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A Novel Method for the Determination of Biliary Clearance in Humans

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ABSTRACT

Biliary excretion is an important route of elimination and the biliary tract is a potential site of toxicity for many drugs and xenobiotics. Quantification of biliary excretion in healthy human volunteers is logistically challenging and is rarely defined during drug development. The current study uses a novel oroenteric tube coupled with a specialized clinical protocol to examine the pharmacokinetics of ^{99m}Technetium (Tc-99m) mebrofenin, a compound that undergoes rapid hepatic uptake and extensive biliary excretion. A custom-made multilumen oroenteric tube was positioned in the duodenum of healthy human volunteers. Subjects were positioned under a gamma camera and 2.5 mCi of Tc-99m mebrofenin was administered intravenously. Duodenal aspirates, blood samples, and urine were collected periodically for 3 hours. Two hours after Tc-99m mebrofenin administration, the gallbladder was contracted with an intravenous infusion of cholecystokinin-8. Gamma scintigraphy was used to determine the gallbladder ejection fraction in each subject. Total systemic clearance of Tc-99m mebrofenin approximated liver blood flow (Cl_{total} 17.3 ± 1.7 mL/min/kg), and 35% to 84% of the Tc-99m mebrofenin dose was recovered in bile. However, when the data were corrected for the gallbladder ejection fraction, 71% to 92% of the *excreted* Tc-99m mebrofenin dose was recovered. This novel oroenteric tube and clinical protocol provide a useful method to quantify biliary excretion of xenobiotics in healthy human volunteers.

KEYWORDS: oroenteric tube, gallbladder, Tc-99m mebrofenin, biliary excretion, biliary clearance.

INTRODUCTION

With the advent of combinatorial chemistry, an increasing number of new chemical entities characterized by high molecular weight and lipophilicity are being synthesized and screened during the drug discovery process.[1] Drugs with these physicochemical characteristics often are associated with significant hepatic metabolism and excretion via the biliary route.[2] Therefore, new tools and techniques are needed to investigate and predict biliary excretion of investigational drugs in healthy human volunteers. Currently, limited information is available regarding the extent of biliary elimination of drugs and metabolites, primarily because of the difficulty in obtaining bile samples from healthy human subjects. Biliary excretion data would be extremely valuable in evaluating the contribution of biliary clearance to total systemic clearance, elucidating potential mechanisms of hepatobiliary toxicity, and examining enterohepatic recirculation. In addition, the Food and Drug Administration (FDA) recently published guidelines detailing the requirements for drug-drug interaction studies necessary in New Drug Application submissions. These guidelines suggest that mechanisms of interactions should be explored, rather than simply performing observational studies.[3] Therefore, a method to investigate potential drug interactions that occur during biliary elimination may increase the quality of information provided to registration agencies and help avoid the withdrawal of drugs from the market as a result of a serious, but foreseeable, drug interaction.

One valuable preclinical tool to investigate biliary clearance of drugs is the sandwich-cultured hepatocyte model. This model has been developed using rat and human hepatocytes to examine biliary excretion in vitro, and has been validated with in vivo data collected from rats.[4] However, the in vivo data required to develop an in vitro–in vivo correlation of biliary clearance for humans is lacking. This shortcoming is primarily a result of the difficulty in obtaining biliary excretion data in humans, particularly in healthy volunteers.

Historically, there has been little success in the development of valid and reliable techniques to quantify biliary excretion of drugs or endogenous compounds in humans. Such data may be obtained from patients suffering from gallbladder disease requiring removal of the gallbladder. Typically, patients are administered the drug of interest prior to surgery, and the amount of drug found in the gallbladder after removal is quantified.[5,6] This approach only allows a single time point determination of drug content in bile. More commonly, studies employ patients that require a temporary bile shunt (T-tube) that diverts bile from the liver to a transcutaneous port for external collection.[7,8] With this method, biliary excretion may be determined over time while the shunt is in place.

