recommended that if the daily dose exceeds 15 mg (maximum 20 mg) it should be given in 2 or 3 doses.

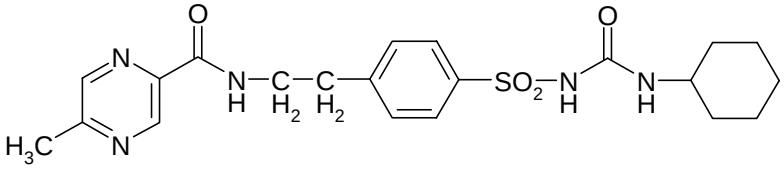

Figure 3. Chemical structure of glipizide.

Pharmacokinetics. Glipizide is completely absorbed from the gastrointestinal tract and has an oral bioavailability of nearly 100% (Wåhlin-Boll et al 1982, Pentikäinen et al 1983). Its peak plasma concentration is nearly 200 ng/ml after the oral administration of 2.5 mg glipizide, and is generally reached within 1 to 2 hours (Huupponen et al 1982, Wåhlin-Boll et al 1982). Like glibenclamide, glipizide is also highly bound to plasma protein (92% to 99%) (Pentikäinen et al 1983, Wensing 1989) and has a low volume of distribution (about 10 liters) (Wåhlin-Boll et al 1982, Pentikäinen et al 1983). Glipizide is extensively metabolized in the liver, and its main metabolites are 4-trans-hydroxyglipizide,

3-cis-hydroxyglipizide, and N-(2-acetyl-amino-ethyl-phenyl-sulfonyl) N-cyclo-hexylurea (DCAA) (Wensing 1989). CYP2C9 is probably the main CYP enzyme involved in the oxidative metabolism of glipizide, because in an individual homozygous for the defective CYP2C9*3 allele, the oral clearance of glipizide was drastically lower than in other subjects (Kidd et al 1999). The metabolites of glipizide are pharmacologically inactive and are excreted primarily in the urine (Pentikäinen et al 1983, Wensing 1989); its $t_{½}$ is between 2 and 4 hours (Wåhlin-Boll et al 1982, Pentikäinen et al 1983). Glipizide pharmacokinetics are unaffected by aging (Kradjan et al 1989).

Adverse effects. The main adverse effect of glipizide is hypoglycemia (Wensing 1989), which is potentially dangerous (Seltzer 1972). Other common adverse effects include gastrointestinal reactions such as nausea, vomiting, or epigastric pain, and skin reactions such as pruritus, erythema, urticaria, or morbilliform or maculopapular rash (Wensing 1989). Hepatic, renal, and hematological adverse effects are rare (Wensing 1989).

Pharmacokinetic interactions. Glipizide is very similar to glibenclamide with respect to its chemical structure, pharmacokinetic profile, and metabolism. One would therefore expect to see similar metabolic drug-drug interactions. However, relatively few have been reported. The CYP2C9 inhibitor fluconazole, 100 mg daily for 7 days, raised the AUC of glipizide by 49% (Stockley 1999a). As could be expected on the basis of current knowledge of the metabolism of glipizide, a single 20-mg oral dose of the CYP3A4 inhibitor nifedipine failed to affect significantly the pharmacokinetics of glipizide (Connacher et al 1987). In a study in patients with NIDDM, cimetidine (400 mg) and ranitidine (150 mg), taken together with a normal morning dose of glipizide, raised the AUC of glipizide by 23% and 34%, respectively (Feely et al 1993). In that study, the hypoglycemic activity of glipizide was also significantly increased. The cyclooxygenase inhibitor indobufen (200 mg twice daily for 5 days) elevated the AUC of glipizide by 25% (Elvander-Ståhl et al 1984). Magnesium hydroxide (850 mg) taken together with glipizide accelerated the absorption of glipizide and enhanced the early insulin and glucose responses (Kivistö & Neuvonen 1991a). Sodium bicarbonate, but not aluminum hydroxide, may also accelerate glipizide absorption (Kivistö & Neuvonen 1991b). The dietary fiber guar gum used for NIDDM patients to treat hyperglycemia does not affect the absorption of glipizide (Huupponen et al 1985).

2.2.5. Repaglinide

Repaglinide, a benzoic acid derivative, is a structural analogue of meglitinide, the nonsulfonylurea moiety of glibenclamide (Landgraf 2000). It is the first of the meglitinide analogues that has reached clinical use and is indicated for the treatment of NIDDM. Repaglinide is shorter-acting than any of the sulfonylureas and may prove useful in lowering postprandial glucose excursions (Landgraf et al 1999), which are relatively poorly controlled by the sulfonylureas. Repaglinide is administered preprandially in a single dose ranging between 0.5 mg and 4 mg. The maximum recommended daily dose is 16 mg.

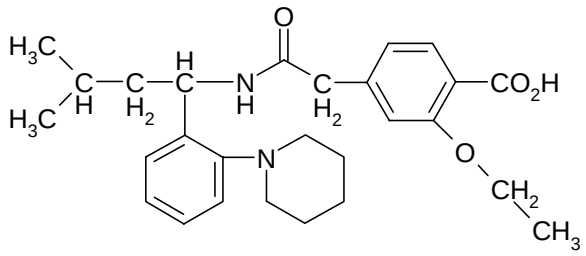

Figure 4. Chemical structure of repaglinide.

Pharmacokinetics. Repaglinide has an oral bioavailability of about 60%, mainly because of first-pass metabolism (Hatorp et al 1998). The peak plasma concentrations of about 10 ng/ml after the administration of a single 0.5 mg dose are reached in approximately 1 hour (Prandin prescribing information 1998). Repaglinide is highly (more than 98%) bound to serum proteins, and its volume of distribution is about 31 liters (Guay 1998). Repaglinide is metabolized in the liver to three major inactive metabolites, an aromatic amine (M1), a dicarboxylic acid (M2), and an acyl glucuronide (M7) (Guay 1998). M2 is the major metabolite in humans, and in vitro studies indicate that CYP3A4 is involved in the formation of M1 and M2 metabolites (Prandin prescribing information 1998). The metabolites are excreted primarily in the feces, with only about 8% of the total repaglinide dose found in the urine (van Heiningen et al 1999). Consequently, repaglinide was well tolerated in subjects with renal impairment (Marbury et al 2000). On the other hand, the clearance of repaglinide is significantly reduced in patients with hepatic impairment (Hatorp et al 2000). The $t_{½}$ of repaglinide is about 1 hour (Hatorp et al 1999). The pharmacokinetics of repaglinide are similar in healthy subjects both young and old (Hatorp et al 1999).

Adverse effects. The most common adverse effect of repaglinide is hypoglycemia (Guay 1998), but the risk of hypoglycemia seems to be slightly smaller with repaglinide than with glibenclamide or glipizide (Guay 1998). Other common adverse effects include gastrointestinal reactions such as nausea and vomiting, diarrhea, constipation and dyspepsia, and headache (Guay 1998). Allergic reactions are rare (Guay 1998).

Pharmacokinetic interactions. In one study, cimetidine (400 mg twice daily) did not significantly affect the pharmacokinetics of repaglinide (Hatorp & Thomsen 2000). Repaglinide slightly, but significantly, reduced the C_{max} of theophylline but had no effect on its other pharmacokinetic variables (Hatorp & Thomsen 2000). Repaglinide did not affect digoxin pharmacokinetics (Hatorp & Thomsen 2000).

2.2.6. Pharmacodynamic interactions with sulfonylureas and repaglinide

Several drugs can affect glucose metabolism and are therefore often considered potentially harmful for patients with NIDDM (Chan et al 1996). In many cases these drugs are, however, tolerated well enough, and for instance β-adrenergic receptor antagonists are used more often by patients with NIDDM than by other individuals of their age, healthy or ill (Reunanen et al 2000), probably because of the severity and greater incidence of coronary heart disease in patients with NIDDM. Some of the drugs reported to cause pharmacodynamic interactions with sulfonylureas or repaglinide resulting in hypoglycemia or hyperglycemia are summarized in Table II. Most of the hypoglycemic deaths are explained by the combined use of hypoglycemic drugs (e.g., insulin or sulfonylureas) and ethanol (Chan et al 1996). Ethanol inhibits gluconeogenesis, an inhibition particularly dangerous in patients with insufficient glycogen reserves, i.e., in fasted or malnourished subjects or those having just undertaken exhausting sports (Chan et al 1996).

Table II. Examples of drugs associated with hypoglycemic or hyperglycemic pharmacodynamic interactions with sulfonylureas or repaglinide.

Drug class	*Hypoglycemic*	*Hyperglycemic*
Antihyperglycemic drugs	Metformin	
	Thiazolidinediones	
NSAIDs	Acetylsalicylic acid	
Cardiovascular drugs	ACE-inhibitors	β-adrenoceptor antagonists
	β-adrenoceptor antagonists	Diazoxide
	Disopyramide	Diuretics
Sympathomimetic drugs	β-adrenoceptor agonists (salbutamol; overdose)	β-adrenoceptor agonists (salbutamol)
		Theophylline
Hormones	IGF-I	Corticosteroids
	Insulin	Growth hormone
	Octreotide	Oral contraceptive steroids (progestogens)
Psychotropic drugs	Lithium	Caffeine (overdose)
	Monoamine oxidase inhibitors	Chlordiazepoxide
	Tricyclic antidepressants	Clozapine
		Cocaine
		Lithium
		Mianserin
		Phenothiazines
Immunosuppressives and immunomodulators		Ciclosporin
		Interleukin-6
		Tacrolimus
Antimalarial drugs	Chloroquine	
	Mefloquine	
	Quinidine	
	Quinine	
Other	Ethanol	
	Trimethoprim-Sulfamethoxazole	

Adapted from Chan et al 1996.

2.2.7. Role of sulfonylureas and repaglinide in the management of type 2 diabetes mellitus

The UK Prospective Diabetes Study (UKPDS; began 1977) investigated whether intensive blood-glucose control reduced the risk of macrovascular or microvascular complications in patients with NIDDM. This study included patients treated with metformin, insulin, the first-generation sulfonylurea chlorpropamide, or either of the second-generation sulfonylureas glibenclamide or glipizide, or a combination of a sulfonylurea with metformin or insulin. The UKPDS demonstrated that intensive blood-glucose control by the use of sulfonylureas or insulin markedly reduces the risk of microvascular complications, but seems not to reduce the risk of macrovascular disease (UK Prospective Diabetes Study Group 1998). Treatment of hyperglycemia can therefore be considered one of the cornerstones of the management of NIDDM.

Hyperglycemia in patients with NIDDM is primarily treated with diet, because many patients are overweight. However, the blood glucose levels are often not sufficiently lowered by diet alone. The second-line treatment is the use of oral antidiabetic drugs. When the disease proceeds, blood glucose levels may not be sufficiently controlled by the oral antidiabetic drugs, so that insulin therapy becomes necessary.

Because macrovascular disease seems not to be effectively prevented by the lowering of high blood glucose levels, recent research has investigated the possible means of its prevention. Intervention trials have suggested that in NIDDM patients, effective treatment of hypertension and of hypercholesterolemia, and small doses of acetylsalicylic acid may prevent macrovascular disease (Yki-Järvinen 2000). The most common form of dyslipidemia in these patients is a combination of high serum triglyceride and low high-density lipoprotein cholesterol levels. Several studies are currently investigating whether treatment of dyslipidemias with either the HMG-CoA reductase inhibitors or fibrates or their combination is effective in the primary or secondary prevention of macrovascular disease in such patients (Yki-Järvinen 2000). Because of the severity of the disease and its associated comorbidities, patients with NIDDM need to use almost all drugs more often than do other individuals of their age (Reunanen et al 2000) and are thus more likely to experience harmful drug interactions.

3. Rifampicin

Rifampicin was developed in the 1960s and was the first synthetic derivative of the antibiotic rifamycin B to be active when given orally. It inhibits bacterial RNA polymerase by forming a stable drug-enzyme complex (Wehrli 1983). Rifampicin is used primarily in the treatment of tuberculosis (Douglas & McLeod 1999), in which case it is always administered in combination with other drugs such as isoniazid, ethambutol, or pyrazinamide to prevent development of resistance. The dosage of rifampicin in adults in treatment of tuberculosis is usually between 450 and 600 mg once daily (Douglas & McLeod 1999). Rifampicin is a useful orally active alternative also in the treatment of both methicillin-sensitive and methicillin-resistant staphylococcal infections (Turnidge & Grayson 1993).

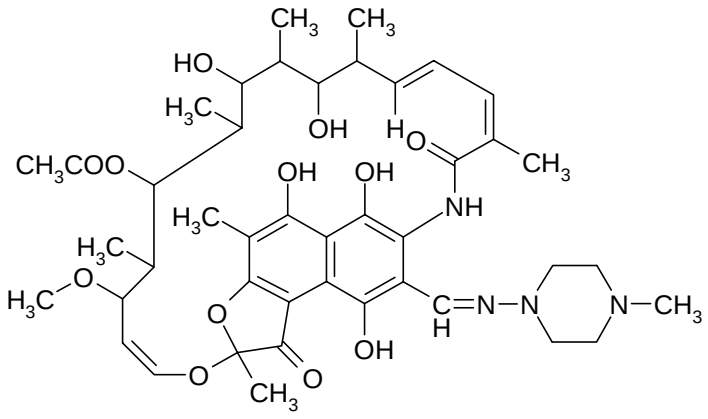

Figure 5. Chemical structure of rifampicin.

Pharmacokinetics. Rifampicin is well absorbed after oral administration and reaches its peak plasma concentrations of about 4 to 32 μg/ml in 2 to 4 hours after administration of 600 mg (Acocella 1978, Holdiness 1984). Its oral bioavailability is about 68% during continuous treatment (Loos et al 1987). Rifampicin is approximately 80% protein bound in the plasma (Acocella 1978), and its volume of distribution is about 50 to 60 liters (Holdiness 1984). Its main metabolites in man are desacetylrifampicin, which is formed by desacetylation, and formylrifampicin, which is formed by hydrolysis (Acocella 1978, Holdiness 1984). Formylrifampicin is found only in the urine, but desacetylrifampicin and parent rifampicin, both pharmacologically active, are excreted primarily in the bile (Acocella 1978). The $t_{½}$ of rifampicin after an initial dose is about 2 to 5

hours (Holdiness 1984) and decreases during continuous treatment because of autoinduction (Loos et al 1987).

Adverse effects. Significant adverse drug reactions to rifampicin at therapeutic dosages are rare. However, rifampicin may cause hepatotoxicity, especially in patients with chronic liver disease, alcoholism, or poor nutrition, or in the elderly (Douglas & McLeod 1999). Intermittent high-dose rifampicin therapy or the reinstitution of rifampicin therapy has been associated with an immune-mediated reaction with flu-like symptoms, which may lead to acute renal failure (Douglas & McLeod 1999).

Interactions. Rifampicin is a potent inducer of several of the human drug-metabolizing enzymes. It strongly induces CYP3A4 both in the liver and in the intestine (Combalbert et al 1989, Kolars et al 1992) and therefore reduces the plasma concentrations and effects of numerous CYP3A4 substrates (Venkatesan 1992, Backman et al 1996a, Villikka et al 1997, Lamberg et al 1998a, Kyrklund et al 2000, Branch et al 2000). In cell cultures, rifampicin has also been shown to induce CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A5, but not CYP2C18 (Sumida et al 2000, Dalet-Beluche et al 1992, Chang et al 1997, Morel et al 1990, Gerbal-Chaloin et al 2001, Wrighton & Thummel 2000). Drug interaction studies show that rifampicin induces CYP2C enzymes also in vivo (Syvälahti et al 1976, Feng et al 1998, Branch et al 2000) and suggest that rifampicin may also induce CYP1A2 in vivo (Bachmann & Jauregui 1993, Branch et al 2000). In cell cultures, rifampicin also induces some phase II enzymes such as UGT and sulfotransferase (Doostdar et al 1993, Kern et al 1997), and increases in vivo the glucuronidation of propafenone (Dilger et al 2000) but not that of morphine (Fromm et al 1997). It therefore seems that, as with the CYP-enzymes, rifampicin has isoenzyme-specific inducing effects on phase II enzymes. Apart from the CYP enzymes, rifampicin is also a potent inducer of the P-glycoprotein and may reduce the plasma concentrations of P-glycoprotein substrates (Schuetz et al 1996). In addition, a recent study showed that rifampicin also induces intestinal MRP2 (multidrug resistance protein 2), which is known to be involved in the efflux of drug conjugates (Fromm et al 2000).

4. CYP inhibitors studied

4.1. Fluconazole

Fluconazole was synthesized in 1982 and belongs to the triazole group of the azole antimycotics. Fluconazole, by binding to the fungal CYP enzyme lanosterol 14α-demethylase, inhibits fungal synthesis of ergosterol (Grant & Clissold 1990), an essential compound of the fungal cell membrane. Inhibition of ergosterol synthesis leads to abnormalities in membrane permeability, in the activity of membrane-bound enzymes, and in coordination of chitin synthesis (Grant & Clissold 1990). Fluconazole, in oral and intravenous formulations, is used in the treatment of superficial and deep candidiasis, dermatophytosis, and also cryptococcal meningitis; dosage is between 50 mg and 400 mg once daily, depending on severity of infection (Grant & Clissold 1990, Debruyne 1997).

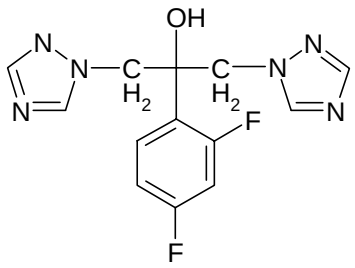

Figure 6. Chemical structure of fluconazole.

Pharmacokinetics. Fluconazole is well absorbed after oral administration and, after a single dose, the peak plasma concentrations of about 2 μg/ml (after 100 mg orally) to 7 μg/ml (after 400 mg orally) are reached within 1 to 2 hours (Grant & Clissold 1990). Its oral bioavailability exceeds 90% (Grant & Clissold 1990). It is only slightly bound to plasma proteins (about 11%) and has a relatively large volume of distribution of some 50 to 60 liters. Fluconazole is excreted primarily unaltered in the urine (up to 80% of the total dose), and its $t_{½}$ ranges between 27 and 37 hours (Grant & Clissold 1990). Because fluconazole is eliminated by renal excretion, its elimination may be prolonged in patients with impaired renal function. In patients whose glomerular filtration rate is < 20 ml/min, its $t_{½}$ may be prolonged, up to 100 hours (Grant & Clissold 1990).

Adverse effects. Fluconazole is generally well tolerated. The most common adverse effects include various gastrointestinal symptoms: nausea, abdominal pain, vomiting, and diarrhea. Headache and skin rash have also been associated with its use. Abnormalities in hematological, renal, or liver function parameters have been observed in rare cases in patients with an underlying severe disease such as AIDS (Grant & Clissold 1990). There have also been rare reports of anaphylaxia.

Interactions. Fluconazole is a relatively potent inhibitor of CYP2C9 both in vitro and in vivo (Backman et al 2000a). Accordingly, 400 mg fluconazole daily for 6 days inhibited the hydroxylation of S-warfarin, a CYP2C9-catalyzed reaction, by 70% (Black et al 1996), and 200 mg daily fluconazole for 14 days boosted the AUC of intravenous phenytoin, a substrate of CYP2C9, by 75% (Blum et al 1991). In addition, fluconazole (400 mg on day 1 and 200 mg on days 2-4) boosted the AUC(0-∞) of fluvastatin, an HMG-CoA reductase inhibitor that is primarily metabolized by CYP2C9, by 84% (Kantola et al 2000) and reduced the AUC(0-∞) of the active E-3174 metabolite of losartan by 53% (Kaukonen et al 1998). Fluconazole also inhibits CYP3A4 (Backman et al 2000a): fluconazole (400 mg on day 1 and 200 mg on days 2-6) boosted the AUC(0-∞) of oral midazolam, a CYP3A4 probe drug, to 3.6-fold that of the control (Olkkola et al 1996). In comparison, the known potent CYP3A4 inhibitor itraconazole (200 mg daily for 4 days) raised the AUC(0-∞) of oral midazolam 10- to 15-fold (Olkkola et al 1994). In vitro studies have shown that fluconazole can also inhibit CYP2C19 (Backman et al 2000a).

4.2. Fluvoxamine

Fluvoxamine is an antidepressant drug that specifically inhibits neuronal uptake of serotonin (5-hydroxytryptamine) (Perucca et al 1994). It is used mainly in the treatment of major depression, with doses in adults ranging between 50 and 300 mg daily (Perucca et al 1994). Other therapeutic indications include obsessive-compulsive disorder, anxiety, and eating disorders (Perucca et al 1994, Figgitt & McClellan 2000). Fluvoxamine is as effective in the treatment of depression as are the tricyclic antidepressant drugs (Perucca et al 1994). Compared with the tricyclic antidepressants, fluvoxamine has a low incidence of anticholinergic adverse effects and decreased cardiotoxicity.

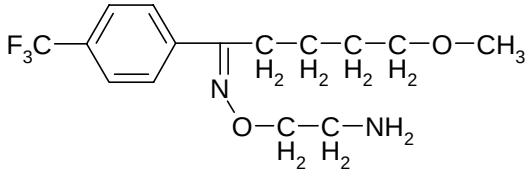

Figure 7. Chemical structure of fluvoxamine.

Pharmacokinetics. Fluvoxamine is almost completely but relatively slowly absorbed from the gastrointestinal tract (Figgitt & McClellan 2000). The peak plasma concentration of about 70 ng/ml (after repeated administration of 100 mg daily) is reached within 2 to 8 hours after its administration (Figgitt & McClellan 2000). It is only 77% bound to plasma protein (Perucca et al 1994). Because it has not been given to humans intravenously, its oral bioavailability and volume of distribution are unknown. Fluvoxamine is eliminated mainly by oxidative metabolism, with only less than 4% excreted unchanged in the urine (Perucca et al 1994). The $t_{½}$ of fluvoxamine is about 15 to 20 hours (Perucca et al 1994).

Adverse effects. Fluvoxamine is generally well tolerated; nausea is the most common adverse effect. Other, less common, adverse effects include somnolence, asthenia, headache, dry mouth and insomnia (Figgitt & McClellan 2000). Some patients may experience excitation, agitation, or increased anxiety at the initiation of fluvoxamine therapy (Figgitt & McClellan 2000). Because fluvoxamine inhibits serotonin reuptake, it may, although rarely, cause serotonin syndrome (Figgitt & McClellan 2000).

Interactions. Fluvoxamine is a potent inhibitor of CYP1A2 (Brøsen 1995, Rasmussen et al 1995) and has also been shown to inhibit CYP2C19, CYP2D6, and CYP3A4 (Rasmussen et al 1998, Kashuba et al 1998, Lamberg et al 1998b), and may therefore interact with substrates of these enzymes. In vitro studies show that fluvoxamine can also inhibit CYP2C9 (Schmider et al 1997, Hemeryck et al 1999, Olesen & Linnet 2000). This was confirmed recently by an in vivo study demonstrating that fluvoxamine inhibits the CYP2C9-mediated biotransformation of tolbutamide (Madsen et al 2001).

4.3. Gemfibrozil

Gemfibrozil is a fibric acid derivative used in the treatment of dyslipidemias. It effectively lowers plasma triglyceride concentrations and raises high density lipoprotein (HDL) cholesterol levels (Todd & Ward 1988). It enhances lipoprotein lipase activity, reduces hepatic triglyceride production, and enhances the clearance of triglycerides from plasma (Todd & Ward 1988). This, at least in part, may be caused by activation of the PPARα receptor by gemfibrozil (Fuhr 2000).

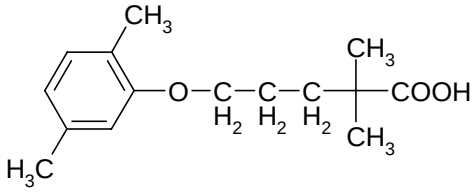

Figure 8. Chemical structure of gemfibrozil.

Pharmacokinetics. Gemfibrozil is well absorbed after oral administration and has an oral bioavailability of nearly 100% (Miller & Spence 1998). The peak plasma concentrations are approximately 15 μg/ml to 25 μg/ml at a dosage of 600 mg of immediate-release gemfibrozil twice daily (Todd & Ward 1988). Gemfibrozil is highly bound to plasma proteins (about 98%) (Todd & Ward 1988). It is metabolized in man to several compounds, its principal metabolite being a benzoic acid derivative; all the metabolites except one and the parent gemfibrozil form glucuronide conjugates and are then excreted mainly in the urine (Todd & Ward 1988). The $t_{½}$ of gemfibrozil is about 1.5 hours (Miller & Spence 1998).

Adverse effects. Gemfibrozil is well tolerated in most patients. The most common adverse effects are gastrointestinal, including abdominal pain, diarrhea, nausea, vomiting, and flatulence, and also rash. Gemfibrozil may also lead to an increased incidence of gallstone formation and occasionally increased transaminase levels (Todd & Ward 1988). In rare cases, gemfibrozil use has been associated with myositis or potentially life-threatening rhabdomyolysis, most often when it is used in combination with an HMG-CoA reductase inhibitor (Miller & Spence 1998). Cerivastatin seems to be one of the most susceptible HMG-CoA reductase inhibitors of this interaction, and the

concomitant use of gemfibrozil with cerivastatin has recently become contraindicated (Anonymous 2001).

Interactions. Case reports have suggested that gemfibrozil and some other fibrates may increase the effects of sulfonylureas and warfarin (Ahmad 1991, Girardin et al 1992, Ahmad 1990, Rindone & Keng 1998). In recent studies, gemfibrozil has been shown to raise plasma concentrations of the active HMG-CoA reductase inhibitors simvastatin acid and lovastatin acid (Backman et al 2000b, Kyrklund et al 2001), but not those of the parent simvastatin and lovastatin, both of which are substrates of CYP3A4 (Prueksaritanont et al 1997, Wang et al 1991). The interactions of gemfibrozil with some HMG-CoA reductase inhibitors therefore seem to be at least partially pharmacokinetic. Gemfibrozil does not inhibit CYP3A4 in vitro (Backman et al 2000b), nor does it seem to inhibit the P-glycoprotein (Fehrman-Ekholm et al 1996, Pisanti et al 1998). On the other hand, recent results from in vitro studies indicate that gemfibrozil is a potent inhibitor of CYP2C9 (Wang et al 2001a, Wen et al 2001).

4.4. Clarithromycin

Clarithromycin is a semisynthetic macrolide antibiotic. Like the macrolide erythromycin, it has a 14-membered ring, but differs from erythromycin by the methylation of a hydroxyl group (Rodvold 1999). This modification increases the acid stability of clarithromycin and also reduces gastrointestinal adverse effects compared with the actions of erythromycin (Rodvold 1999). Clarithromycin binds to bacterial 23S ribosomal RNA and inhibits bacterial protein synthesis (Zhanel et al 2001). It is used mainly in the treatment of upper and lower respiratory tract infections (Zhanel et al 2001), and skin and soft tissue infections. Clarithromycin may also be used in the treatment of leprosy, and opportunistic mycobacterial infections and in the eradication of *Helicobacter pylori*.

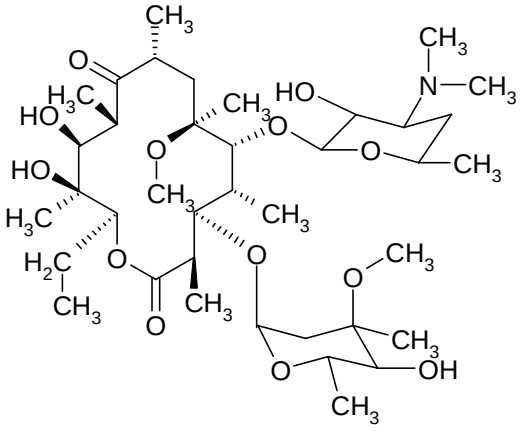

Figure 9. Chemical structure of clarithromycin.

Pharmacokinetics. Clarithromycin, in tablet form, is usually rapidly absorbed with the C_{max} (0.46 to 0.65 μg/ml after a single 250-mg oral dose) occurring between 1.8 and 2.8 hours (Rodvold 1999). It has an oral bioavailability of only about 55% (Chu et al 1992a) because of first-pass metabolism, a bioavailability which may be slightly increased when the tablets are taken with food (Chu et al 1992b). Clarithromycin is between 42 and 72% bound to plasma proteins and has an apparent volume of distribution of between 200 and 400 liters (Rodvold 1999). Clarithromycin is extensively metabolized in the liver, with CYP3A4 playing an important role in its metabolism (Rodrigues et al 1997). The elimination kinetics of clarithromycin depend on the dose: the $t_{½}$ is between 2 and 4 hours with a dosage of 250 mg twice daily and about 5 hours at higher doses (Rodvold 1999). The $t_{½}$ of the active 14-hydroxy metabolite of clarithromycin is about 5 hours (Rodvold 1999).

Adverse effects. Clarithromycin is generally well-tolerated, and significant adverse drug reactions are rare. Gastrointestinal intolerance is the most common adverse effect associated with the use of such macrolide antibiotics and is dose-related (Zhanel et al 2001): the macrolide erythromycin is associated with a high frequency of gastrointestinal intolerance (20% to 50% of patients), whereas the newer derivatives such as clarithromycin seem to cause gastrointestinal adverse effects less frequently (Chien et al 1993). Clarithromycin may cause hepatotoxicity, although the risk is low (Zhanel et al 2001).

Interactions. Drugs that modulate CYP3A4 activity can affect clarithromycin plasma concentrations, because clarithromycin is metabolized by CYP3A4 (Rodrigues et al 1997). The mean serum levels of clarithromycin were reported to be 13% of the control value during 600 mg rifampicin daily (Wallace et al 1995). Fluconazole (400 mg on day 1 and 200 mg on days 2 to 4) elevated the C_{max} and AUC(0-12) of clarithromycin by 12% and 18%, respectively (Gustavson et al 1996). Apart from being a substrate of CYP3A4, clarithromycin is also a potent inhibitor of CYP3A4, as has been shown in vitro (Gascon & Dayer 1991, Mayhew et al 2000) and in vivo (Westphal 2000). Clarithromycin forms an inactive iron-metabolite complex with the enzyme and thus inhibits its catalytic activity. It increases the plasma concentrations of several CYP3A4 substrates, for example those of midazolam, triazolam, and cisapride (Westphal 2000). The effect of clarithromycin on CYP3A4 activity in vivo is dose-dependent: clarithromycin 250 mg twice daily boosted the AUC of the CYP3A4 probe drug midazolam 3.6-fold (Yeates et al 1996), whereas 500 mg clarithromycin twice daily boosted it approximately 7-fold (Gorski et al 1998). Clarithromycin does not seem to inhibit CYP1A2, CYP2C9, or CYP2D6 activities in vivo (Bruce et al 2001) but can raise plasma concentrations of digoxin, probably by inhibiting the P-glycoprotein-mediated renal and biliary excretion of digoxin (Wakasugi et al 1998).

Table III. Inhibitory potencies of fluconazole, fluvoxamine, gemfibrozil, and clarithromycin on selected CYP enzymes (expressed as K_i in μmol/l), based on in vitro studies with human hepatic microsomes.

Drug	*CYP1A2*	*CYP2C9*	*CYP2C19*	*CYP2D6*	*CYP3A4*
Fluconazole[1]	> 800	7-17	2	-	1.27- > 80
Fluvoxamine[2,3]	0.041-0.24	2.2-6.0	0.087	3.9-16.6	10.0-40
Gemfibrozil[4]	82	5.8	24	*	*
Clarithromycin[5]	-	-	-	-	K_I = 5.49, k_{inact} = 0.072 †

K_i, inhibition constant; K_I (μmol/l), concentration of mechanism-based inhibitor required for half-maximal inactivation; k_{inact} (min^{-1}), maximal rate of mechanism-based inactivation at saturation

* lack of significant effect in the concentration range 5-250 μmol/l

† mechanism-based inhibition

Sources: [1]Backman et al 2000a, [2]Olesen & Linnet 2000, [3]Shad & Preskorn 2000, [4]Wen et al 2001, [5]Mayhew et al 2000

AIMS OF THE STUDY

The sulfonylureas glibenclamide, glimepiride, and glipizide are metabolized by the CYP2C9 enzyme. In addition, glibenclamide is a well-established substrate of the P-glycoprotein drug transporter. Repaglinide, on the other hand, is a substrate of CYP3A4. Although there is some information on the effects of modulators of CYP enzymes and P-glycoprotein on these drugs, no controlled studies exist on the effects of inducers of CYP enzymes or of P-glycoprotein, on the pharmacokinetics and pharmacodynamics of these drugs. Nor are there any published studies on the effects of potent CYP inhibitors on glimepiride and repaglinide. Induction and inhibition of CYP enzymes can have clinically significant effects on the plasma concentrations and effects of such antidiabetic drugs. To test this hypothesis, these studies were conducted to examine the effects of induction and inhibition of CYP enzymes on the pharmacokinetics and pharmacodynamics of these oral antidiabetic drugs.

The specific aims of the studies were:

1. Investigation of the effects of induction of CYP enzymes, with rifampicin serving as the model inducer, on the pharmacokinetics and pharmacodynamics of glibenclamide, glimepiride, glipizide, and repaglinide (Studies I-III)

2. Investigation of the effects of the CYP2C9 inhibitors fluconazole, fluvoxamine, and gemfibrozil on the pharmacokinetics and pharmacodynamics of glimepiride (Studies IV and V)

3. Investigation of the effects of the CYP3A4 inhibitor clarithromycin on the pharmacokinetics and pharmacodynamics of repaglinide (Study VI)

MATERIALS AND METHODS

1. Subjects

A total of 43 (29 female and 14 male) healthy volunteers participated in the studies. They were ascertained to be healthy by a medical history, a physical examination, and routine laboratory tests, including fasting serum glucose, before they were entered in the studies. None of the subjects was using continuous medication, except for several female subjects on oral contraceptive steroids. When the pretreatment was rifampicin, these women were advised to use other methods of contraception starting one week before the first pre-medication day and continuing until the end of the first full menstrual cycle after the study. This was because the use of rifampicin or other CYP-inducing drugs increases the metabolism of steroid hormones and thus reduces the effects of oral contraceptives. Use of other drugs and grapefruit or grapefruit juice was prohibited starting 2 weeks before each study day. Participation in any other trial and blood donation within one month before and after the study were also prohibited. The subjects are described in Table IV.

Table IV. Characteristics of the subjects.

Study No.	*Subjects (male/female)*	*Age (years)*	*Weight (kg)*	*Smoker (yes/no)*	*Users of oral contraceptives*
I	5/5	23 ± 2	63 ± 8	3/7	5
II a)	4/6	24 ± 5	68 ± 11	1/9	6
II b)	2/8	23 ± 2	67 ± 8	1/9	4
III	4/5	23 ± 2	66 ± 11	2/7	2
IV	6/6	24 ± 2	63 ± 10	3/9	4
V	2/8	23 ± 2	65 ± 10	1/9	8
VI	5/4	24 ± 2	67 ± 10	3/6	3
		23 ± 3	66 ± 10		

Age and weight data are mean ± SD.

2. Study designs

All the six were randomized, placebo-controlled crossover studies and were carried out in the Department of Clinical Pharmacology, University of Helsinki.

Details of the designs are given in Table V. Studies I, III, V, and VI consisted of two phases and Study IV of three phases. Study II comprised two substudies with two phases each and an identical design. The washout period was 4 weeks in all studies except Study V, in which it was 2 weeks.

The pretreatment medications and matched placebos were supplied, packed, and labeled according to a randomization list for each subject by the Pharmacy of the Helsinki University Central Hospital. The study drugs (sulfonylureas and repaglinide) were also supplied by the Pharmacy of the Helsinki University Central Hospital. In Studies IV, V, and VI, glimepiride and repaglinide tablets were halved by the investigators. Glimepiride 1 mg tablets have double bisects to facilitate the division of the tablet into equal halves. Repaglinide 0.5 mg tablets do not contain bisects, and the tablets were weighed before and after halving. The mean weight of the original tablets was 98.1 mg (n=30; coefficient of variation, CV, 1.2%) and of the halved tablets 49.1 mg (n=30; CV 0.7%). The greatest percentage deviation from the mean weight of the halved tablets was less than 2%. The study drugs were given after an overnight fast at 8:30 in the morning in Studies I, II, and III and at 9:00 in Studies IV, V, and VI. The volunteers ingested the study drugs with 150 ml water while seated, and spent the next 3 hours seated. Each received a standardized breakfast precisely 15 minutes after administration of the study drug, a standardized warm meal after 3 hours and a standardized light meal after 7 hours. The breakfast was eaten within 10 minutes and contained about 370 kcal energy, 70 g carbohydrates, 8 g protein, and 6 g fat. Food intake was identical in all study phases. In Study VI, the subjects received in addition two standard snacks precisely 1 and 2 hours after the administration of the study drug. The snacks were identical, eaten within 5 minutes and containing about 200 kcal energy, 45 g carbohydrates, 2 g protein, and 1 g fat.

Table V. Structure of the studies.

Study No.	*Pretreatment medication and dose*	*Duration of pretreatment*	*Washout period (weeks)*	*Study drug and dose*	*Administration of study drug*
I	Each with rifampicin 600 mg or placebo once daily	5 days (at 20:00)	4	Glimepiride 1 mg	Each given on day 6 at 8:30-9:00
II a)			4	Glibenclamide 1.75 mg	
b)			4	Glipizide 2.5 mg	
III			4	Repaglinide 0.5 mg	
IV	Fluconazole (400 mg on day 1 and 200 mg on days 2-4), fluvoxamine 100 mg or placebo once daily	4 days (at 8:00)	4	Glimepiride 0.5 mg	On day 4 at 9:00-9:30
V	Gemfibrozil 600 mg or placebo twice daily	3 days (at 8:00 and 20:00)	2	Glimepiride 0.5 mg	On day 3 at 9:00-9:30
VI	Clarithromycin 250 mg or placebo twice daily	5 days (at 8:00 and 20:00)	4	Repaglinide 0.25 mg	On day 5 at 9:00-9:30

3. Blood sampling

On the days of administration of the study drug, a plastic cannula was inserted into a forearm vein of each subject and kept patent with an obturator. Timed blood samples were drawn just before administration of the study drug and ½, 1, 1½, 2, 2½, 3, 4, 5, 7, 9, and 12 hours later in Studies I, II, IV, and V. In Study III, the blood samples were collected before the administration of repaglinide and 15, 30, and 45 minutes and 1, 1½, 2, 2½, 3, 4, 5, and 7 hours later. In Study VI, the blood samples were taken before the administration of repaglinide and 20, 40, 60, 80, and 100 minutes and 2, 2½, 3, 4, 5, and 7 hours later. The blood samples (10 ml each) were taken into tubes that contained 0.1 ml ethylenediaminetetraacetic acid (0.47 mol/l; EDTA) in all studies. In Studies V and VI, additional 5 ml blood samples were taken at all time-points into tubes with an inert gel barrier and a clot activator disc for serum samples. The serum tubes in Studies V and VI were placed on ice, and serum was separated by centrifugation with a relative centrifugal force of 1900g immediately after blood sampling. The plasma samples were separated by centrifugation with a relative

centrifugal force of 1900g within 30 minutes after blood sampling. The samples were stored at -40°C (Studies I and III-VI) or at -80°C (Study II) until analysis.

4. Determination of plasma drug concentrations

4.1. Sulfonylureas

In Studies I, II, IV, and V, the plasma concentrations of three sulfonylureas: glimepiride, glibenclamide, and glipizide, were determined by liquid chromatography-tandem mass spectrometry. The system was equipped with a Perkin Elmer Series 200 injector and binary pumps (Perkin Elmer, Concord, Ontario, Canada), a Hypersil BDS-C_{18} column (4.0 mm x 100 mm, particle size 3 μm; Hewlett-Packard, Waldbronn, Germany), and a Perkin Elmer SCIEX API 3000 LC/MS/MS System (Sciex Division of MDS Inc, Toronto, Ontario, Canada). Glibenclamide served as internal standard in Studies I, IV, and V and glimepiride in Study II. Internal standard was added to tubes that contained 1.0 ml plasma and 1.0 ml extraction solution (0.1 mol/l hydrochloric acid and potassium chloride, i.e., 8.28 ml 37% hydrochloric acid plus 7.45 g potassium chloride per liter). After the mixture was vortexed, 5.0 ml diethyl ether was added, and the tubes underwent shaking for 20 minutes. After centrifugation, the diethyl ether phase was evaporated to dryness under a stream of nitrogen at 30 °C. The evaporation residues were reconstituted with 0.1 ml of the mobile phase, which consisted of acetonitrile (57%) and 10 mmol/l ammonium formate (pH 3.5; 43%). A 5- to 10-μl aliquot of the final preparation was injected into the column. A flow rate of 0.5 ml/min was used in chromatography.

The samples were analyzed by use of liquid chromatography, followed by atmospheric pressure chemical ionization at 375 °C for glibenclamide and glipizide or 330 °C for glimepiride and tandem mass spectrometry. Nitrogen flow rate in the nebulizer was approximately 0.7 l/min, and the flow rate of the curtain gas nitrogen was approximately 2.0 l/min. The collision energies for glibenclamide, glimepiride, and glipizide were 20.0 eV, and nitrogen served as the collision gas. The ion transitions monitored were *m/z* 494 → 369 for glibenclamide, *m/z* 491 → 352 for glimepiride, and *m/z* 446 → 321 for glipizide. These transitions represent the formation of the $(M+H)^+$ ions. The standard curve was determined and the samples quantified by the Perkin Elmer SCIEX

Macquan program (Perkin Elmer SCIEX Instruments, Concord, Ontario, Canada). The concentrations were computed on the basis of the area of the peak in the chromatogram of the $(M+H)^+$ ions (Study II) or on the basis of the height of the peak (Studies I, IV, and V). The standard curves were determined by linear regression analysis; the standard curves for glibenclamide, glimepiride, and glipizide were linear in the concentration-range of 0 to 100 ng/ml ($r > 0.999$, weighting 1/x). The limits of quantification of the methods were 0.1 ng/ml (Study I), 0.04 ng/ml (Study IV), and 0.05 ng/ml (Study V) for glimepiride, and 0.05 ng/ml for glibenclamide and 0.1 ng/ml for glipizide in Study II. Results from quality control assays in Studies I, II, IV, and V for the methods are presented in Table VI. The extraction recoveries of glibenclamide, glimepiride, and glipizide exceeded 75%. The hormones or their metabolites used in oral contraceptive preparations or the plasma matrix did not interfere with the determination of glibenclamide, glimepiride, or glipizide plasma concentrations.