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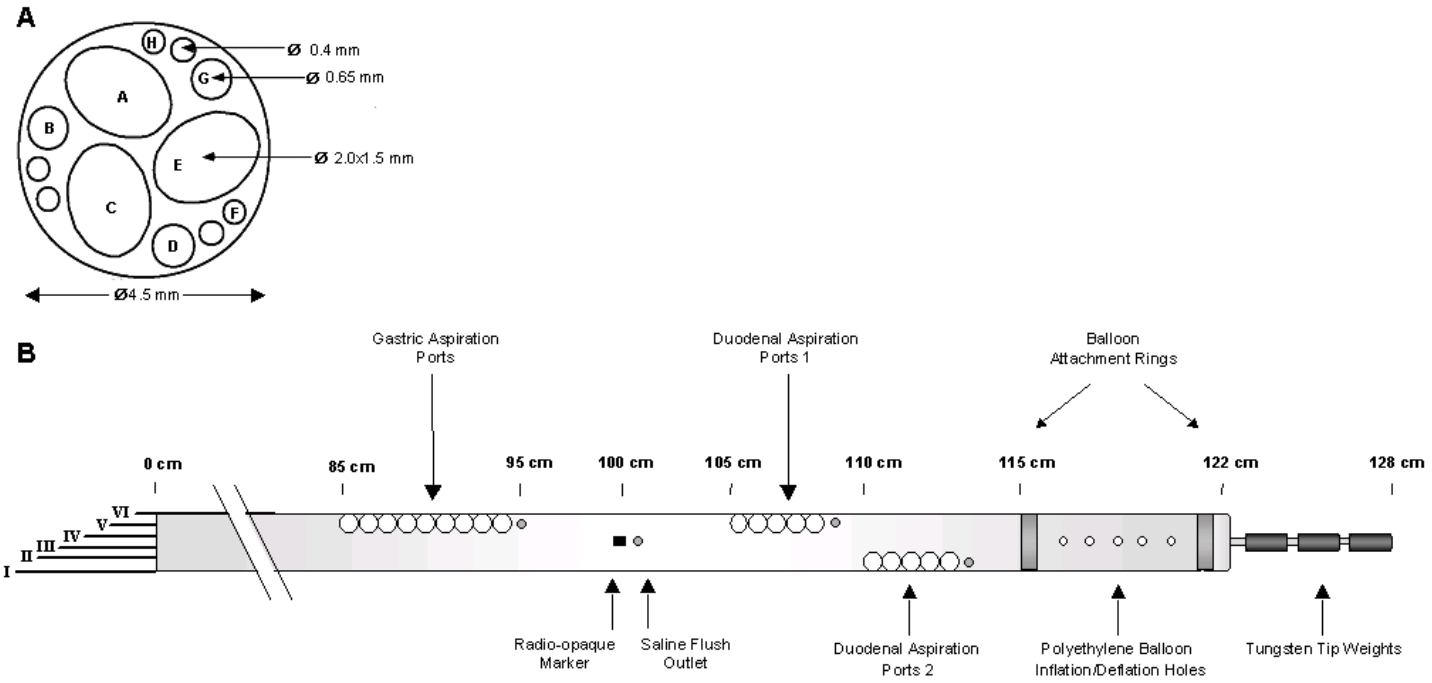

Figure 1. (A) Cross-sectional diagram of the oroenteric tube. Lumen labels correspond to the following functions: A, duodenal aspiration 1; B, duodenal aspiration 1 vacuum relief; C, duodenal aspiration 2; D, duodenal aspiration 2 vacuum relief; E, gastric aspiration; F, gastric aspiration vacuum relief; G, balloon inflation; H, saline flush. (B) Schematic of oroenteric tube. Syringe connector labels correspond to the following channels: I, duodenal aspiration 1 and 2; II, gastric aspiration; III, duodenal aspiration 1 and 2 vacuum relief; IV, gastric aspiration vacuum relief; V, balloon inflation/deflation; VI, saline flush.

The major limitation of both of the above techniques is the use of patients with significant hepatobiliary disease as study subjects. Several studies have employed healthy human volunteers and oroenteric tubes to withdraw pancreatic-biliary secretions from the duodenum.[9] These studies have used occlusive balloons to facilitate more complete bile collection,[10] or have perfused nonabsorbable markers into the duodenum to evaluate recovery.[11,12] A significant advantage of these techniques is the ability to conduct studies in healthy human volunteers, particularly in light of the role of hepatobiliary transport proteins in hepatic drug disposition. Certain disease states may significantly influence transport protein expression, function, localization, and/or bile flow along the bile canaliculi, thereby altering drug secretion and excretion patterns.[13-15]

Previous studies conducted in healthy human volunteers have resulted in incomplete and highly variable recovery of compounds excreted in bile.[10,16] This is primarily a result of difficulties in obtaining and assessing the completeness of bile collection, and a lack of control over gallbladder contraction. Described here is a method using a novel oroenteric tube and specialized clinical protocol that facilitates efficient recovery of biliary secretions and optimal gallbladder refilling status and contractibility. The use of the gamma emitter ^{99m}Technetium (Tc-99m) mebrofenin as a probe compound in this study allowed real-time assessment of biliary excretion, gallbladder ejection fraction, and the efficiency of bile collection. This compound is available for intravenous administration, is not significantly metabolized, and is principally excreted unchanged in bile.