4.2. Repaglinide

The plasma concentrations of repaglinide (Studies III and VI) were determined by liquid chromatography-tandem mass spectrometry. The system was equipped with a Perkin Elmer Series 200 injector and binary pumps, a Waters C_8 Guard column (10 mm x 2.1 mm, particle size 3.5 µm; Waters Corp, Milford, MA, USA; Study VI), a Purospher STAR RP-18 column (55 mm x 2 mm, particle size 3 µm; Merck KGaA, Darmstadt, Germany; Studies III and VI), and a Perkin-Elmer SCIEX API 3000 LC/MS/MS System. The internal standard indomethacin was added to tubes that contained 1.0 ml plasma and 1.0 ml extraction buffer (0.1 mol/l potassium phosphate; pH 5.9). After the mixture was vortexed, 6.0 ml diethyl ether (containing 1.5% isoamylalcohol) was added, and the tubes were shaken for 30 minutes.

After centrifugation, the diethyl ether phase was transferred into new tubes and evaporated to dryness under a stream of nitrogen at 30 °C. The evaporation residues were reconstituted with 0.15 ml of the mobile phase, which consisted in Study III of 50% of acetonitrile and 50% of 10 mmol/l ammonium formate (pH 3.5) and in Study VI of 80% of acetonitrile and 20% of 10 mmol/l ammonium formate (pH 3.5). A 10-µl aliquot of the final preparation was injected into the column. A flow rate of 0.375 ml/min was used in chromatography in both studies. The eluting compounds of interest were analyzed by liquid

chromatography, followed by atmospheric pressure chemical ionization at 420 °C and tandem mass spectrometry. Nitrogen flow rate in the nebulizer was approximately 0.7 l/min and the flow rate of the curtain gas nitrogen approximately 2.0 l/min. The collision energy for repaglinide was 40.0 eV, with nitrogen serving as the collision gas. The ion transitions monitored were *m/z* 453 → 230 for repaglinide and *m/z* 358 → 139 for indomethacin. The standard curve was determined and the samples quantified with the Perkin Elmer SCIEX Macquan program. Concentrations were computed on the basis of the area of the peak in the chromatogram of the $(M+H)^+$ ion. The standard curve was determined by linear regression analysis, and the standard curve for repaglinide was linear in the concentration-range of 0 to 10 ng/ml ($r > 0.999$, weighting 1/x). The quantification limit was 0.05 ng/ml. Results from quality control assays in Studies III and VI for the method are shown in Table VI. The extraction recovery of repaglinide exceeded 90%. The hormones or their metabolites used in oral contraceptive preparations or the plasma matrix did not interfere with the determination of repaglinide plasma concentrations.

4.3. CYP inhibitors

The plasma concentrations of fluconazole (Study IV) were determined with high-performance liquid chromatography (HPLC) and ultraviolet detection by a modification of a previously described method (Inagaki et al 1992). The system was equipped with a HP 1100 Series Liquid Chromatography System (Hewlett-Packard) and a Novapak C_{18} column (3.9 mm x 150 mm, particle size 4 µm; Waters Corp). The internal standard UK 54373 was added to tubes that contained 0.5 ml plasma and 1.0 ml extraction buffer (0.1 mol/l sodium phosphate; pH 6.0). After vortexing, the analyte and internal standard were extracted from plasma into methanol by use of Varian Bond Elut C_{18} solid-phase extraction columns (Varian, Harbour City, CA, USA). After evaporation to dryness under nitrogen at 40 °C, the residue was reconstituted with 0.2 ml of mobile phase, which consisted of methanol (35%) and 0.025 mol/l sodium phosphate buffer (pH 7.0; 65%). A 5-µl aliquot of the final preparation was injected into the column. A flow rate of 0.5 ml/min was used in chromatography. The extraction recovery of fluconazole by the original method was 97% (Inagaki et al 1992). The limit of quantification of fluconazole was 0.20 µg/ml; the results from quality control assays for the method are shown in Table VI.

MATERIALS AND METHODS

The plasma concentrations of fluvoxamine (Study IV) were determined with HPLC and ultraviolet detection by a modification of a previously described method (van der Meersch-Mougeot & Diquet 1991). The system was equipped with a HP 1050 Autosampler (Hewlett-Packard), a Pharmacia LKB-HPLC Pump 2248 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), a Zorbax SIL column (4.6 mm x 250 mm, particle size 5 µm; Rockland Technologies/Dupont Company, Chaddsford, PA, USA), and a Perkin Elmer Model 785A ultraviolet detector (Perkin Elmer, Norwalk, CT, USA). The internal standard haloperidol was pipeted into tubes and the solvent evaporated. Thereafter 1.0 ml plasma, 0.125 ml sodium hydroxide (2 mol/l), and 5.0 ml hexane were added. The mixture was shaken for 15 minutes. After centrifugation, hexane extraction was repeated with a new amount of 5.0 ml hexane. The combined hexane solutions were evaporated to dryness under nitrogen in a dry-bath set at 30 °C. The residue was reconstituted with 0.2 ml of mobile phase, which consisted of methanol (985 ml), acetonitrile (10 ml), tetrahydroflurane (2 ml), water (2 ml), and diethylamine (0.1 ml). A 50-µl aliquot of the final preparation was injected into the column. A flow rate of 0.8 ml/min was used in chromatography. The extraction recovery of fluvoxamine by the original method was 88% (Inagaki et al 1992). The limit of quantification of fluvoxamine was 10.0 ng/ml; the results from quality control assays for the method are shown in Table VI.

Plasma gemfibrozil concentrations (Study V) were determined with HPLC and ultraviolet detection by a modification of a previously described method (Hengy & Kölle 1985). The system was equipped with a HP 1050 Autosampler, a Pharmacia LKB-HPLC Pump 2248, a Novapak C_{18} column, and a Perkin Elmer Model 785A ultraviolet detector. A volume of 0.5 ml plasma was placed in tubes and mixed with the internal standard ibuprofen. The mixture was acidified with 0.1 ml hydrochloric acid (1 mol/l). After the mixture was vortexed, 5.0 ml cyclohexane was added, and the tubes were shaken for 20 minutes. After centrifugation, the cyclohexane phase was evaporated to dryness under nitrogen at 40 °C. The evaporation residue was reconstituted with 0.1 ml of mobile phase, which consisted of acetonitrile (500 ml), water (500 ml), and 85% phosphoric acid (1.38 ml). A 2- to 10-µl aliquot of the final preparation was injected into the column. A flow rate of 0.7 ml/min was used in chromatography. The extraction recovery of gemfibrozil was 90%. The limit of quantification was 0.1 µg/ml, and the results from quality control assays for the method are shown in Table VI.

The plasma clarithromycin concentrations (Study VI) were determined with HPLC and electrochemical detection by a modification of previously described methods (Laakso et al 1990, Grgurinovich & Matthews 1988). The system was equipped with a HP 1050 Autosampler, a Pharmacia LKB-HPLC Pump 2248, a Novapak C_{18} column, and a ESA Model 5100 A Coulochem electrochemical detector (ESA Inc, Chelmsford, MA, USA). Internal standard roxithromycin was added to tubes that contained 0.5 ml plasma and 50 µl acetonitrile. After vortexing, 50 µl saturated sodium carbonate and 2.5 ml methyl-tert-buthylether were added. The mixture was shaken for 15 minutes. After centrifugation, 2 ml of the upper phase was transferred into new tubes and evaporated to dryness under a stream of nitrogen (35 °C). The residue was reconstituted with 0.15 ml of mobile phase, which consisted of 0.1 mol/l acetic acid buffer (pH 5.0; 58%), acetonitrile (32%), and methanol (10%). A 20-µl aliquot of the final preparation was injected into the column. A flow rate of 0.7 ml/min was used in chromatography. The extraction recovery of clarithromycin was 97%. The quantification limit was 0.2 µg/ml; the results from quality control assays for the method are presented in Table VI.

No studies were performed on the stability of any of the analytes. However, the plasma samples were stored at -40 °C (Studies I and III-VI) or -80 °C (Study II) and were analyzed at a maximum 5 months after completion of the studies.

Table VI. Results from quality control assays for determinations of plasma drug concentrations.

Drug (study)	*n*	*Inter-assay coefficient of variation (CV) at*		
		Low concentration	*Medium concentration*	*High concentration*
Clarithromycin (VI)	8	10.2% (0.9 µg/ml)	6.4% (4.4 µg/ml)	3.9% (8.6 µg/ml)
Fluconazole (IV)	11	2.3% (0.39 µg/ml)	1.2% (4.4 µg/ml)	2.0% (14.3 µg/ml)
Fluvoxamine (IV)	7	9.2% (16.9 ng/ml)	4.5% (85.7 ng/ml)	5.4% (324 ng/ml)
Gemfibrozil (V)	6	9.7% (2.1 µg/ml)	6.8% (14.6 µg/ml)	2.6% (29.6 µg/ml)
Glibenclamide (II)	5	5.9% (0.1 ng/ml)	3.8% (5 ng/ml)	1.8% (150 ng/ml)
Glimepiride (I)	4	6.8% (0.1 ng/ml)	5.7% (6.4 ng/ml)	2.0% (98.7 ng/ml)
Glimepiride (IV)	5	7.2% (0.5 ng/ml)	5.9% (5.3 ng/ml)	1.5% (52.9 ng/ml)
Glimepiride (V)	5	6.9% (0.1 ng/ml)	2.7% (5.0 ng/ml)	4.6% (50 ng/ml)
Glipizide (II)	5	3.5% (0.1 ng/ml)	3.6% (5.0 ng/ml)	6.5% (150 ng/ml)
Repaglinide (III)	5	6.2% (0.05 ng/ml)	6.0% (1.0 ng/ml)	3.9% (10.0 ng/ml)
Repaglinide (VI)	5	5.5% (0.1 ng/ml)	6.6% (1.0 ng/ml)	4.4% (10.4 ng/ml)

5. Pharmacokinetic calculations

The pharmacokinetics of the study drugs were characterized by peak concentration in plasma (C_{max}), time to C_{max} (t_{max}), elimination half-life ($t_{½}$), and areas under the plasma concentration-time curve from time 0 to t (AUC(0-t) where t refers to the time-point of the last blood sample taken) or 0 to infinity (AUC(0-∞)). The terminal log-linear part of the plasma drug concentration-time curve was visually identified for each individual drug concentration curve. The elimination-rate constant (k_{el}) was determined by linear regression analysis of the log-linear part of the plasma concentration-time curve. The $t_{½}$ was calculated by the equation $t_{½} = \ln 2/k_{el}$. The AUC values were calculated by the linear trapezoidal rule in Study I and by the linear trapezoidal rule for the rising phase of the plasma drug concentration-time curve and by the log-linear trapezoidal rule for the descending phase in Studies II to VI. When appropriate, the extrapolation to infinity was accomplished by dividing the last determined concentration by k_{el}.

The pharmacokinetics of the CYP inhibitors fluconazole, fluvoxamine, gemfibrozil, and clarithromycin (Studies IV-VI) were characterized by C_{max} and AUC(1 - t+1), that is the AUC from 1 hour after the last dose of the inhibitor up to the time-point of the last blood sample taken. All pharmacokinetic

calculations were performed with the program MK-model, version 5.0 (Biosoft, Cambridge, UK).

6. Pharmacodynamic measurements

The pharmacodynamic effects of the sulfonylureas and repaglinide were characterized by the blood glucose (all studies) and serum insulin (Studies V and VI) responses.

6.1. Blood glucose

In all studies, blood glucose concentrations of the EDTA-treated blood samples were measured immediately after each sampling by the glucose oxidase method using the Precision G Blood Glucose Testing System (Medisense, Bedford, MA, USA), with its electrochemical detection technique. The results from blood glucose control solution testing are shown in Table VII.

To adjust for variation in baseline blood glucose values, the blood glucose response was characterized by determining the decremental area (net area below baseline) under the concentration-time curve of blood glucose from time 0 to 3 hours (decremental AUC(0-3); Studies I and III-VI), from 0 to 5 hours (decremental AUC(0-5); Study III), or from 0 to 7 hours (decremental AUC(0-7); Studies I, II and IV-VI) after the administration of the study drug. The decremental AUC values were calculated by the linear trapezoidal rule. The maximum increase and the maximum decrease in blood glucose concentrations from baseline blood glucose values were also calculated in all studies for the time-period 0 to 3 hours after administration of the study drug.

Table VII. Results from blood glucose control solution testing.

		Between-day coefficient of variation (CV) at concentrations		
Study No.	*n*	*Low (mmol/l)*	*Medium (mmol/l)*	*High (mmol/l)*
Study I	4	9.8% (2.7)	6.5% (4.9)	5.9% (15.2)
Study II	8	10.1% (2.5)	4.8% (4.6)	5.3% (14.5)
Study III	4	10.0% (2.6)	12.7% (4.7)	7.9% (15.7)
Study IV	6	11.0% (2.7)	9.6% (4.6)	4.3% (15.2)
Study V	4	3.8% (3.0)	8.4% (5.4)	2.5% (16.8)
Study VI	4	5.1% (2.6)	3.7% (4.7)	4.8% (14.8)

6.2. Serum insulin

In Studies V and VI, serum insulin concentrations were also measured in the serum samples for the time period 0 to 7 hours after administration of the study drug. Serum insulin concentrations were quantified by a fluoroimmunoassay method with a commercially available kit, AutoDELFIA™ Insulin (Wallac Oy, Turku, Finland). The inter-assay CV for this method with a full standard curve was 2.3% at 5.7 mU/l, 3.0% at 13.7 mU/l, and 3.5% at 30.2 mU/l (AutoDELFIA™ Insulin kit package insert, 1998).

The insulin response was characterized by determining the incremental area (net area above baseline) under the concentration-time curve of serum insulin for the time periods 0 to 3 hours (incremental AUC(0-3)) and 0 to 7 hours (incremental AUC(0-7)) after administration of the study drug. The incremental AUC values were calculated by the linear trapezoidal rule. The maximum increase in serum insulin concentration from the baseline value was also calculated.

7. Statistical analysis

Results of the studies are expressed as mean values ± SEM (Studies I-III), mean ± SD (Study IV), or as mean ± SD in Results and tables and as mean ± SEM in figures (Studies V and VI). T_{max} data are expressed as median with range. The chosen level of statistical significance in all studies was $P < .05$. Pharmacokinetic variables were log-transformed before the statistical analysis, when appropriate. In Study I, 95% confidence intervals (CI) were calculated for the mean differences of all variables (except t_{max}) between placebo and rifampicin phases. The pharmacokinetic variables, except t_{max}, and the blood glucose variables after the pretreatments were compared with the Student *t*-test for paired values in Studies I and III. The Wilcoxon signed-rank test was used in Studies I to III and V to VI for analysis of the t_{max} values. In Study IV (3 phases), the t_{max} data was analyzed by Friedman's two-way analysis of variance (ANOVA) followed by the Wilcoxon signed-rank test with the Bonferroni correction. ANOVA followed by a posteriori testing with the paired *t*-test with the Bonferroni correction was used for analysis of the other variables in Study IV. In Studies II, V, and VI, the pharmacokinetic and pharmacodynamic variables between pretreatments were compared with ANOVA. In Study II, the difference in the ratio of the glibenclamide or glipizide AUC(0-∞) during the

rifampicin phase between the glibenclamide and glipizide studies was compared by the two-sample *t*-test. Pearson's correlation coefficient was used in Studies IV to VI to investigate the possible relationship between the pharmacokinetic variables of the CYP inhibitors and the study drugs. All the data were analyzed with the statistical program Systat for Windows, version 6.0.1 (SPSS Inc, IL, USA).

8. Ethical considerations

All the study protocols were approved by the appropriate ethics committees according to current regulations. The study protocols of Studies I, III, and IV were approved by the Ethics Committee of the Department of Clinical Pharmacology, University of Helsinki. The protocols of Studies II, V, and VI were approved by the Ethics Committee for Studies in Healthy Volunteers of the Hospital District of Helsinki and Uusimaa. In addition, all the study protocols were approved by the Finnish National Agency for Medicines. All the subjects received both oral and written information and gave their written informed consent before they were entered in the studies.

RESULTS

1. Effects of rifampicin on oral antidiabetic drugs

1.1. Glimepiride (Study I)

A 5-day treatment with 600 mg of rifampicin once daily moderately decreased plasma glimepiride concentrations (Fig 10). The mean AUC(0-∞) of glimepiride was decreased by 34% ($P < .001$) by rifampicin and a reduction in the AUC(0-∞) (range, 14% to 41%) appeared in every subject. The mean C_{max} of glimepiride was reduced by 14% ($P = .06$) by rifampicin, and the mean $t_{½}$ was shortened from 2.6 to 2.0 hours ($P < .05$).

No statistically significant differences were evident in the blood glucose variables between the placebo and rifampicin phases (Fig 11), but two subjects experienced mild symptomatic hypoglycemia during the placebo phase and none during the rifampicin phase.

1.2. Glibenclamide (Study II)

A 5-day pretreatment with 600 mg of rifampicin once daily significantly reduced plasma concentrations of glibenclamide (Fig 10). Rifampicin reduced the mean AUC(0-∞) of glibenclamide by 39% ($P < .001$) and the C_{max} by 22% ($P = .01$) compared to placebo. The mean $t_{½}$ of glibenclamide was shortened from 2.0 to 1.7 hours ($P < .05$) by rifampicin. A reduction in the AUC(0-∞) was seen in every subject.

Here, the significant reduction in plasma glibenclamide concentrations resulted also in significantly higher blood glucose concentrations in the rifampicin phase than in the placebo phase (Fig 11). The blood glucose decremental AUC(0-7) was reduced from 5.9 to 3.3 mmol · h/l ($P = .05$) and the maximum decrease in blood glucose concentration from 2.2 to 1.4 mmol/l ($P < .001$) by rifampin. A reduction in the maximum decrease in blood glucose was seen in every subject, and 4 subjects received additional carbohydrates orally during the placebo phase to correct significant hypoglycemia (that is, a blood glucose concentration < 2.0

mmol/l). Only 1 subject received additional carbohydrates during the rifampicin phase. None of the subjects experienced severe hypoglycemic symptoms.

1.3. Glipizide (Study II)

The 5-day treatment with 600 mg of rifampicin once daily significantly affected the pharmacokinetics of glipizide (Fig 10). Rifampicin reduced the mean AUC(0-∞) of glipizide by 22% ($P < .05$), but elevated the C_{max} by 18% ($P < .05$). The mean $t_{½}$ of glipizide was shortened from 3.0 to 1.9 hours ($P = .01$) by rifampicin. A reduction in the $t_{½}$, but not in the AUC(0-∞), was seen in every subject. The relative decrease in the AUC(0-∞) of glibenclamide was significantly greater than that in the AUC(0-∞) of glipizide ($P < .05$).

Statistically non-significant decreases in the decremental AUC(0-7) and maximum decrease in blood glucose were obvious during the rifampicin phase compared to those of the placebo phase (Fig 11). On the other hand, 4 subjects received additional oral carbohydrates during the placebo phase to correct significant hypoglycemia, but only 1 subject received additional carbohydrates during the rifampicin phase. None of the subjects experienced severe hypoglycemic symptoms.

1.4. Repaglinide (Study III)

In this study, 600 mg of rifampicin once daily for 5 days considerably reduced plasma repaglinide concentrations (Fig 10). Rifampicin reduced the mean AUC(0-∞) of repaglinide by 57% ($P < .001$) and the C_{max} by 41% ($P = .001$) compared with placebo. The mean $t_{½}$ of repaglinide was shortened from 1.5 to 1.1 hours ($P < .01$) by rifampicin. Reductions in the AUC(0-∞) (range, 30% to 78%), C_{max} (range, 15% to 73%) and $t_{½}$ (range, 1% to 37%) values were seen in every subject.

In agreement with the effects of rifampicin on the pharmacokinetics of repaglinide, the blood glucose response to repaglinide was also significantly attenuated by rifampicin (Fig 11). Rifampicin reduced the mean decremental AUC(0-3) of blood glucose from 0.94 to -0.23 mmol · h/l ($P < .05$) and the mean maximum decrease in blood glucose concentration by 35% ($P < .05$)

compared with placebo. The decremental AUC(0-5) of blood glucose and the maximum increase in blood glucose concentration after breakfast were not significantly changed. The lowest measured blood glucose concentration was 1.6 mmol/l, which was observed in one subject during the placebo phase. She had only mild symptoms of hypoglycemia, but received carbohydrate supplementation.

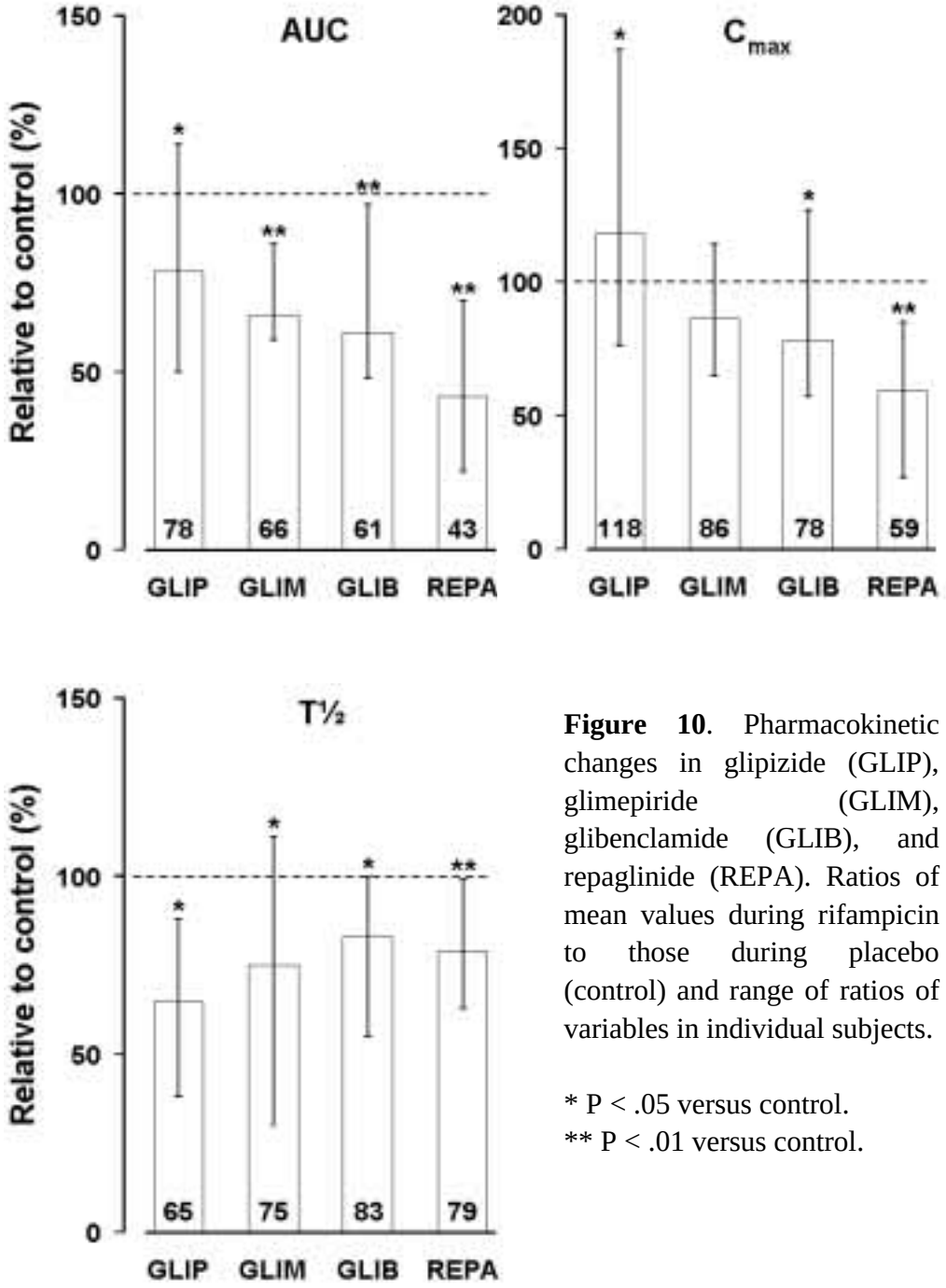

Figure 10. Pharmacokinetic changes in glipizide (GLIP), glimepiride (GLIM), glibenclamide (GLIB), and repaglinide (REPA). Ratios of mean values during rifampicin to those during placebo (control) and range of ratios of variables in individual subjects.

* P < .05 versus control.
** P < .01 versus control.

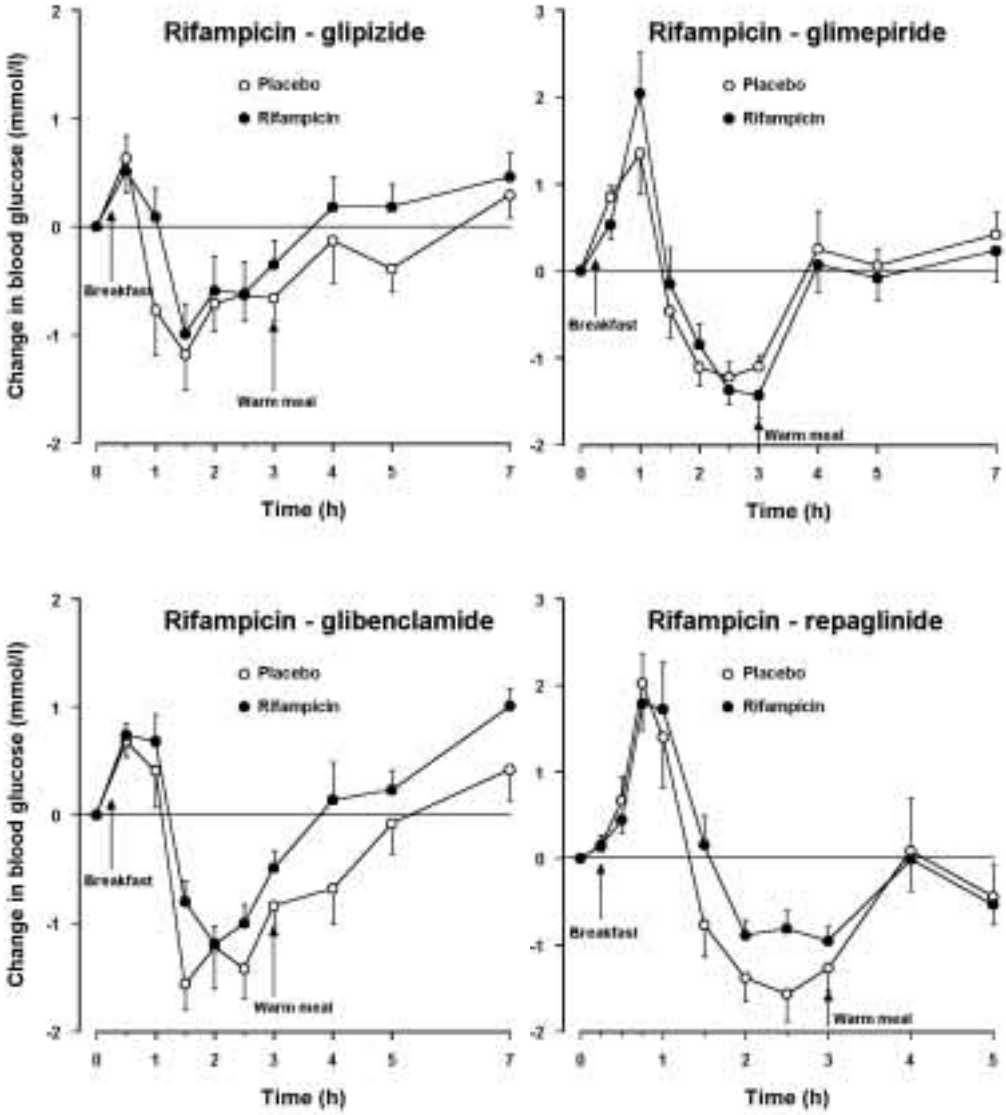

Figure 11. Mean (± SEM) change in blood glucose concentrations after administration of 2.5 mg glipizide, 1 mg glimepiride, 1.75 mg glibenclamide, or 0.5 mg repaglinide after a pretreatment with 600 mg rifampicin or placebo once daily for 5 days.

2. Effects of inhibitors of CYP enzymes on glimepiride and repaglinide

2.1. Fluconazole effects on glimepiride (Study IV)

A 4-day treatment with fluconazole once daily (400 mg on day 1 and 200 mg on days 2-4) significantly raised plasma glimepiride concentrations (Fig 12). Fluconazole raised the mean AUC(0-∞) and the C_{max} of glimepiride by 138% and 51% ($P < .0001$), respectively, compared with the placebo phase. The mean $t_{½}$ of glimepiride was prolonged from 2.0 to 3.3 hours ($P < .0001$) by fluconazole.

There were no statistically significant differences in blood glucose variables between the fluconazole and placebo phases (Fig 13). None of the subjects experienced symptomatic hypoglycemia.

2.2. Fluvoxamine effects on glimepiride (Study IV)

A 4-day treatment with 100 mg fluvoxamine once daily significantly elevated plasma glimepiride concentrations (Fig 12). Fluvoxamine raised the mean C_{max} of glimepiride by 43% ($P < .05$) compared with placebo and prolonged the $t_{½}$ from 2.0 to 2.3 hours ($P < .01$). However, only a non-significant increase in the mean AUC(0-∞) of glimepiride (33%) occurred after fluvoxamine. Fluconazole had a significantly ($P < .0005$) greater effect on both the $t_{½}$ and AUC(0-∞) of glimepiride than did fluvoxamine.

There were no statistically significant differences in blood glucose concentrations between the fluvoxamine and placebo phases (Fig 13). None of the subjects experienced symptomatic hypoglycemia.

2.3. Gemfibrozil effects on glimepiride (Study V)

The 5-dose pretreatment with 600 mg gemfibrozil administered twice daily modestly elevated plasma glimepiride concentrations (Fig 12). The mean AUC(0-∞) of glimepiride was raised by 23% ($P < .005$), and the mean $t_{½}$ was

prolonged from 2.1 to 2.3 hours ($P < .05$) by gemfibrozil. An increase in the AUC(0-∞) (range, 6% to 56%) was evident in every subject. A statistically non-significant increase (14%) in the C_{max} of glimepiride occurred after gemfibrozil compared to that after placebo. There was a tendency towards a positive correlation between the AUC(1-13) of gemfibrozil and the increase in the AUC(0-12) of glimepiride after gemfibrozil compared with that after placebo (r = .58, P =.08).

No statistically significant differences in serum insulin or blood glucose variables occurred between placebo and gemfibrozil phases (Fig 13). None of the subjects experienced symptomatic hypoglycemia.

2.4. Clarithromycin effects on repaglinide (Study VI)

The 9-dose pretreatment with 250 mg clarithromycin administered twice daily significantly raised plasma concentrations of repaglinide (Fig 12). Clarithromycin raised the mean AUC(0-∞) of repaglinide by 40% ($P < .0001$) and the C_{max} by 67% ($P < .005$) compared with placebo. The mean $t_{½}$ of repaglinide was prolonged from 1.4 to 1.7 hours ($P < .05$) by clarithromycin. Increases in the C_{max} and AUC values were seen in every subject. There was a significant negative correlation between clarithromycin C_{max} and the ratio of the AUC(0-7) of repaglinide after clarithromycin to that after placebo (r = -.66, P = .05).

The higher plasma repaglinide concentrations caused by clarithromycin resulted in a significantly increased serum insulin response. Clarithromycin raised the incremental AUC(0-3) of serum insulin and the maximum increase in the serum insulin concentration by 51% ($P < .05$) and 61% ($P < .01$), respectively. Statistically non-significant increases in the incremental AUC(0-7) of serum insulin and decremental AUC values of blood glucose occurred after clarithromycin compared to after placebo (Fig 13). None of the subjects experienced symptomatic hypoglycemia.

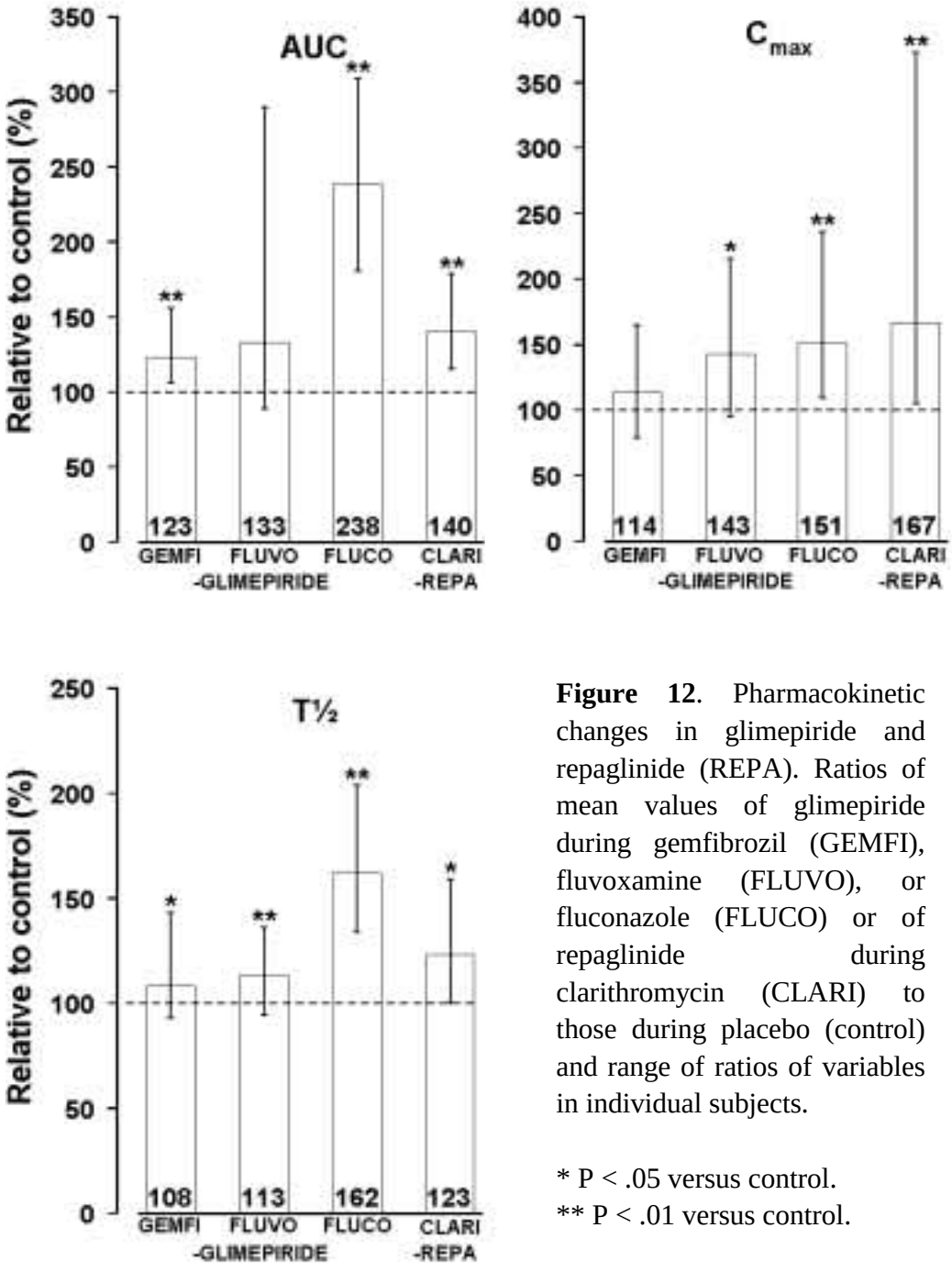

Figure 12. Pharmacokinetic changes in glimepiride and repaglinide (REPA). Ratios of mean values of glimepiride during gemfibrozil (GEMFI), fluvoxamine (FLUVO), or fluconazole (FLUCO) or of repaglinide during clarithromycin (CLARI) to those during placebo (control) and range of ratios of variables in individual subjects.

* P < .05 versus control.
** P < .01 versus control.

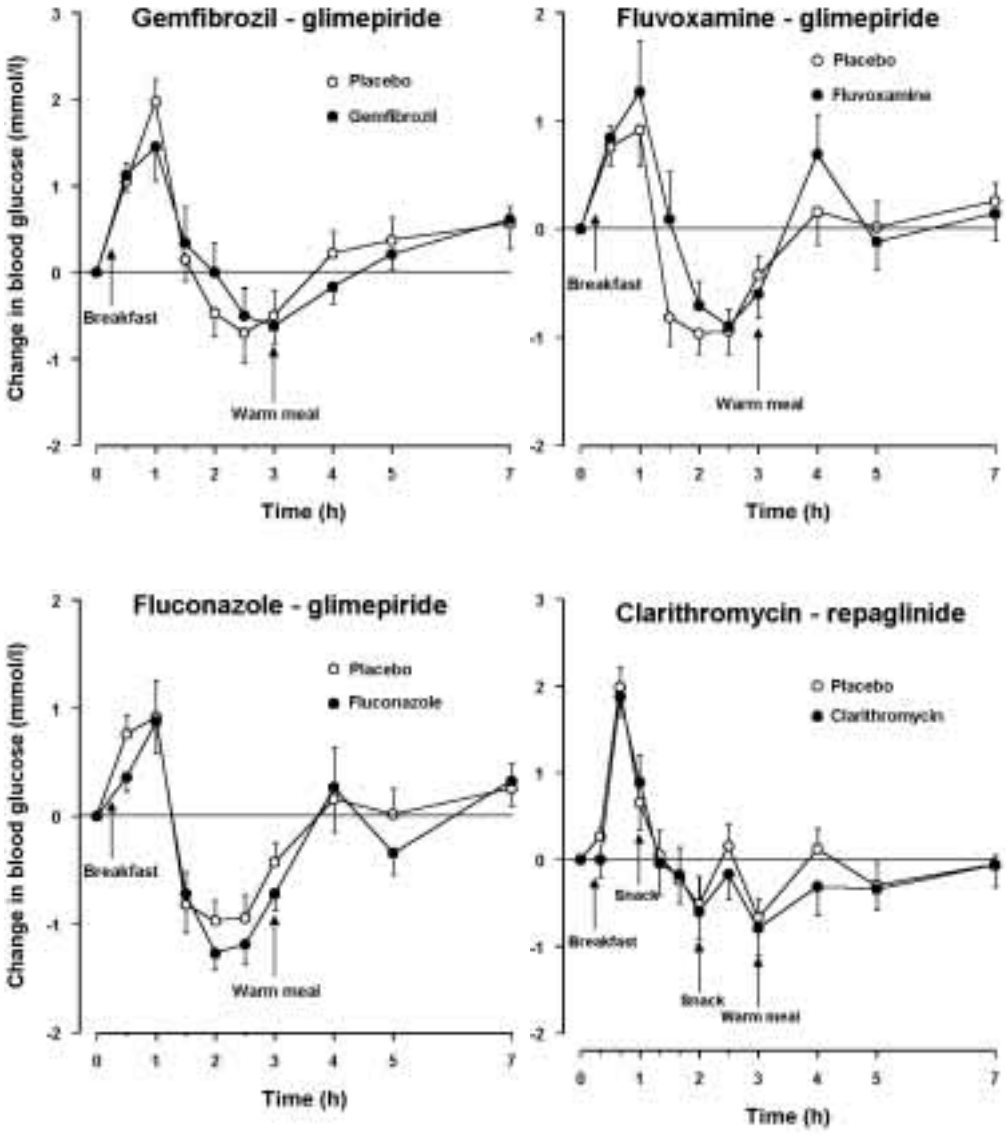

Figure 13. Mean (± SEM) change in blood glucose concentrations after administration of 0.5 mg glimepiride after a pretreatment with gemfibrozil (600 mg twice daily for 2.5 days), fluvoxamine (100 mg daily for 4 days), or fluconazole (400 mg on day 1 and 200 mg on days 2 to 4) or placebo, and mean (± SEM) change in blood glucose concentrations after administration of 0.25 mg repaglinide after 9 doses of 250 mg clarithromycin or placebo administered twice daily.

3. Summary of results

Rifampicin (600 mg once daily for 5 days) reduced plasma concentrations of the sulfonylureas glibenclamide, glimepiride, and glipizide (Fig 10). Rifampicin had a significantly greater effect on the AUC(0-12) of both glibenclamide ($P < .05$, two-sample *t*-test) and glimepiride ($P < .05$, two-sample *t*-test) than on that of glipizide. Rifampicin also significantly reduced the blood glucose-lowering effect of glibenclamide (Fig 11).

The plasma concentrations of repaglinide were reduced considerably by rifampicin (Fig 10). Rifampicin also reduced its blood glucose-lowering effect (Fig 11). The effect of rifampicin on the AUC(0-∞) of repaglinide was significantly greater than its effects on the AUC(0-∞) of glibenclamide ($P < .05$, two-sample *t*-test), glimepiride ($P < .05$, two-sample *t*-test), or glipizide ($P < .01$, two-sample *t*-test).

Fluconazole (400 mg on day 1 and 200 mg on days 2 to 4), fluvoxamine (100 mg daily for 4 days), and gemfibrozil (600 mg twice daily for 2.5 days) all enhanced the plasma concentrations of glimepiride (Fig 12). The AUC(0-∞) of glimepiride was enhanced to the greatest extent by fluconazole, whereas both fluvoxamine and gemfibrozil only moderately raised the AUC(0-∞) of glimepiride. Fluconazole had a significantly greater effect on the AUC(0-∞) of glimepiride than did either fluvoxamine ($P < .0005$, ANOVA followed by paired *t*-test with the Bonferroni correction) or gemfibrozil ($P < .0001$, two-sample *t*-test). Neither fluconazole, fluvoxamine, nor gemfibrozil significantly affected the blood glucose-lowering effect of glimepiride (Fig 13).