Materials and Methods

Catheter Design

A commercially available oroenteric catheter extrusion was modified according to the design in Figure 1 to aspirate secretions from the gastric and duodenal regions of the human gastrointestinal tract (Dentsleeve, Wayville, Australia). Briefly, the tube is a silicone multilumen extrusion 4.5 mm in diameter, 128 cm long, with 3 tungsten tip weights to facilitate passage of the tube through the pyloric sphincter. The distal/terminal end of the tube was fitted with a welded polyethylene balloon to facilitate migration of the tube during placement, and to ensure occlusion of the intestine during the bile collection period. The balloon (9 cm long × 4.7 cm wide, uninflated) was single use and fitted to metal balloon attachment rings using 1.0 silk suture. Two of the large lumens of the extrusion (Figure 1, Panel A, lumens A and C) were dedicated to aspiration of secretions from the duodenal region, while lumen E was used for aspiration from the gastric region. Aspiration vacuum relief was provided by lumens B, D, and F. Lumen G was used to inflate/deflate the polyethylene balloon and lumen H provided a saline flush to

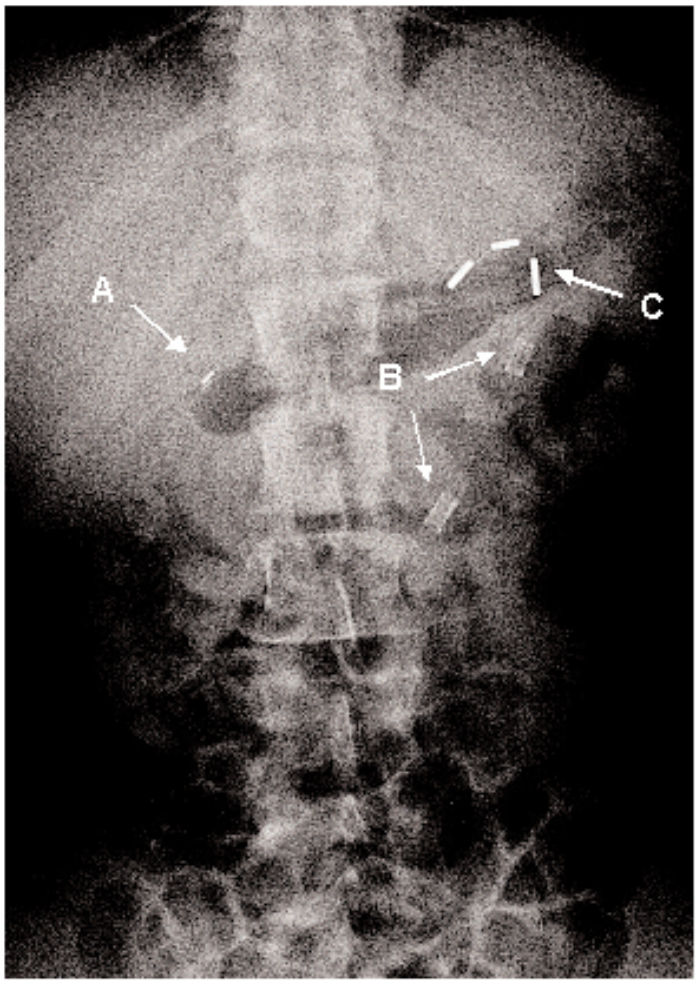

Figure 2. A representative fluoroscopic image of the oroenteric tube correctly positioned. Arrows indicate the following: A, radio-opaque marker at the pyloric sphincter; B, balloon attachment rings; C, tungsten tip weights positioned at the Ligament of Treitz.

reduce viscosity of the duodenal secretions. A radio-opaque pyloric marker was positioned 100 cm from the syringe connectors to facilitate correct placement of the tube under fluoroscopy.