Clarithromycin (250 mg twice daily for 4.5 days) raised the plasma concentrations of repaglinide (Fig 12). The insulin response was also significantly enhanced by clarithromycin pretreatment. In this study, the blood glucose-lowering effect of repaglinide remained unaffected (Fig 13).

DISCUSSION

1. Methodological considerations

This series of studies investigated the effects of induction and inhibition of CYP enzymes on the pharmacokinetics and pharmacodynamics of oral antidiabetic drugs. The antidiabetic drugs of these studies act by enhancing insulin secretion in the pancreas and thereby reducing blood glucose concentrations.

All 6 studies were carried out with healthy volunteers. Each received a short-term pretreatment with either rifampicin or one of the inhibitors of CYP enzymes or placebo, and thereafter a single oral dose of the antidiabetic drug. Highly specific and sensitive LC/MS/MS assay methods were developed for the antidiabetic drugs to allow the use of low, well-tolerated doses of the drugs. In addition, the volunteers received standardized meals during the days of administration of the antidiabetic drugs and were under direct medical supervision. Their blood glucose concentrations were frequently measured during the study days. Medication for the treatment of possible severe hypoglycemia was available at the study site but was not needed. In addition, snacks rich in carbohydrates were available, and the volunteers received carbohydrate supplementation when considered necessary.

All were placebo-controlled, randomized crossover studies with a washout period of 2 to 4 weeks. With the crossover design, the subjects served as their own controls, which helped to minimize variation and reduced the number of subjects needed to show a significant interaction according to statistical criteria. Balanced randomization and adequate washout periods minimized the risk of any possible carry-over effect.

Because of the low doses of antidiabetic drugs, glimepiride and repaglinide tablets had to be halved in Studies IV to VI. Dividing tablets may, however, increase interindividual variation in dosage and thus increase variation in drug plasma concentrations. Glimepiride 1 mg tablets have double bisects to facilitate the division of the tablet accurately. Repaglinide 0.5 mg tablets, lacking bisects, were halved, then weighed, and these halved tablets met the criteria of the European Pharmacopoeia for tablets of that size (European Pharmacopoeia Third Edition 1997).

DISCUSSION

The number of subjects in each study was relatively small (9-12). However, based on variations in pharmacokinetic variables of these drugs in previous studies and on information from previous drug interaction studies, this number of subjects seemed sufficient to reveal any clinically significant pharmacokinetic interaction (about a 30% change in AUC) at a statistical significance level of $P < .05$.

The volunteers participating in the studies were healthy and relatively young. Because the prevalence of most diseases increases with age, these findings may thus not be directly extrapolated to older subjects with NIDDM. The pharmacokinetics of glimepiride, glipizide, and repaglinide are not, however, significantly affected by age, and aging has only minor effects on the pharmacokinetics of glibenclamide. Furthermore, aging does not impair the enzyme induction caused by rifampicin (Dilger et al 2000). On the other hand, patients with NIDDM probably exhibit characteristics that increase variation in the extent of drug interaction.

Disturbances in insulin secretion and in the blood glucose-lowering effect of insulin play a central role in the pathogenesis of NIDDM. Thus, extrapolation of pharmacodynamic responses from healthy volunteers to patients with NIDDM should be made with caution. In any case, the main focus of these studies was to detect possibly clinically significant pharmacokinetic drug interactions.

The pretreatment period in the studies with inhibitors of CYP enzymes was between 2 and 5 days (significant inhibition of CYP enzymes occurs even after a single dose of an inhibitor, according to Neuvonen et al 1996). The steady state concentrations of clarithromycin and gemfibrozil were probably reached within this period. In the fluconazole study, a loading dose of fluconazole was given on day 1 (twice the dose administered on days 2 to 4). With this dosing regimen, 90% of the steady state concentration was achieved by the second treatment day. Thus it is unlikely that the effect of fluconazole on glimepiride would have been much greater after a pretreatment period longer than 4 days. Considering the $t_{½}$ of fluvoxamine (about 15 to 20 hours), it is also unlikely that the effects of fluvoxamine on glimepiride would have been much greater after a longer pretreatment period. In addition, the same pretreatments have been used in some previous interaction studies; sharing the same design facilitates comparison of results across different studies.

Enzyme inhibition takes place when the inhibitor is present, whereas enzyme induction by rifampicin requires the synthesis of new enzyme protein and thus requires a longer time. Rifampicin was given for a relatively short period (5 days). Results of one study suggest that only about half of the maximum inducing effect of rifampicin is reached during this period (Lee et al 1993), but in another study the inducing effect of rifampicin reached a plateau after 6 days, and the extent of induction after 5 days was close to the plateau level (Tran et al 1999). A 5-day pretreatment with rifampicin was chosen because such pretreatment has often been shown to reveal potentially clinically significant interactions and to prevent unnecessary exposure of healthy volunteers to rifampicin.

The studies were not balanced with respect to subjects' gender. No balance was considered necessary, because the volunteers served as their own controls. In addition, the physiological changes during the menstrual cycle seem to have only minor effects on pharmacokinetics (Kashuba & Nafziger 1998). Furthermore, the washout period in all studies except V was 4 weeks, which equals the average menstrual cycle. Several of the female volunteers were using oral contraceptives, and the steroids in these preparations are potent inhibitors of CYP3A4 in vitro (Back et al 1991, Guengerich 1988, Guengerich 1990). However, they seem to have only minor effects on the pharmacokinetics of CYP3A4 substrates (Palovaara et al 2000). Although several of the drugs studied in this work were CYP2C9 substrates, and the effects of oral contraceptive steroids on the pharmacokinetics of such substrates have not been specifically studied, the risk of significant inhibition of CYP2C9 by these steroids was considered to be very low. Consequently, the use of oral contraceptive steroids was not considered an exclusion criteria. Oral contraceptive steroids did not seem in our studies to have significant effects on the pharmacokinetics of the CYP2C9 substrates.

The volunteers fasted overnight before administration of each antidiabetic drug to reduce possible variation in drug absorption (meals during the study days were standardized and identical) and also to reduce variation in baseline blood glucose and serum insulin concentrations.

2. Effects of induction of CYP enzymes on oral antidiabetic drugs

2.1. Effects of induction on the CYP2C9 substrates glimepiride, glibenclamide, and glipizide

These studies (I-II) demonstrated that induction of CYP2C9 by rifampicin reduces the plasma concentrations of the sulfonylureas glibenclamide, glimepiride, and glipizide. The blood glucose-lowering effect of glibenclamide was also significantly reduced by rifampicin, whereas in healthy volunteers, rifampicin had no statistically significant effects on the blood glucose responses to glimepiride and glipizide. Rifampicin dropped the total AUC of glibenclamide, glimepiride, and glipizide by 39%, 34%, and 22%, respectively. Conversely, rifampicin had its greatest effect on the $t_{½}$ of glipizide (35% reduction) and its smallest effect on that of glibenclamide (17% reduction). The C_{max} value of glibenclamide was significantly reduced by rifampicin, and a statistically non-significant reduction in the C_{max} of glimepiride occurred after rifampicin. On the other hand, rifampicin significantly elevated the C_{max} of glipizide, suggesting that it increased the systemic availability of glipizide.

Because of significant hypoglycemia, several subjects received carbohydrate supplementation during the placebo phase in the rifampicin-glibenclamide and rifampicin-glipizide studies, whereas only one subject needed additional carbohydrates in those studies during the rifampicin phase. The extra carbohydrates obviously reduced the difference in blood glucose variables between the placebo and rifampicin phases in those studies, and may partly explain the lack of statistical significance in the blood glucose variables in the glipizide study. Because additional carbohydrates were mostly needed in the placebo phases, results of Study II are more likely to underestimate than overestimate the true pharmacodynamic interactions of rifampicin with glibenclamide and glipizide. Additional carbohydrates were not needed in the rifampicin-glimepiride study, and it is not clear why the rifampicin-caused reduction in plasma glimepiride concentrations did not significantly alter the blood glucose-lowering effect of glimepiride in healthy volunteers.

The sulfonylureas glibenclamide, glimepiride, and glipizide are eliminated primarily by CYP2C9-mediated metabolism (Brian 2000, Langtry & Balfour

1998, Kidd et al 1999). P-glycoprotein seems to be an important determinant of the disposition of glibenclamide, as glibenclamide is a substrate of the P-glycoprotein (Goldstein et al 1999), and a single 120 mg dose of verapamil, a known inhibitor of P-glycoprotein, elevated the C_{max} and AUC(0.5-6) of glibenclamide by 28% and 26%, respectively (Semple et al 1986). Because rifampicin potently induces CYP2C9, among other CYP enzymes (Gerbal-Chaloin et al 2001, Sumida et al 2000), the most likely explanation for these interactions seems to be induction of the CYP2C9-mediated metabolism of these sulfonylureas by rifampicin. However, rifampicin may also have reduced the oral bioavailability of glibenclamide by inducing the intestinal and hepatic P-glycoprotein (Greiner et al 1999, Westphal et al 2000). It is not yet known whether glimepiride or glipizide is a substrate of the P-glycoprotein.

Of interest is the finding that rifampicin raised the mean C_{max} of glipizide, despite a considerable decrease in the $t_{½}$. Furthermore, a great interindividual variation occurred in the effect of rifampicin on the C_{max} of glipizide (an effect ranging from a 24% reduction to an 87% increase) and an increased blood glucose-lowering effect of glipizide occurred in the subject with the greatest increase. Moreover, the t_{max} of glipizide was significantly shorter in the rifampicin phase. The increase in C_{max} suggests that rifampicin enhanced either the rate of absorption of glipizide or its systemic availability. The explanation may be increased activity of an uptake transporter in the small intestine, such as an organic anion-transporting polypeptide (OATP). If the uptake transporter were induced also in hepatocytes, this would explain the fact that rifampicin reduced the $t_{½}$ of glipizide to the greatest extent of all these antidiabetic drugs, because hepatic clearance of drugs depends not only on the rate of hepatic metabolism but also on the rate of uptake of drugs into the liver. Unfortunately, it is not yet known whether glipizide is a substrate of any uptake transporter, and further studies are needed to clarify the mechanism of the apparent increased systemic availability of glipizide after rifampicin treatment.

Some drugs other than rifampicin are also potent inducers of CYP enzymes. These include the widely used antiepileptic drugs carbamazepine and phenytoin (Brodie 1992, Anderson 1998) and the herbal antidepressant St John's wort (Ernst 1999, Piscitelli et al 2000, Roby et al 2000). Carbamazepine and phenytoin seem capable of inducing CYP2C9 (Anderson 1998) and may therefore reduce the plasma concentrations of glibenclamide, glimepiride, and glipizide. St John's wort is a relatively potent inducer of CYP3A4 and P-

glycoprotein (Piscitelli et al 2000, Roby et al 2000, Dürr et al 2000). Although some case reports suggest that St John's wort may also induce CYP2C9 (Qun-Ying et al 2000), preliminary results of a recent study indicate that in fact it does not (Wang et al 2001b). It is, however, possible that St John's wort reduces the plasma concentrations of the P-glycoprotein substrate glibenclamide.

The interactions of CYP2C9-inducing drugs with glibenclamide are clinically significant, as has been demonstrated for the rifampicin-glibenclamide interaction in patients with NIDDM (Surekha et al 1997). Glimepiride and especially glipizide seem to be less susceptible to interaction with inducing agents. However, because of the variation in the effects of rifampicin on glimepiride and on glipizide and because elderly patients with NIDDM may exhibit additional characteristics affecting the interaction, the blood glucose-lowering effects of glimepiride and glipizide may, in some patients, also be reduced by rifampicin. Blood glucose concentrations should therefore be closely monitored if rifampicin or another potent inducer of CYP2C9 is added to the therapy of a patient with NIDDM who is treated with sulfonylureas or if the inducing agent is discontinued. The dosage of the sulfonylurea should be adjusted accordingly.

2.2. Effects of induction on the CYP3A4 substrate repaglinide

Rifampicin reduced the total AUC of repaglinide by 57% and also significantly reduced its C_{max} and $t_{½}$ values. Despite the fact that one subject received additional carbohydrates during the placebo phase and no subjects during the rifampicin, a statistically significant reduction in the blood glucose-lowering effect of repaglinide was seen after rifampicin.

The oral bioavailability of repaglinide is only about 60% because of first-pass metabolism (Hatorp et al 1998). Repaglinide is metabolized to three major inactive metabolites, M1, M2, and M7 (Guay 1998), and M2 is the major human metabolite (van Heiningen et al 1999). According to in vitro studies conducted by the manufacturer, CYP3A4 is involved in the formation of M1 and M2 metabolites (Prandin prescribing information 1998). Because CYP3A4 is present in significant quantities in the small intestinal mucosa (Kivistö et al 1996), it is probable that the intestinal CYP3A4 is important for the first-pass metabolism of repaglinide. Intestinal CYP3A4 is inducible by rifampicin (Kolars et al 1992),

and it plays an important role in the interactions of rifampicin with several drugs, e.g., ciclosporin, verapamil, and nifedipine (Hebert et al 1992, Fromm et al 1996, Holtbecker et al 1996). Because rifampicin reduced both the $t_{½}$ and C_{max} of repaglinide, it seems that it induced the CYP3A4-mediated metabolism of repaglinide during both first-pass and elimination phases. It is not yet known whether repaglinide is a substrate of the P-glycoprotein, and the possibility that induction of P-glycoprotein by rifampicin contributed to the interaction observed cannot be ruled out.

Of the antidiabetic drugs studied, induction of CYP enzymes by rifampicin had the greatest effect on the CYP3A4 substrate repaglinide. The effects of rifampicin on the pharmacokinetics of many other CYP3A4 substrates are also greater than those on the pharmacokinetics of CYP2C9 substrates. In comparison with the effects of rifampicin on glibenclamide, glimepiride, and glipizide, the same treatment with rifampicin lowered the AUC of midazolam, triazolam, buspirone, and simvastatin (all substrates of CYP3A4) to 4%, 5%, 10%, and 13% of control values, respectively (Backman et al 1996a, Villikka et al 1997, Lamberg et al 1998a, Kyrklund et al 2000). This may be explained in part by the large effect of rifampicin on the first-pass metabolism of these drugs, reflecting the small intestine's greater amount of CYP3A4 than of CYP2C9 (Zhang et al 1999), and the greater inducibility in cell cultures of CYP3A4 than of CYP2C9 (Sumida et al 2000).

Carbamazepine and phenytoin are as potent inducers of CYP3A4 as is rifampicin (Backman et al 1996b) and are therefore likely to cause similar effects on repaglinide, as was demonstrated in Study III with rifampicin. St John's wort is a relatively potent inducer of CYP3A4 (Piscitelli et al 2000, Roby et al 2000, Dürr et al 2000) and therefore probably also reduces the plasma concentrations of repaglinide.

The interactions of inducers of CYP3A4 with repaglinide are likely to be clinically significant, and blood glucose concentrations should be closely monitored if rifampicin or another potent inducer of CYP3A4 is added to the therapy of a patient treated with repaglinide, or if the inducing agent is discontinued. The dose of repaglinide should be adjusted according to blood glucose measurements. In some patients, repaglinide may be virtually ineffective during their treatment with potent inducers of CYP3A4, and thus it may be wise to change the oral antidiabetic therapy to another compound.

3. Effects of inhibition of CYP enzymes on oral antidiabetic drugs

3.1. Effects of inhibition of CYP2C9 on glimepiride

These studies (IV and V) showed that CYP2C9 inhibitors can elevate the plasma concentrations of the sulfonylurea glimepiride. Fluconazole raised the total AUC of glimepiride to almost 2.4-fold the control value, whereas fluvoxamine caused a statistically non-significant increase of 33% in the total AUC. Gemfibrozil raised the total AUC of glimepiride by 23%. Fluconazole raised the C_{max} of glimepiride by 51%, fluvoxamine by 43%, and gemfibrozil by 14% (n.s.). The $t_{½}$ of glimepiride was prolonged to the greatest extent by fluconazole, but fluvoxamine and gemfibrozil also significantly, although slightly, prolonged the $t_{½}$. Marked variation occurred in the extent of all these interactions, but variation seemed to be the greatest with fluvoxamine. For example, the ratio of the glimepiride AUC(0-∞) during the fluvoxamine phase to that during the placebo phase ranged from 0.89 to 2.90. No significant differences in blood glucose variables were found in either study, which may in part be explained by the food intake which probably increased variation in blood glucose variables, thereby interfering with the analysis of the pharmacodynamic response.

Glimepiride is completely absorbed after oral administration and, as a low clearance drug, has a high oral bioavailability that approaches 100% (Langtry & Balfour 1998). It is metabolized in the liver by CYP2C9 to a hydroxy metabolite, with further dehydrogenation by the cytosolic alcohol and aldehyde dehydrogenase enzymes to a carboxy metabolite (Langtry & Balfour 1998). It is not yet known whether glimepiride is a substrate of the P-glycoprotein.

Fluconazole has been shown to be a potent inhibitor of CYP2C9 both in vitro (Back et al 1988, Kunze et al 1996, Hargreaves et al 1994) and in vivo (Kazierad et al 1997, Blum et al 1991, Black et al 1996, Kaukonen et al 1998, Kantola et al 2000), and the mechanism of the fluconazole-glimepiride interaction is probably inhibition of the CYP2C9-mediated biotransformation of glimepiride. Although fluconazole also significantly inhibits CYP3A4 (Hargreaves et al 1994, Varhe et al 1996, Olkkola et al 1996, Jurima-Romet et al 1994), this enzyme probably plays no role in the biotransformation of glimepiride (Langtry & Balfour 1998). It is also unlikely that P-glycoprotein is involved, because, unlike ketoconazole and itraconazole, fluconazole does not seem to inhibit the P-glycoprotein

(Woodland et al 1998). The increase in plasma glimepiride concentrations caused by fluconazole did not cause significant effects on the glucose variables in healthy volunteers; however, because the effect of glimepiride is dose-dependent, concomitant use of fluconazole with glimepiride is likely to increase the risk of hypoglycemia.

In comparison with the effects of fluconazole on the pharmacokinetics of glimepiride, a 7-day treatment with 100 mg fluconazole daily raised the AUC of glipizide and glibenclamide by 49% and 44% (Stockley 1999a). These increases in AUC would probably have been greater at a higher fluconazole dosage. The interaction of fluconazole with sulfonylureas is potentially dangerous; at least one death in association with hypoglycemia was reported to be a result of the concomitant use of fluconazole and glibenclamide (Diflucan prescribing information 1998), and one case report described a patient with NIDDM who developed hypoglycemic coma during concomitant use of 200 mg fluconazole daily and 2.5 mg glipizide 3 times daily (Fournier et al 1992).

Fluvoxamine is a potent inhibitor of CYP1A2 (Brøsen 1995, Rasmussen et al 1995) and has been shown also to inhibit CYP2C19, CYP2D6, and CYP3A4 (Rasmussen et al 1998, Kashuba et al 1998, Lamberg et al 1998b). In addition, fluvoxamine was recently shown to inhibit CYP2C9 both in vitro and in vivo (Schmider et al 1997, Hemeryck et al 1999, Olesen & Linnet 2000, Madsen et al 2001). Because the AUC(0-∞) of glimepiride was not significantly elevated and the $t_{½}$ was only slightly prolonged by fluvoxamine in the present study, the moderate increase (43%) in the C_{max} of glimepiride may be explained by increased rate of absorption of glimepiride. A possible mechanism may be accelerated gastric emptying caused by fluvoxamine, as the activation of the gastric serotonin receptor (5-HT_4) accelerates emptying (Hedge et al 1995). However, inhibition of the metabolism of glimepiride by fluvoxamine probably also contributed to the increase in the C_{max}. Although there was considerable interindividual variation in the extent of the fluvoxamine-glimepiride interaction at both the pharmacokinetic and the pharmacodynamic level, in most cases, this interaction is possibly of limited clinical significance. Fluvoxamine was recently shown to induce hyperglycemia in mice (Yamada et al 1999). This effect is probably mediated by serotonin and caused by inhibition of insulin secretion. In line with this, in the fluvoxamine-glimepiride study the blood glucose concentrations tended to be higher after fluvoxamine than after placebo,

although in the fluvoxamine phase the plasma glimepiride concentrations were higher.

Recent in vitro studies indicate that gemfibrozil, as well, is an inhibitor of CYP2C9 (Wang et al 2001a, Wen et al 2001). Inhibition of the CYP2C9-mediated biotransformation of glimepiride by gemfibrozil seems to be the most likely explanation for the interaction observed between gemfibrozil and glimepiride. Although it is not yet known whether glimepiride is a substrate of the P-glycoprotein, inhibition of the P-glycoprotein cannot explain the present interaction, because gemfibrozil does not seem to inhibit the P-glycoprotein (Fehrman-Ekholm et al 1996, Pisanti et al 1998).

The interaction of gemfibrozil with glimepiride is probably of limited clinical significance in most patients. However, because gemfibrozil can improve insulin sensitivity in hypertriglyceridemic patients (Avogaro et al 1995, Mussoni et al 2000) and because of variation in the extent of the interaction, in some patients the blood glucose-lowering effect of glimepiride may be greater during concomitant treatment with gemfibrozil.

Acetylsalicylic acid (1 g daily) reduced the AUC and C_{max} of glimepiride by 34% and 4% (Amaryl prescribing information 2000). This was probably the result of acetylsalicylic acid's displacing glimepiride from plasma proteins. Glimepiride is a low clearance drug but is highly bound to plasma proteins and has a very low volume of distribution. Therefore, displacement of glimepiride from plasma proteins can lead to an increased free fraction, leading to an increased clearance of the drug. Because gemfibrozil is also highly bound to plasma proteins, this same effect may have lessened the extent of the gemfibrozil-glimepiride interaction. Thus, the relatively low degree of pharmacokinetic interaction between gemfibrozil and glimepiride should not cause one to conclude that gemfibrozil only modestly inhibits CYP2C9 in vivo.

In conclusion, fluconazole considerably elevated the plasma concentrations of glimepiride and prolonged its $t_{½}$. This was probably the result of inhibition of the CYP2C9-mediated biotransformation of glimepiride by fluconazole. Concomitant use of glimepiride with fluconazole or other potent inhibitors of CYP2C9 such as amiodarone (Heimark et al 1992) or miconazole (O'Reilly et al 1992) may increase the risk of hypoglycemia. Fluvoxamine and gemfibrozil moderately elevate the plasma concentrations and slightly prolong the $t_{½}$ of

glimepiride and may, in some patients, enhance the blood glucose-lowering effect of glimepiride.

3.2. Effects of inhibition of CYP3A4 on repaglinide

Study VI demonstrated that inhibition of CYP3A4 can raise the plasma concentrations and the effects of repaglinide. In this study, 250 mg clarithromycin twice daily boosted the C_{max} and AUC(0-∞) of repaglinide by 67% and 40%, respectively. In line with this, the insulin response was significantly enhanced by clarithromycin. There was marked interindividual variation in the extent of the effect of clarithromycin on the C_{max} of repaglinide (the relative increase in the C_{max} of repaglinide ranged from 10 to 270%). No significant differences appeared in the blood glucose variables between the clarithromycin and placebo phases. This may be explained by the relatively low degree of pharmacokinetic interaction and by the repeated food intake during the study days (for safety reasons) which probably amplified variation in blood glucose values.

The oral bioavailability of repaglinide is only about 60%, because of first-pass metabolism (Hatorp et al 1998). According to in vitro studies, CYP3A4 is the principal CYP enzyme involved in the metabolism of repaglinide to its main metabolites (Prandin prescribing information 1998). The metabolites are excreted almost exclusively in the feces (van Heiningen et al 1999). Because CYP3A4 is expressed in marked quantities in the small intestinal mucosa (Kivistö et al 1996, Zhang et al 1999), it is likely that the intestinal CYP3A4 is involved in repaglinide first-pass metabolism. A previous study failed to show any significant effect of cimetidine (400 mg twice daily) – a relatively weak inhibitor of CYP3A4 and of some other CYP enzymes (Martínez et al 1999) – on repaglinide pharmacokinetics (Hatorp & Thomsen 2000).

Clarithromycin forms inactive iron-metabolite complexes with CYP3A4 and strongly inhibits its catalytic activity (Gascon & Dayer 1991, Spicer et al 1997). Clarithromycin considerably increases the plasma concentrations and effects of several CYP3A4 substrates (Westphal 2000), for example those of the CYP3A4 probe drug midazolam (Yeates et al 1996, Gorski et al 1998). The mechanism of the interaction between clarithromycin and repaglinide is probably inhibition of the CYP3A4-mediated metabolism of repaglinide. Because clarithromycin raised

considerably the C_{max} of repaglinide but only slightly prolonged the $t_{½}$, it seems that clarithromycin inhibited the CYP3A4-mediated metabolism of repaglinide mainly during the first pass. Intestinal CYP3A4 has been shown to play a major role in drug interactions with CYP3A4 inhibitors, for instance in the interaction between clarithromycin and midazolam (Gorski et al 1998), and it is probable that intestinal CYP3A4 plays an important role also in the interaction of clarithromycin with repaglinide. Apart from CYP3A4, clarithromycin seems to inhibit the P-glycoprotein, as well (Wakasugi et al 1998). Because it is not yet known whether repaglinide is a substrate of the P-glycoprotein, the possibility cannot be ruled out that inhibition of the P-glycoprotein by clarithromycin contributed to this interaction.

The effect of clarithromycin on CYP3A4 activity in vivo is clearly dose-dependent (Yeates et al 1996, Gorski et al 1998). It is therefore probable that a higher dosage of clarithromycin would have had a greater effect on the plasma concentrations and effects of repaglinide than was seen in this study with 250 mg clarithromycin twice daily.

The C_{max} of clarithromycin correlated inversely with the relative increase in the AUC(0-7) of repaglinide (i.e., subjects with the greatest increases in repaglinide AUC(0-7) had the lowest clarithromycin C_{max} values). Because clarithromycin is itself a substrate of CYP3A4 (Rodrigues et al 1997), clarithromycin C_{max} values are probably inversely related to CYP3A4 activity during the first pass of clarithromycin. Thus it seems evident that the potential for an increase in plasma repaglinide concentrations after inhibition of CYP3A4 is greater in subjects with higher CYP3A4 activity. Therefore, some patients who initially need higher doses of repaglinide because of their higher CYP3A4 activity may be more susceptible to the interaction between CYP3A4 inhibitors and repaglinide than are patients whose blood glucose levels are sufficiently controlled with lower doses of repaglinide.

The interactions of oral antidiabetic drugs, ones acting by increasing insulin secretion, with inhibitors of their metabolism are potentially dangerous. In addition to clarithromycin, a number of other drugs such as diltiazem, erythromycin, itraconazole, nefazodone, and verapamil, as well as grapefruit juice are potent inhibitors of CYP3A4 (Dresser et al 2000) and may therefore elevate the plasma concentrations and enhance the effects of repaglinide. The effects of erythromycin on the hypoglycemic activity of repaglinide may be

particularly pronounced, since erythromycin enhances insulin secretion and lowers blood glucose levels, probably through its effects on the motilin receptor (Ueno et al 2000). In any case, because the effects of CYP3A4 inhibitors are substrate-dependent (Wang et al 2000), one must be cautious in extrapolating results obtained with other CYP3A4 substrates to repaglinide.

In conclusion, clarithromycin elevates the plasma concentrations and enhances the effects of repaglinide, probably by inhibiting the CYP3A4-mediated biotransformation of repaglinide mainly during the first-pass phase. The concomitant use of clarithromycin or other potent inhibitors of CYP3A4 with repaglinide may enhance its blood glucose-lowering effect and increase the risk of hypoglycemia.

CONCLUSIONS

From these six studies the following conclusions can be drawn:

1. Rifampicin, and probably also other potent inducers of CYP2C9, reduce the plasma concentrations of the sulfonylureas glibenclamide, glimepiride, and glipizide. Glibenclamide is the most susceptible sulfonylurea to induction, and glipizide least affected; induction of the P-glycoprotein may also contribute to this interaction between rifampicin and glibenclamide. The concomitant use of rifampicin with a sulfonylurea may thus worsen blood glucose control.

2. Rifampicin, and probably also other potent inducers of CYP3A4, reduce the plasma concentrations and the blood glucose-lowering effect of repaglinide. Rifampicin has greater effects on the CYP3A4 substrate repaglinide than on any of the sulfonylureas.

3. Inhibition of CYP2C9 elevates plasma concentrations of glimepiride. The concomitant use of fluconazole or other potent inhibitors of CYP2C9 with glimepiride may markedly increase the risk of hypoglycemia. Glimepiride is also significantly, but less affected by fluvoxamine, gemfibrozil, and other less potent inhibitors of CYP2C9.

4. Inhibition of CYP3A4 enhances the plasma concentrations of and insulin response to repaglinide. The concomitant use of potent inhibitors of CYP3A4 with repaglinide may enhance its blood glucose-lowering effect and increase the risk of hypoglycemia.

ACKNOWLEDGEMENTS

This work was carried out at the Department of Clinical Pharmacology, University of Helsinki, during the years 1999 to 2001. I am grateful to all who helped me during this period.

I am especially grateful to Professor Pertti Neuvonen, Head of the Department of Clinical Pharmacology, for his encouraging supervision. His broad knowledge in clinical pharmacology and in science has made a great impression on me. He has created with his warmth an inspiring environment for productive scientific work.

I am very grateful to Docent Kari Kivistö, my other supervisor, for scientific guidance and friendly encouragement during these years. Inspiring discussions with him about clinical pharmacology and science, and about life in general have been most valuable.

I wish to express my sincere thanks to Docent Risto Huupponen and Professor Olavi Tokola for their thorough review and constructive criticism concerning this thesis.

Warm thanks are due to Docent Janne Backman for collaboration and for his invaluable counsel in statistical issues.

I express my gratitude to Jouko Laitila and Mikko Neuvonen for their skilled mass spectrometric determinations of plasma drug concentrations. Kerttu Mårtensson, Eija Mäkinen-Pulli, Lisbet Partanen, and Anja Närväinen are also warmly acknowledged for their determining of plasma drug concentrations and their skillful technical assistance. Without their efforts these studies would not have been possible. Airi Järvinen is also gratefully acknowledged.

I wish to thank all my colleagues at the department of Clinical Pharmacology for their support and friendship. Kari Raaska, Carl Kyrklund, Laura Juntti-Patinen, Kati Ahonen, Teemu Kantola, Jari Lilja, Tiina Varis, Jun-Sheng Wang, Xia Wen, Outi Lapatto-Reiniluoto, Harri Luurila, Lasse Lehtonen, Mika Jokinen, Kirsti Villikka, Maija Kaukonen, Elina Saarenmaa, and Matti Kivikko have created a cozy but stimulating atmosphere at the department. Tuija Itkonen is also gratefully acknowledged for her help in many practical matters.

ACKNOWLEDGEMENTS

I also want to thank Docent Kalle Hoppu and the staff of the Poison Information Centre.

Carol Norris is warmly acknowledged for author-editing the English language of this thesis.

This work was financially supported by the Clinical Drug Research Graduate School, the Helsinki University Central Hospital Research Fund, the National Technology Agency of Finland (TEKES), the Finnish Medical Society Duodecim, and the Emil Aaltonen Foundation, all of which are gratefully acknowledged.

I am also grateful to all the volunteers who participated in the studies.

My warm thanks are due to my friends for enriching my life with other important things, such as singing. Every Tuesday's singing in the Tavastia Nation Singers and free time spent with the Singers during the past 6 years have been most rewarding. Unfortunately, I have not been able to attend as much as I would have liked.

My warmest thanks are due to my father Timo Niemi, my mother Liisa Larkas, to my sister Paula Arvilommi and her husband Jaakko, as well as their lovely children Lotta and Ronja, and to my sister Laura Niemi, for their support and encouragement during these years.

Espoo, November 22nd, 2001

Mikko Niemi

REFERENCES

Abdel-Rahman SM, Gotschall RR, Kauffman RE, Leeder JS, Kearns GL. Investigation of terbinafine as a CYP2D6 inhibitor in vivo. *Clin Pharmacol Ther* 1999;65:465-72.

Acocella G. Clinical pharmacokinetics of rifampicin. *Clin Pharmacokinet* 1978;3:108-127.

Aguilar-Bryan L, Clement JP 4th,Gonzalez G, Kunjilwar K, Babenko A, Bryan J. Toward understanding the assembly and structure of K_{ATP} channels. *Physiol Rev* 1998;78:227-45.

Ahmad S. Gemfibrozil interaction with warfarin sodium. *Chest* 1990;98:1041-2.

Ahmad S. Gemfibrozil: interaction with glyburide. *South Med J* 1991;84:102.

Aithal GP, Day CP, Kesteven PJL, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999;353:717-9.

Amaryl prescribing information. Hoechst-Roussel Pharmaceuticals, Kansas City, MO, USA, 2000

Anderson GD. A mechanistic approach to antiepileptic drug interactions. *Ann Pharmacother* 1998;32:554-63.

Anonymous. Prescribing information revised for cerivastatin in the US and UK. *Reactions* 2001;859:2.

Appel S, Rüfenacht T, Kalafsky G, Tetzloff W, Kallay Z, Hitzenberger G, Kutz K. Lack of interaction between fluvastatin and oral hypoglycemic agents in healthy subjects and in patients with non-insulin-dependent diabetes mellitus. *Am J Cardiol* 1995;76:29A-32A.

Asplund K, Wiholm BE, Lithner F. Glibenclamide-associated hypoglycemia: a report on 57 cases. *Diabetologia* 1983;24:412-7.

AutoDELFIA™ Insulin kit package insert. Wallac Oy, Turku, Finland 1998.

Avogaro A, Beltramello P, Marin R, Zambon S, Bonanome A, Biffanti S, Confortin L, Manzato E, Crepaldi G, Tiengo A. Insulin action and glucose metabolism are improved by gemfibrozil treatment in hypertriglyceridemic patients. *Atherosclerosis* 1995;113:117-24.

Bachmann KA, Jauregui L. Use of single sample clearance estimates of cytochrome P450 substrates to characterize human hepatic CYP status in vivo. *Xenobiotica* 1993;23:307-15.

Back DJ, Houlgrave R, Tjia JF, Ward S, Orme ML. Effect of the progestogens, gestodene, 3-keto desogestrel, levonorgestrel, norethisterone and norgestimate on the oxidation of ethinylestradiol and other substrates by human liver microsomes. *J Steroid Biochem Mol Biol* 1991;38:219-25.

Back DJ, Tjia JF, Karbwang J, Colbert J. In vitro inhibition studies of tolbutamide hydroxylase activity of human liver microsomes by azoles, sulphonamides and quinolines. *Br J Clin Pharmacol* 1988;26:23-9

Backman JT, Kivistö KT, Wang JS, Neuvonen PJ. Antifungals. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000a. p. 623-646.

REFERENCES

Backman JT, Kyrklund C, Kivistö KT, Wang JS, Neuvonen PJ. Plasma concentrations of active simvastatin acid are increased by gemfibrozil. *Clin Pharmacol Ther* 2000b;68:122-9.

Backman JT, Olkkola KT, Neuvonen PJ. Rifampin drastically reduces plasma concentrations and effects of oral midazolam. *Clin Pharmacol Ther* 1996a;1:7-13.

Backman JT, Olkkola KT, Ojala M, Laaksovirta H, Neuvonen PJ. Concentrations and effects of oral midazolam are greatly reduced in patients treated with carbamazepine and phenytoin. *Epilepsia* 1996b;37:253-7.

Badian M, Korn A, Lehr KH, Malerczyk V, Waldhausl W. Pharmacokinetics and pharmacodynamics of the hydroxymetabolite of glimepiride (Amaryl) after intravenous administration. *Drug Metabol Drug Interact* 1996;13:69-85.

Balfour JA, McTavish D. Acarbose. An update of its pharmacology and therapeutic use in diabetes mellitus. *Drugs* 1993;46:1025-54.

Balfour JA, Plosker GL. Rosiglitazone. *Drugs* 1999;57:921-30.

Bertilsson L, Carrillo JA, Dahl ML, Llerena A, Alm C, Bondesson U, Lindström L, de la Rubia IR, Ramos S, Benitez J. Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test. *Br J Clin Pharmacol* 1994;38:471-3.

Bertz RJ, Granneman GR. Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin Pharmacokinet* 1997;32:210-58.

Black DJ, Kunze KL, Wienkers LC, Gidal BE, Seaton TL, McDonnell ND, Evans JS, Bauwens JE, Trager WF. Warfarin-fluconazole; II: a metabolically based drug interaction: in vivo studies. *Drug Metab Dispos* 1996;24:422-8.

Blum RA, Wilton JH, Hilligloss DM, Gardner MJ, Henry EB, Harrison NJ, Schentag JJ. Effect of fluconazole on the disposition of phenytoin. *Clin Pharmacol Ther* 1991;49:420-5.

Branch RA, Adedoyin A, Frye RF, Wilson JW, Romkes M. In vivo modulation of CYP enzymes by quinidine and rifampin. *Clin Pharmacol Ther* 2000;68:401-11.

Brian WR. Hypoglycemic agents. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 529-543.

Brodie MJ. Drug interactions in epilepsy. *Epilepsia* 1992;33(suppl 1):S13-22.

Bruce MA, Hall SD, Haehner-Daniels BD, Gorski JC. In vivo effect of clarithromycin on multiple cytochrome P450s. *Drug Metab Dispos* 2001;29:1023-8.

Brøsen K. Drug interactions and the cytochrome P450 system: The role of cytochrome P450 1A2. *Clin Pharmacokinet* 1995;29(suppl 1):20-5.

Chan JC, Cockram CS, Critchley JA. Drug-induced disorders of glucose metabolism. Mechanisms and management. *Drug Safety* 1996;15:135-57.

Chang TK, Yu L, Maurel P, Waxman DJ. Enhanced cyclophosphamide and ifosfamide activation in primary human hepatocyte cultures: response to cytochrome P-450 inducers and autoinduction by oxazaphosphorines. *Cancer Res* 1997;57:1946-54.

Chien SM, Pichotta P, Siepman N, Chan CK, The Canada-Sweden clarithromycin-pneumonia study group. Treatment of community-acquired pneumonia. A multicenter, double-blind, randomized study comparing clarithromycin with erythromycin. *Chest* 1993;103:697-701.

Chu SY, Deaton R, Cavanaugh J. Absolute bioavailability of clarithromycin after oral administration in humans. *Antimicrob Agents Chemother* 1992a;36:1147-50.

Chu SY, Park Y, Locke C, Wilson DS, Cavanaugh JC. Drug-food interaction potential of clarithromycin, a new macrolide antimicrobial. *J Clin Pharmacol* 1992b;32:32-6.

Combalbert J, Fabre I, Fabre G, Dalet I, Drancourt J, Cano JP, Maurel P. Metabolism of cyclosporin A. IV. Purification and identification of the rifampicin-inducible human liver cytochrome P-450 (cyclosporin A oxidase) as a product of P450IIIA gene subfamily. *Drug Metab Dispos* 1989;17:197-207.

Connacher AA, El Debani AH, Isles TE, Stevenson IH. Disposition and hypoglycaemic action of glipizide in diabetic patients given a single dose of nifedipine. *Eur J Clin Pharmacol* 1987;33:81-3.

Coppack SW, Lant AF, McIntosh CS, Rodgers AV. Pharmacokinetic and pharmacodynamic studies of glibenclamide in non-insulin dependent diabetes mellitus. *Br J Clin Pharmacol* 1990;29:673-84.

Creutzfeldt W. Current views on the mode of action of hypoglycemic sulfonamides. *Acta Diabet Lat* 1969;6(suppl 1):201-15.

Dabrowski M, Wahl P, Holmes WE, Ashcroft FM. Effect of repaglinide on cloned beta cell, cardiac and smooth muscle types of ATP-sensitive potassium channels. *Diabetologia* 2001;44:747-56.

Dalet-Beluche I, Boulenc X, Fabre G, Maurel P, Bonfils C. Purification of two cytochrome P450 isozymes related to CYP2A and CYP3A gene families from monkey (baboon, Papio papio) liver microsomes. Cross reactivity with human forms. *Eur J Biochem* 1992;204:641-48.

Debruyne D. Clinical pharmacokinetics of fluconazole in superficial and systemic mycoses. *Clin Pharmacokinet* 1997;33:52-77.

Diabeta prescribing information. Aventis Pharmaceuticals Products Inc, Bridgewater, NJ, USA, 2000

Diflucan prescribing information. Pfizer Inc, New York, NY, USA, 1998

Dilger K, Hofmann U, Klotz U. Enzyme induction in the elderly: effect of rifampin on the pharmacokinetics and pharmacodynamics of propafenone. *Clin Pharmacol Ther* 2000;67:512-20.

Doostdar H, Grant MH, Melvin WT, Wolf CR, Burke MD. The effects of inducing agents on cytochrome P450 and UDP-glucuronyltransferase activities in human HEPG2 hepatoma cells. *Biochem Pharmacol* 1993;46:629-35.

Douglas JG, McLeod MJ. Pharmacokinetic factors in the modern drug treatment of tuberculosis. *Clin Pharmacokinet* 1999;37:127-46.

Dresser GK, Spence JD, Bailey DG. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet* 2000;38:41-57.

Dunn CJ, Faulds D. Nateglinide. *Drugs* 2000;60:607-15.

Dunn CJ, Peters DH. Metformin. A review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus. *Drugs* 1995;49:721-49.

REFERENCES

Dürr D, Stieger B, Kullack-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, Fattinger K. St John's wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin Pharmacol Ther* 2000;68:598-604.

Elvander-Ståhl E, Melander A, Wåhlin-Boll E. Indobufen interacts with the sulphonylurea, glipizide, but not with the β-adrenergic receptor antagonists, propranolol and atenolol. *Br J Clin Pharmacol* 1984;18:773-8.

Ernst E. Second thoughts about safety of St John's wort. *Lancet* 1999;354:2014-6.

European Pharmacopoeia, Third Edition. Strasbourg: Council of Europe; 1997. p. 133

Feely J, Collins WCJ, Cullen M, El Debani AH, MacWalter RS, Peden NR, Stevenson IH. Potentiation of the hypoglycaemic response to glipizide in diabetic patients by histamine H2-receptor antagonists. *Br J Clin Pharmacol* 1993;35:321-3.

Fehrman-Ekholm I, Jogestrand T, Angelin B. Decreased cyclosporine levels during gemfibrozil treatment of hyperlipidemia after kidney transplantation. *Nephron* 1996;72:483.

Feng HJ, Huang SL, Wang W, Zhou HH. The induction effect of rifampicin on activity of mephenytoin 4'-hydroxylase related to M1 mutation of CYP2C19 and gene dose. *Br J Clin Pharmacol* 1998;45:27-9.

Figgitt DP, McClellan KJ. Fluvoxamine. An updated review of its use in the management of adults with anxiety disorders. *Drugs* 2000;60:925-54.

Fleishaker JC, Phillips JP. Evaluation of a potential interaction between erythromycin and glyburide in diabetic volunteers. *J Clin Pharmacol* 1991;31:259-62.

Fournier JP, Schneider S, Matinez P, Mahagne MH, Ducoeur S, Haffner M, Thiercelin D, Chichmanian RM, Bertrand F. Coma hypoglycemique chez une patiente traitee par glipizide et fluconazole: une possible interaction? *Therapie* 1992;47:446-7.

Fromm MF, Busse D, Kroemer HK, Eichelbaum M. Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. *Hepatology* 1996;24:796-801.

Fromm MF, Eckhardt K, Li S, Schänzle G, Hofmann U, Mikus G, Eichelbaum M. Loss of analgesic effect of morphine due to coadministration of rifampin. *Pain* 1997;72:261-7.

Fromm MF, Kauffman HM, Fritz P, Burk O, Kroemer HK, Warzok RW, Eichelbaum M, Siegmund W, Schrenk D. The effect of rifampin treatment on intestinal expression of human MRP transporters. *Am J Pathol* 2000;157:1575-80.

Fuhlendorff J, Rorsman P, Kofod H, Brand CL, Rolin B, MacKay P, Shymko R, Carr RD. Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. *Diabetes* 1998;47:345-51.

Fuhr U, Anders EM, Mahr G, Sorgel F, Staib AH. Inhibitory potency of quinolone antibacterial agents against cytochrome P4501A2 activity in vivo and in vitro. *Antimicrob Agents Chemother* 1992;36:942-8.

Fuhr U. Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. *Clin Pharmacokinet* 2000;38:493-504.

Funck-Bretano C, Becquemont L, Lenevu A, Roux A, Jaillon P, Beaune P. Inhibition by omeprazole of proguanil metabolism: mechanism of the interaction in vitro and prediction of in vivo results from the in vitro experiments. *J Pharmacol Exp Ther* 1997;280:730-8.

Gascon MP, Dayer P. Comparative effects of macrolide antibiotics on liver monoxygenases. *Clin Pharmacol Ther* 1991;49:158.

Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* 2001;276:14581-7.

Gerbal-Chaloin S, Pascussi J-M, Pichard-Garcia L, Daujat M, Waechter F, Fabre J-M, Carrère N, Maurel P. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab Dispos* 2001;29:242-51.

Gillies PS, Dunn CJ. Pioglitazone. *Drugs* 2000;60:333-343.

Girardin E, Vial T, Pharm E, Evreux J-C. Hypoglycémies induites par les sulfamides hypoglycémiants. *Ann Med Interne* 1992;143:11-7.

Goldstein PE, Boom A, van Geffel J, Jacobs P, Masereel B, Beauwens R. P-glycoprotein inhibition by glibenclamide and related compounds. *Pflugers Arch* 1999;437:652-60.

Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM Jr., Hall SD. The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. *Clin Pharmacol Ther* 1998;64:133-43.

Grant SM, Clissold SP. Fluconazole. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial and systemic mycoses. *Drugs* 1990;39:877-916.

Greiner B, Eichelbaum M, Fritz P, Kreichgauer H-P, von Richter O, Zundler J, Kroemer HK. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* 1999;104;147-53.

Grgurinovich N, Matthews A. Analysis of erythromycin and roxithromycin in plasma or serum by high-performance liquid chromatography using electrochemical detection. *J Chromatogr* 1988;433:298-304.

Gribble FM, Tucker SJ, Seino S, Ashcroft FM. Tissue specificity of sulfonylureas: studies on cloned cardiac and beta-cell K_{ATP} channels. *Diabetes* 1998;47:1412-8.

Gu DF, Hinks LJ, Morton NE, Day IN. The use of long PCR to confirm three common alleles at the CYP2A6 locus and the relationship between genotype and smoking habit. *Ann Hum Genet* 2000;64:383-90.

Guay DR. Repaglinide, a novel, short-acting hypoglycemic agent for type 2 diabetes mellitus. *Pharmacotherapy* 1998;18:1195-204.

Guengerich FP. Mechanism-based inactivation of human liver microsomal cytochrome P-450 IIIA4 by gestodene. *Chem Res Toxicol* 1990;3:363-71.

Guengerich FP. Oxidation of 17 α-ethynylestradiol by human liver cytochrome P-450. *Mol Pharmacol* 1988;33:500-8.

Gustavson LE, Shi H, Palmer RN, Siepman NC, Craft JC. Drug interaction between clarithromycin and fluconazole in healthy subjects. *Clin Pharmacol Ther* 1996;59:185

REFERENCES

Hamman MA, Bruce MA, Haehner-Daniels BD, Hall SD. The effect of rifampin administration on the disposition of fexofenadine. *Clin Pharmacol Ther* 2001;69:114-21.

Hargreaves JA, Jezequel S, Houston JB. Effect of azole antifungals on human microsomal metabolism of diclofenac and midazolam. *Br J Clin Pharmacol* 1994;38:175P

Hatorp V, Huang WC, Strange P. Repaglinide pharmacokinetics in healthy young adult and elderly subjects. *Clin Ther 1999*;21:702-10.

Hatorp V, Oliver S, Su CA. Bioavailability of repaglinide, a novel antidiabetic agent, administered orally in tablet or solution form or intravenously in healthy male volunteers. *Int J Clin Pharmacol Ther* 1998;36:636-41.

Hatorp V, Thomsen MS. Drug interaction studies with repaglinide: repaglinide on digoxin or theophylline pharmacokinetics and cimetidine on repaglinide pharmacokinetics. *J Clin Pharmacol* 2000;40:184-92.

Hatorp V, Walther KH, Christensen MS, Haug-Pihale G. Single-dose pharmacokinetics of repaglinide in subjects with chronic liver disease. *J Clin Pharmacol* 2000;40:142-52.

Hebert MF, Roberts JP, Prueksaritanont T, Benet LZ. Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. *Clin Pharmacol Ther* 1992;52:453-7.

Hedge SS, Wong AG, Perry MR, Ku P, Moy TM, Loeb M, Eglen RM. 5-HT4 receptor mediated stimulation of gastric emptying in rats. *Naunyn Schmiedebergs Arch Pharmacol* 1995;351:589-95.

Heimark LD, Wienkers L, Kunze K, Gibaldi M, Eddy AC, Trager WF, O'Reilly RA, Goulart DA. The mechanism of the interaction between amiodarone and warfarin in humans. *Clin Pharmacol Ther* 1992;51:398-407.

van Heiningen PN, Hatorp V, Kramer Nielsen K, Hansen KT, van Lier JJ, van De Merbel NC, Oosterhuis B, Jonkman JHG. Absorption, metabolism and excretion of a single oral dose of 14C-repaglinide during repaglinide multiple dosing. *Eur J Clin Pharmacol* 1999;55:521-5.

Hemeryck A, De Vriendt C, Belpaire FM. Inhibition of CYP2C9 by selective serotonin reuptake inhibitors: in vitro studies with tolbutamide and (S)-warfarin using human liver microsomes. *Eur J Clin Pharmacol* 1999;54:947-51.

Hengy H, Kölle EU. Determination of gemfibrozil in plasma by high performance liquid chromatography. *Arzneimittelforschung* 1985;35:1637-9.

Hirohito S, Akimitsu T, Nobuhiro Y. Ibuprofen-related hypoglycemia in a patient receiving sulfonylurea. *Ann Intern Med* 2001;134:344.

Holdiness MR. Clinical pharmacokinetics of the antituberculosis drugs. *Clin Pharmacokinet* 1984;9:511-44.

Holtbecker N, Fromm MF, Kroemer HK, Ohnhaus EE, Heidemann H. The nifedipine-rifampin interaction. Evidence for induction of gut wall metabolism. *Drug Metab Dispos* 1996;24:1121-3.

Hukkanen J, Lassila A, Päivärinta K, Valanne S, Sarpo S, Hakkola J, Pelkonen O, Raunio H. Induction and regulation of xenobiotic-metabolizing cytochrome P450s in the human A549 lung adenocarcinoma cell line. *Am J Respir Cell Mol Biol* 2000;22:360-6.

Huupponen R, Karhuvaara S, Seppälä P. Effect of guar gum on glipizide absorption in man. *Eur J Clin Pharmacol* 1985;28:717-9.

Huupponen R, Seppälä P, Iisalo E. Glipizide pharmacokinetics and response in diabetics. *Int J Clin Pharmacol Ther Toxicol* 1982;20:417-22.

Inagaki K, Takagi J, Lor E, Okamoto MP, Gill MA. Determination of fluconazole in human serum by solid-phase extraction and reversed-phase high-performance liquid chromatography. *Ther Drug Monit* 1992;14:306-11.

Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H, Sugiyama Y. Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* 1998;50:387-411.

Jalava KM, Partanen J, Neuvonen PJ. Itraconazole decreases renal clearance of digoxin. *Ther Drug Monit* 1997;19:609-13.

Jurima-Romet M, Crawford K, Cyr T, Inaba T. Terfenadine metabolism in human liver. In vitro inhibition by macrolide antibiotics and azole antifungals. *Drug Metab Dispos* 1994;22:849-57.

Kalbag JB, Walter YH, Nedelman JR, McLeod JF. Mealtime glucose regulation with nateglinide: comparison with repaglinide and placebo. *Diabetes Care* 2001;24:73-7.

Kantola T, Backman JT, Niemi M, Kivistö KT, Neuvonen PJ. Effect of fluconazole on plasma fluvastatin and pravastatin concentrations. *Eur J Clin Pharmacol* 2000;56:225-9.

Kashuba AD, Nafziger AN, Kearns GL, Leeder JS, Gotschall R, Rocci ML Jr, Kulawy RW, Beck DJ, Bertino JS Jr. Effect of fluvoxamine therapy on the activities of CYP1A2, CYP2D6 and CYP3A as determined by phenotyping. *Clin Pharmacol Ther* 1998;64:257-68.

Kashuba AD, Nafziger AN. Physiological changes during the menstrual cycle and their effects of the pharmacokinetics and pharmacodynamics of drugs. *Clin Pharmacokinet* 1998;34:203-18.

Kaukonen KM, Olkkola KT, Neuvonen PJ. Fluconazole but not itraconazole decreases the metabolism of losartan to E-3174. *Eur J Clin Pharmacol* 1998;53:445-9.

Kazierad DJ, Martin DE, Blum RA, Tenero DM, Ilson B, Boike SC, Etheredge R, Jorkasky DK. Effect of fluconazole on the pharmacokinetics of eprosartan and losartan in healthy male volunteers. *Clin Pharmacol Ther* 1997;62:417-25.

Kern A, Bader A, Pichlmayr R, Sewing KF. Drug metabolism in hepatocyte sandwich cultures of rats and humans. *Biochem Pharmacol* 1997;54:761-72.

Kharasch ED, Hankins DC, Taraday JK. Single-dose methoxsalen effects on human cytochrome P-450 2A6 activity. *Drug Metab Dispos* 2000;28:28-33.

Kidd RS, Straugh AB, Meyer MC, Blaisdell J, Goldstein JA, Dalton JT. Pharmacokinetics of chlorpheniramine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. *Pharmacogenetics* 1999;9:71-80.

Kivistö KT, Brookjans G, Fromm MF, Griese EU, Munzel P, Kroemer HK. Expression of CYP3A4, CYP3A5 and CYP3A7 in human duodenal tissue. *Br J Clin Pharmacol* 1996;42:387-9.

REFERENCES

Kivistö KT, Neuvonen PJ. Enhancement of absorption and effect of glipizide by magnesium hydroxide. *Clin Pharmacol Ther* 1991a;49:39-43.

Kivistö KT, Neuvonen PJ. Differential effects of sodium bicarbonate and aluminium hydroxide on the absorption and activity of glipizide. *Eur J Clin Pharmacol* 1991b;40:383-6.

Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Wilson TM, Zetterstrom RH, Perlmann T, Lehmann JM. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* 1998;92:73-82.

Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB. Indentification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J Clin Invest* 1992;90:1871-8.

Korzekwa KR, Krishnamachary N, Shou M, Ogai A, Parise RA, Rettie AE, Gonzalez FJ, Tracy TS. Evaluation of atypical cytochrome P450 kinetics with two-substrate models: evidence that multiple substrates can simultaneously bind to cytochrome P450 active sites. *Biochemistry* 1998;37:4137-47.

Kradjan WA, Kobayashi KA, Bauer LA, Horn JR, Opheim KE, Wood FJ. Glipizide pharmacokinetics: effects of age, diabetes, and multiple dosing. *J Clin Pharmacol* 1989;29:1121-7.

Kramer W, Muller G, Girbig F, Gutjahr U, Kowalewski S, Hartz D, Summ HD. Differential interaction of glimepiride and glibenclamide with the beta-cell sulfonylurea receptor. II. Photoaffinity labeling of a 65 kDa protein by [3H]glimepiride. *Biochim Biophys Acta* 1994;1191:278-90.

Krishna DR, Klotz U. Extrahepatic metabolism of drugs in humans. *Clin Pharmacokinet* 1994;26:144-60.

Kubacka RT, Antal EJ, Juhl RP, Welshman IR. Effects of aspirin and ibuprofen on the pharmacokinetics and pharmacodynamics of glyburide in healthy subjects. *Ann Pharmacother* 1996;30:20-6.

Kubacka RT, Antal EJ, Juhl RP. The paradoxical effect of cimetidine and ranitidine on glibenclamide pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol* 1987;23:743-51.

Kunze KL, Trager WF. Isoform-selective mechanism-based inhibition of human cytochrome P450 1A2 by furafylline. *Chem Res Toxicol* 1993;6:649-56.

Kunze KL, Wienkers LC, Thummel KE, Trager WF. Warfarin-fluconazole. I. Inhibition of the human cytochrome P450-dependent metabolism of warfarin by fluconazole: in vitro studies. *Drug Metab Dispos* 1996;24:414-21

Kyrklund C, Backman JT, Kivistö KT, Neuvonen M, Laitila J, Neuvonen PJ. Plasma concentrations of active lovastatin acid are markedly increased by gemfibrozil but not by bezafibrate. *Clin Pharmacol Ther* 2001;69:340-5.

Kyrklund C, Backman JT, Kivistö KT, Neuvonen M, Laitila J, Neuvonen PJ. Rifampin greatly reduces plasma simvastatin and simvastatin acid concentrations. *Clin Pharmacol Ther* 2000;68:592-7.

Laakso S, Scheinin M, Anttila M. Determination of erythromycin base and 2-acetylerythromycin in human plasma using high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 1990;526:475-86.

Lamberg TS, Kivistö KT, Neuvonen PJ. Concentrations and effects of buspirone are considerably reduced by rifampicin. *Br J Clin Pharmacol* 1998a;45:381-5.

Lamberg TS, Kivistö KT, Laitila J, Mårtensson K, Neuvonen PJ. The effect of fluvoxamine on the pharmacokinetics and pharmacodynamics of buspirone. *Eur J Clin Pharmacol* 1998b;54:761-6.

Landgraf R, Bilo HJG, Müller PG. A comparison of repaglinide and glibenclamide in the treatment of type 2 diabetic patients previously treated with sulphonylureas. *Eur J Clin Pharmacol* 1999;55:165-71.

Landgraf R. Meglitinide analogues in the treatment of type 2 diabetes mellitus. *Drugs & Aging* 2000;17:411-25.

Langtry HD, Balfour JA. Glimepiride. A review of its use in the management of type 2 diabetes mellitus. *Drugs* 1998;55:563-84.

Lasker JM, Wester MR, Aramsombatdee E, Raucy JL. Characterization of CYP2C19 and CYP2C9 from human liver: respective roles in microsomal tolbutamide, s-mephenytoin, and omeprazole hydroxylations. *Arch Biochem Biophys* 1998;353:16-28

Lee K, Mize R, Lowenstein SR. Glyburide-induced hypoglycemia and ranitidine. *Ann Intern Med* 1987;107:261-2.

Lee KH, Shin JG, Chong WS, Kim S, Lee JS, Jang IJ, Shin SG. Time course of the changes in prednisolone pharmacokinetics after co-administration or discontinuation of rifampin. *Eur J Clin Pharmacol* 1993;45:287-9.

Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, Kliewer SA. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* 1998;102:1016-23.

Lin JH, Lu AYH. Inhibition and induction of cytochrome P450 and the clinical implications. *Clin Pharmacokinet* 1998;35:361-90.

Lindstrom TD, Hanssen BR, Wrighton SA. Cytochrome P-450 complex formation by dirithromycin and other macrolides in rat and human livers. *Antimicrob Agents Chemother* 1993;37:265-9.

Loos U, Musch E, Jensen JC, Schwabe HK, Eichelbaum M. Influence of the enzyme induction by rifampicin on its presystemic metabolism. *Pharmacol Ther* 1987;33:201-4.

Loubatières A. The discovery of hypoglycemic sulfonamides and particularly of their action mechanism. *Acta Diabet lat* 1969;6(suppl 1):20-56.

Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, Brown MB, Huo W, Watkins PB. Grapefruit juice increases felodipine oral bioavailability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* 1997;99:2545-53.

Madsen H, Enggaard TP, Hansen LL, Klitgaard NA, Brøsen K. Fluvoxamine inhibits the CYP2C9 catalyzed biotransformation of tolbutamide. *Clin Pharmacol Ther* 2001;69:41-7.

REFERENCES

Marbury TC, Ruckle JL, Hatorp V, Andersen MP, Kramer Nielsen K, Huang WC, Strange P. Pharmacokinetics of repaglinide in subjects with renal impairment. *Clin Pharmacol Ther* 2000;67:7-15.

Martínez C, Albet C, Agúndez J, Herrero E, Carrillo JA, Márquez M, Benítez J, Ortiz JA. Comparative in vitro and in vivo inhibition of cytochrome P450 CYP1A2, CYP2D6, and CYP3A by H2-receptor antagonists. *Clin Pharmacol Ther* 1999;65:369-76.

Mayhew BS, Jones DR, Hall SD. An in vitro model for predicting in vivo inhibition of cytochrome P450 3A4 by metabolic intermediate complex formation. *Drug Metab Dispos* 2000;28:1031-7.

van der Meersch-Mougeot V, Diquet B. Sensitive one-step extraction procedure for column liquid chromatographic determination of fluvoxamine in human and rat plasma. *J Chromatogr* 1991;567:441-9.

Meyer UA. Overview of enzymes of drug metabolism. *J Pharmacokinet Biopharm* 1996;24:449-59.

Miller DB, Spence JD. Clinical pharmacokinetics of fibric acid derivatives (fibrates). *Clin Pharmacokinet* 1998;34:155-62.

Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 1998;45:525-38.

Miners JO, McKinnon RA. CYP1A. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 61-73.

Mocanu MM, Maddock HL, Baxter GF, Lawrence CL, Standen NB, Yellon DM. Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. *Circulation* 2001;103:3111-6.

von Moltke LL, Greenblatt DJ, Schmider J, Duan SX, Wright CE, Harmatz JS, Shader RI. Midazolam hydroxylation by human liver microsomes in vitro: inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents. *J Clin Pharmacol* 1996;36:783-91.

Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL, Kliewer SA. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc Natl Acad Sci U S A* 2000;97:7500-2.

Morel F, Beaune PH, Ratanasavanh D, Flinois J-P, Yang CS, Guengerich FP, Guillouzo A. Expression of cytochrome P-450 enzymes in cultured human heptocytes. *Eur J Biochem* 1990;191:437-444.

Mudaliar S, Henry RR. New oral therapies for type 2 diabetes mellitus: the glitazones or insulin sensitizers. *Annu Rev Med* 2001;52:239-57.

Mussoni L, Mannucci L, Sirtori C, Pazzucconi F, Bonfardeci G, Cimminiello C, Notarbartolo A, Scafidi V, Bittolo Bon G, Alessandrini P, Nenci G, Parise P, Colombo L, Piliego T, Tremoli E. Effects of gemfibrozil on insulin sensitivity and on haemostatic variables in hypertriglyceridemic patients. *Atherosclerosis* 2000;148:397-406.

Mustajoki P, Himberg JJ, Tokola O, Tenhunen R. Rapid normalization of antipyrine oxidation by heme in variegate porphyria. *Clin Pharmacol Ther* 1992;51:320-4.

Mück W. Rational assessment of the interaction profile of cerivastatin supports its low propensity for drug interactions. *Drugs* 1998;56(Suppl 1):15-23.

Nakajima M, Kwon JT, Tanaka N, Zenta T, Yamamoto Y, Yamamoto H, Yamazaki H, Yamamoto T, Kuroiwa Y, Yokoi T. Relationship between interindividual differences in nicotine metabolism and CYP2A6 genetic polymorphism in humans. *Clin Pharmacol Ther* 2001;68:72-8.

Nakamura K, Goto F, Ray WA, McAllister CB, Jacqz E, Wilkinson GR, Branch RA. Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin Pharmacol Ther* 1985;38:402-8.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 1996;6:1-42.

Nelson DR. Cytochrome P450 and the individuality of species. *Arch Biochem Biophys* 1999;369:1-10.

Neuvonen PJ, Kivistö KT. The effects of magnesium hydroxide on the absorption and efficacy of two glibenclamide preparations. *Br J Clin Pharmacol* 1991;32:215-20.

Neuvonen PJ, Varhe A, Olkkola KT. The effect of ingestion time interval on the interaction between itraconazole and triazolam. *Clin Pharmacol Ther* 1996;60:326-31.

Nuttall FQ. Dietary fiber in the management of diabetes. *Diabetes* 1993;42:503-8.

Ohmori S, Ishii I, Kuriya S, Taniguchi T, Rikihisa T, Hirose S, Kanakubo Y, Kitada M. Effects of clarithromycin and its metabolites an the mixed function oxidase system in hepatic microsomes of rats. *Drug Metab Dispos* 1993;21:358-63.

Olesen OV, Linnet K. Fluvoxamine-clozapine drug interaction: inhibition in vitro of five cytochrome P450 isoforms involved in clozapine metabolism. *J Clin Psychopharmacol* 2000;20:35-42.

Olkkola KT, Ahonen J, Neuvonen PJ. The effect of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Anesth Analg* 1996;82:511-6.

Olkkola KT, Backman JT, Neuvonen PJ. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* 1994;55:481-5.

Ong CE, Coulter S, Birkett DJ, Bhasker R, Miners JO. The xenobiotic inhibitor profile of cytochrome P4502C8. *Br J Clin Pharmacol* 2000;50:573-80.

O'Reilly RA, Goulart DA, Kunze KL, Neal J, Gibaldi M, Eddy AC, Trager WF. Mechanisms of the stereoselective interaction between miconazole and racemic warfarin in human. *Clin Pharmacol Ther* 1992;51:656-67.

Oscarson M. Genetic polymorphisms in the cytochrome P450 2A6 (CYP2A6) gene: implications for interindividual differences in nicotine metabolism. *Drug Metab Dispos* 2001;29:91-5.

REFERENCES

Palovaara S, Kivistö KT, Tapanainen P, Manninen P, Neuvonen PJ, Laine K. Effect of an oral contraceptive preparation containing ethinylestradiol and gestodene on CYP3A4 activity as measured by midazolam 1'-hydroxylation. *Br J Clin Pharmacol* 2000;50:333-7.

Pascussi JM, Gerbal-Chaloin S, Fabre JM, Maurel P, Vilarem MJ. Dexamethasone enhances constitutive androstane receptor expression in human hepatocytes: consequences on cytochrome P450 gene regulation. *Mol Pharmacol* 2000a;58:1441-50.

Pascussi JM, Drocourt L, Fabre JM, Maurel P, Vilarem MJ. Dexamethasone induces pregnane X receptor and retinoid X receptor-α expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol Pharmacol* 2000b;58:361-72.

Payen L, Delugin L, Courtois A, Trinquart Y, Guillouzo A, Fardel O. The sulphonylurea glibenclamide inhibits multidrug resistance protein (MRP1) activity in human lung cancer cells. *Br J Pharmacol 2001*;132:778-84.

Pearson JG, Antal EJ, Raehl CL, Gorsch HK, Craig WA, Albert KS, Welling PG. Pharmacokinetic disposition of 14C-glyburide in patients with varying renal function. *Clin Pharmacol Ther* 1986;39:318-24.

Pearson JG. Pharmacokinetics of glyburide. *Am J Med* 1985;79(suppl 3B):67-71.

Peart GF, Boutagy J, Shenfield GM. The metabolism of glyburide in subjects of known debrisoquin phenotype. *Clin Pharmacol Ther* 1989;45:277-84.

Pelkonen O, Mäenpää J, Taavitsainen P, Rautio A, Raunio H. Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica* 1998;28:1203-1253

Pelkonen O, Rautio A, Raunio H, Pasanen M. CYP2A6: a human coumarin 7-hydroxylase. *Toxicology* 2000;144:139-47.

Pentikäinen PJ, Neuvonen PJ, Penttilä A. Pharmacokinetics and pharmacodynamics of glipizide in healthy volunteers. *Int J Clin Pharmacol Ther Toxicol* 1983;21:98-107.

Perucca E, Gatti G, Spina E. Clinical pharmacokinetics of fluvoxamine. *Clin Pharmacokinet* 1994;27:175-90.

Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking. *Nature* 1998;393:750.

Pisanti N, Stanziale P, Imperatore P, D'Alessandro R, De Marino V, Capone D, De Marino V. Lack of effect of gemfibrozil on cyclosporine blood concentrations in kidney-transplanted patients. *Am J Nephrol* 1998;18:199-203.

Piscitelli SC, Burstein AH, Chait D, Alfaro RM, Judith F. Indinavir concentrations and St John's wort. *Lancet* 2000;355:547-8.

Prandin prescribing information. Novo Nordisk Pharmaceuticals, Inc, Princeton, NJ, USA, 1998

Prueksaritanont T, Gorham LM, Ma B, Liu L, Yu X, Zhao JJ, Slaughter DE, Arison BH, Vyas KP. In vitro metabolism of simvastatin in humans – identification of metabolizing enzymes and effect of the drug on hepatic P450s. *Drug Metab Dispos* 1997;25:1191-9.

Qun-Ying Y, Bergquist C, Gerdén B. Safety of St John's wort (Hypericum perforatum). *Lancet* 2000;355:576-7.

Rasmussen BB, Mäenpää J, Pelkonen O, Loft S, Poulsen HE, Lykkesfeldt J, Brøsen K. Selective serotonin reuptake inhibitors and thephylline metabolism in human liver microsomes: potent inhibition by fluvoxamine. *Br J Clin Pharmacol* 1995;39:151-9.

Rasmussen BB, Nielsen TL, Brøsen K. Fluvoxamine inhibits the CYP2C19-catalysed metabolism of proguanil in vitro. *Eur J Clin Pharmacol* 1998;54:735-40.

Relling MV, Aoyama T, Gonzalez FJ, Meyer UA. Tolbutamide and mephenytoin hydroxylation by human cytochrome P450s in the CYP2C subfamily. *J Pharmacol Exp Ther* 1990;252:442-7.

Rettie AE, Koop DR, Haining RL. CYP2C. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 75-86.

Reunanen A, Kangas T, Martikainen J, Klaukka T. Natiowide survey of comorbidity, use, and costs of all medications in Finnish diabetic individuals. *Diabetes Care* 2000;23:1265-71.

Rindone JP, Keng HC. Gemfibrozil-warfarin drug interaction resulting in profound hypoprothrombinemia. *Chest* 1998;114:641-2.

Roberge RJ, Kaplan R, Frank R, Fore C. Glyburide-ciprofloxacin interaction with resistant hypoglycemia. *Ann Emerg Med* 2000;36:160-3.

Roby CA, Anderson GD, Kantor E, Dryer DA, Burstein AH. St John's wort: effect on CYP3A4 activity. *Clin Pharmacol Ther* 2000;67:451-7.

Rodrigues AD, Roberts EM, Mulford DJ, Yao Y, Quellet D. Oxidative metabolism of clarithromycin in the presence of human liver microsomes. *Drug Metab Dispos* 1997;25:623-30.

Rodvold KA. Clinical pharmacokinetics of clarithromycin. *Clin Pharmacokinet* 1999;37:385-98.

Rosenkranz B, Profozic V, Metelko Z, Mrzljak V, Lange C, Malerczyk V. Pharmacokinetics and safety of glimepiride at clinically effective doses in diabetic patients with renal impairment. *Diabetologia* 1996;39:1617-24.

Rydberg T, Jönsson A, Karlsson MO, Melander A. Concentration-effect relations of glibenclamide and its active metabolites in man: modelling of pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol* 1997;43:373-81.

Rydberg T, Jönsson A, Melander A. Comparison of the kinetics of glyburide and its active metabolites in humans. *J Clin Pharm Ther* 1995;20:283-95.

Rydberg T, Jönsson A, Røder M, Melander A. Hypoglycemic activity of glyburide (glibenclamide) metabolites in humans. *Diabetes Care* 1994;17:1026-30.

Sadeque JA, Wandel C, He H, Shah S, Wood JA. Increased drug delivery to the brain by P-glycoprotein inhibition. *Clin Pharmacol Ther* 2000;68:231-7.

Schmider J, Greenblatt DJ, von Moltke LL, Karsov D, Shader RI. Inhibition of CYP2C9 by selective serotonin reuptake inhibitors in vitro: studies of phenytoin p-hydroxylation. *Br J Clin Pharmacol* 1997;44:495-8.

REFERENCES

Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol* 1996;49:311-8.

Schuetz JD, Molowa DT, Guzelian PS. Characterization of cDNA encoding a new member of the glucocorticoid-responsive cytochromes P450 in human liver. *Arch Biochem Biophys* 1989;274:355-65.

Schwinghammer TL, Antal EJ, Kubacka RT, Hackimer ME, Johnston JM. Pharmacokinetics and pharmacodynamics of glyburide in young and elderly nondiabetic adults. *Clin Pharm* 1991;10:532-8.

Scott LJ, Spencer CM. Miglitol. A review of its therapeutic potential in type 2 diabetes mellitus. *Drugs* 2000;59:521-49.

Self TH, Tsiu SJ, Bowld WF, Fowler JW. Interaction of rifampin with glyburide. *Chest* 1989;96:1443-4.

Sellers EM, Kaplan HL, Tyndale RF. Inhibition of cytochrome P450 2A6 increases nicotine's oral bioavailability and decreases smoking. *Clin Pharmacol Ther* 2000;68:35-43.

Seltzer HS. Drug-induced hypoglycemia. A review based on 473 cases. *Diabetes* 1972;21:955-66.

Semple CG, Omile C, Buchanan KD, Beastall GH, Paterson KR. Effect of oral verapamil on glibenclamide stimulated insulin secretion. *Br J Clin Pharmacol* 1986;22:187-90.

Shad MU, Preskorn SH. Antidepressants. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000a. p. 563-577.

Sharom FJ. The P-glycoprotein efflux pump: how does it transport drugs? *J Membr Biol* 1997;160:161-75.

Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994;270:414-23.

Shon JH, Yoon YR, Kim MJ, Cha IJ, Shin JG. Involvement of CYP2C9 on chlorpropamide 2-hydroxylation in human: in vitro and in vivo evidence. *Clin Pharmacol Ther* 2001;69:41.

Shou M, Mei Q, Ettore MW, Dai R, Baillie TA, Rushmore TH. Sigmoidal kinetic model for two co-operative substrate-binding sites in a cytochrome P450 3A4 active site: an example of the metabolism of diazepam and its derivatives. *Biochem J* 1999;340:845-53.

Silverman JA. P-glycoprotein. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 135-44.

Sjöberg S, Wiholm BE, Gunnarsson R, Emilsson H, Thunberg E, Christenson I, Ostman J. Lack of pharmacokinetic interaction between glibenclamide and trimethoprim-sulphamethoxazole. *Diabet Med* 1987;4:245-7.

Song DK, Ashcroft FM. Glimepiride block of cloned ss-cell, cardiac and smooth muscle K-ATP channels. *Br J Pharmacol* 2001;133:193-9.

Sotaniemi EA, Rautio A, Bäckström M, Arvela P, Pelkonen O. CYP3A4 and CYP2A6 activities marked by the metabolism of lignocaine and coumarin in patients with liver and kidney diseases and epileptic patients. *Br J Clin Pharmacol* 1995;39:71-6.

Spicer ST, Liddle C, Chapman JR, Barclay P, Nankivell BJ, Thomas P, O'Connell PJ. The mechanism of cyclosporine toxicity induced by clarithromycin. *Br J Clin Pharmacol* 1997;43:194-6.

Stevens JC, Wrighton SA. Interaction of the enantiomers of fluoxetine and norfluoxetine with human liver cytochromes P450. *J Pharmacol Exp Ther* 1993;266:964-71.

Stockley IH. *Drug interactions*. 5th ed. London: Pharmaceutical Press; 1999a. p. 519-20.

Stockley IH. *Drug interactions*. 5th ed. London: Pharmaceutical Press; 1999b. p. 528.

Sturgess NC, Ashford MLJ, Cook DL, Hales CH. The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* 1985;2:474-5.

Sumida A, Fukuen S, Yamamoto I, Matsuda H, Naohara M, Azuma J. Quantitative analysis of constitutive and inducible CYPs mRNA expression in the HepG2 cell line using reverse transcription-competitive PCR. *Biochem Biophys Res Commun* 2000;267:756-60.

Surekha V, Peter JV, Jeyaseelan L, Cherian AM. Drug interaction: rifampicin and glibenclamide. *Nat Med J India* 1997;10:11-2.

Syvälahti E, Pihlajamäki K, Iisalo E. Effect of tuberculostatic agents on the response of serum growth hormone and immunoreactive insulin to intravenous tolbutamide, and on the half-life of tolbutamide. *Int J Clin Pharmacol Biopharm* 1976;13:83-9.

Takahashi H, Kashima T, Nomizo Y, Muramoto N, Shimizu T, Nasu K, Kubota T, Kimura S, Echizen H. Metabolism of warfarin enantiomers in japanese patients with heart disease having different CYP2C9 and CYP2C19 genotypes. *Clin Pharmacol Ther* 1998;63:519-28.

Tang C, Shou M, Rushmore TH, Mei Q, Sandhu P, Woolf EJ, Rose MJ, Gelmann A, Greenberg HE, de Lepeleire I, van Hecken A, de Schepper PJ, Ebel DL, Schwartz JI, Rodrigues AD. In-vitro metabolism of celecoxib, a cyclooxygenase-2 inhibitor, by allelic variant forms of human liver microsomal cytochrome P450 2C9: correlation with CYP2C9 genotype and in-vivo pharmacokinetics. *Pharmacogenetics* 2001;11:223-35.

Tantcheva-Poór I, Zaigler M, Rietbrock S, Fuhr U. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy caucasians using saliva-based caffeine test. *Pharmacogenetics* 1999;9:131-44.

Todd PA, Ward A. Gemfibrozil. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in dyslipidemia. *Drugs* 1988;36:314-39.

Tokola O, Mustajoki P, Himberg JJ. Haem arginate improves hepatic oxidative metabolism in variegate porphyria. *Br J Clin Pharmacol* 1988;26:753-7.

Tran JQ, Kovacs SJ, McIntosh TS, Davis HM, Martin DE. Morning spot and 24-hour urinary 6β-hydroxycortisol to cortisol ratios: intraindividual variability and correlation under basal conditions and conditions of CYP 3A4 induction. *J Clin Pharmacol* 1999;39:487-94.

Turnidge J, Grayson ML. Optimum treatment of staphylococcal infections. *Drugs* 1993;45:353-66.

REFERENCES

Ueng YF, Kuwabara T, Chun YJ, Guengerich FP. Cooperativity in oxidations catalyzed by cytochrome P450 3A4. *Biochemistry* 1997;36:370-81.

Ueno N, Inui A, Asakawa A, Takao F, Tani S, Komatsu Y, Itoh Z, Kasuga M. Erythromycin improves glycaemic control in patients with type II diabetes mellitus. *Diabetologia* 2000;43:411-5.

UK Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;352:837-53.

Varhe A, Olkkola KT, Neuvonen PJ. Fluconazole, but not terbinafine, enhances the effects of triazolam by inhibiting its metabolism. *Br J Clin Pharmacol* 1996;41:319-23

Venkatesan K. Pharmacokinetic drug interactions with rifampicin. *Clin Pharmacokinet* 1992;22:47-65.

Villikka K, Kivistö KT, Backman JT, Olkkola KT, Neuvonen PJ. Triazolam is ineffective in patients taking rifampin. *Clin Pharmacol Ther* 1997;1:8-14.

Wakasugi H, Yano I, Ito T, Hashida T, Futami T, Nohara R, Sasayama S, Inui K. Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. *Clin Pharmacol Ther* 1998;64:123-8.

Wallace RJ Jr., Brown BA, Griffith DE, Girard W, Tanaka K. Reduced serum levels of clarithromycin in patients treated with multidrug regimens including rifampin or rifambutin for Mycobacterium avium-M. intracellulare infection. *J Infect Dis* 1995;171:747-50.

Wang JS, Wen X, Backman JT, Kivistö KT, Neuvonen PJ. Evaluation of gemfibrozil as an inhibitor of cytochrome P450 (CYP) isoforms: inhibition of CYP2C9 in the presence and absence of human serum albumin (HSA). *Pharmacol Toxicol* 2001a;89(Suppl 1):80-81.

Wang RW, Kari PH, Lu AYH, Thomas PE, Guengerich FP, Vyas KP. Biotransformation of lovastatin. IV. Identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. *Arch Biochem Biophys* 1991;290:355-61.

Wang RW, Newton DJ, Liu N, Atkins WM, Lu AY. Human cytochrome P-450 3A4: in vitro drug-drug interaction patterns are substrate-dependent. *Drug Metab Dispos* 2000;28:360-6.

Wang Z, Gorski JC, Honig P, Collins J, Lesko L, Huang SM, Hall SE. St John's wort (SJW) alters CYP3A but not CYP1A2 or CYP2C9 activity in vivo. *Clin Pharmacol Ther* 2001b;69:P71.

Waxman DJ. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. *Arch Biochem Biophys* 1999;369:11-23.

Wedlund PH, Aslanian WS, McAllister CB, Wilkinson GR, Branch RA. Mephenytoin hydroxylation deficiency in Caucasians: frequency of a new oxidative drug metabolism polymorphism. *Clin Pharmacol Ther* 1984;36:773-80.

Wehrli W. Rifampin: mechanisms of action and resistance. *Reviews of Infectious Diseases* 1983;5(Suppl 3):S407-11.

van der Weide J, Steijins LS, van Weelden MJ, de Haan K. The effect of genetic polymorphism of cytochrome P450 CYP2C9 on phenytoin dose requirement. *Pharmacogenetics* 2001;11:287-91.

Wen X, Wang JS, Backman JT, Kivistö KT, Neuvonen PJ. Gemfibrozil is a potent inhibitor of human cytochrome P450 2C9. *Drug Metab Dispos* 2001;29:1359-61.

Wensing G. Glipizide: an oral hypoglycemic drug. *Am J Med Sci* 1989;298:69-71.

Westphal JF. Macrolide-induced clinically relevant drug interactions with cytochrome P-450A (CYP) 3A4: an update focused on clarithromycin, azithromycin and dirithromycin. *Br J Clin Pharmacol* 2000;50:285-295.

Westphal K, Weinbrenner A, Zschiesche M, Franke G, Knoke M, Oertel R, Fritz P, von Richter O, Warzok R, Hachenberg T, Kauffmann HM, Schrenk D, Terhaag B, Kroemer HK, Siegmund W. Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: A new type of drug/drug interaction. *Clin Pharmacol Ther* 2000;68:345-55.

Woodland C, Ito S, Koren G. A model for the prediction of digoxin-drug interactions at the renal tubular cell level. *Ther Drug Monit* 1998;20:134-8.

Wrighton SA, Stevens JC. The human hepatic cytochromes P450 involved in drug metabolism. *Crit Rev Toxicol* 1992;22:1-21.

Wrighton SA, Thummel KE. CYP3A. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 115-33.

Wåhlin-Boll E, Almér LO, Melander A. Bioavailability, pharmacokinetics and effects of glipizide in type 2 diabetics. *Clin Pharmacokinet* 1982;7:363-72.

Yamada J, Sugimoto Y, Inoue K. Selective serotonin reuptake inhibitors fluoxetine and fluvoxamine induce hyperglycemia by different mechanisms. *Eur J Pharmacol* 1999;382:211-5.

Yasar Ü, Eliasson E, Dahl ML, Johansson I, Ingelman-Sunberg M, Sjöqvist F. Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a swedish population. *Biochem Biophys Res Commun* 1999;254:628-31.

Yeates RA, Laufen H, Zimmermann T. Interaction between midazolam and clarithromycin: comparison with azithromycin. *Int J Clin Pharmacol Ther* 1996;34:400-5.

Yki-Järvinen H. Management of type 2 diabetes mellitus and cardiovascular risk. Lessons from intervention trials. *Drugs* 2000;60:975-83.

Yun CH, Shimada T, Guengerich FP. Purification and characterization of human liver microsomal cytochrome P-450 2A6. *Mol Pharmacol* 1991;40:679-85.

Zanger UM, Eichelbaum M. CYP2D6. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 87-94.

Zhanel GG, Dueck M, Hoban DJ, Vercaigne LM, Embil JM, Gin AS, Karlowsky JA. Review of macrolides and ketolides. Focus on respiratory tract infections. *Drugs* 2001;61:443-498.

Zhang QY, Dunbar D, Ostrowska A, Zeisloft S, Yang J, Kaminsky LS. Characterization of human small intestinal cytochromes P-450. *Drug Metab Dispos* 1999;27:804-9.