Clinical Protocol

Four nonsmoking volunteers (20-24 years of age; 3 male, 1 female) within 20% of ideal body weight (64.5-84.4 kg) completed the study. All procedures were approved by the Clinical Research Advisory Committee and the Committee on the Protection of the Rights of Human Subjects of the University of North Carolina (UNC) at Chapel Hill School of Medicine. Subjects provided written informed consent prior to participation in the study and were healthy as indicated by their medical history, physical examination, routine laboratory tests, and electrocardiogram. Subjects were asked to abstain from any medication (with the exception of oral contraceptives) for 2 weeks prior to the study, and to abstain from caffeine and alcohol during the study. Subjects were admitted to the General Clinical Research Center of UNC Hospitals the evening before the study and given a high-fat meal (~55 g of fat) at 18:00 hours and a high-fat snack (~45 g of fat) at 23:00 hours. Subjects then remained fasted until the end of the study. At 06:00 the following morning, the oroenteric tube was passed through the mouth and positioned with the aid of dynamic fluoroscopic radiography (ICONOS R200, Seimens, Hoffman Estates, IL). Correct placement of the tube was judged by the position of the pyloric marker (right-hand margin of spine) and the tip weights (immediately distal to the Ligament of Treitz) (Figure 2). Subjects were then positioned supine under a gamma camera (E-CAM Dual Head Gamma Camera, Siemens); baseline blood, urine, and bile samples were collected; and the occlusive polyethylene balloon was inflated with ~20 mL of air until the subjects experienced mild discomfort. Subjects were then administered a 2.5 mCi IV bolus dose of Tc-99m mebrofenin (Squibb Diagnostics, Princeton, NJ) via a forearm vein indwelling catheter (specific activity 0.8-1.1 mCi/mg at time of preparation). Blood samples (3 mL) were collected at 1, 2.5, 5, 7.5, 10, 20, 40, 60, 80, 100, 120, 140, 165, and 180 minutes postdose from the contralateral arm. Biliary secretions collected from the occluded duodenal region were continuously aspirated and pooled over the following intervals: 0-5, 5-10, 10-30, 30-50, 50-70, 70-90, 90-110, 110-120, 120-130, 130-150, and 150-180 minutes postdose. Anterior gamma scintigraphic images of the abdomen were acquired under dynamic mode at 1-minute intervals and analyzed using ESOFT 2.5 software (Siemens). Two hours post-Tc-99m mebrofenin administration, cholecystokinin-8 (sincalide, 0.02 µg/kg, Bracco Diagnostics, Princeton, NJ) was administered as an IV infusion over 30 minutes to facilitate contraction of the gallbladder. Cholecystokinin-8 is an octa-peptide derived from cholecystokinin and has a very short duration of action (half-life in blood of 2.5 minutes).[17,18] Urine was collected at the conclusion of the sampling period (180 minutes).

Whole blood radioactivity was determined by gamma scintillation counting (Quantum-8 Multichannel Analyzer, Oak Ridge, TN). Bile collected over each interval and an aliquot of urine were analyzed by a CRC-15R dose calibrator (Capintec, Ramsay, NJ). All samples were corrected for decay based on the Tc-99m half-life of 6.01 hours. Linearity of the gamma camera response was assessed using 250-mL saline bags injected with predetermined amounts of radioactivity (0.5-3 mCi) measured with a CRC-15R dose calibrator.

Data Analysis

A duplicate dose of Tc-99m mebrofenin was prepared at the same time as the dose administered to the subject and used for calculation of the "theoretical dose." This reference standard also was used to convert the units of the whole blood radioactivity from counts per minute (cpm) to nCi, in order to obtain a more quantitative measurement of the amount of

radioactivity present in blood. After assessing the radioactivity remaining in the syringe, IV tubing, and catheter, the "administered dose" was then calculated by difference. Biliary recovery and urinary recovery were calculated as the percentage of the administered dose that accumulated in bile and urine, respectively, 180 minutes after dosing. A gallbladder ejection fraction (EF) was calculated from planar scintigraphic images using the following equation:

$$Ejection\ Fraction = \frac{\left(GB^{120\,\mathrm{min}}\right) - \left(GB^{150\,\mathrm{min}}\right)}{\left(GB^{120\,\mathrm{min}}\right)} \quad (1)$$

where *GB* represents the counts per minute in the gallbladder region of the image pre- (time = 120 minutes) and post- (time = 150 minutes) infusion of cholecystokinin-8.

In order to account for the variable contractile response of the gallbladder, the total amount of radioactivity collected from the duodenal region during the 30-minute infusion of cholecystokinin-8 was divided by the ejection fraction. This correction accounted for the Tc-99m mebrofenin remaining in the gallbladder after contraction. Addition of this value to the cumulative amount of Tc-99m mebrofenin collected in the first 120 minutes of the study represented the total amount of Tc-99m mebrofenin transported from the liver into bile, and the amount that would have been collected if the gallbladder were to contract completely.

$$\%\ Recovery\ of\ Excreted\ Dose = \frac{100\%}{X^0} \times \left(X_{Bile}^{0-120\,\mathrm{min}} + \frac{X_{Bile}^{120-150\,\mathrm{min}}}{EF} \right) \quad (2)$$

where X^0 represents the administered dose; X_{Bile}, the amount of Tc-99m mebrofenin recovered in biliary secretions collected over the time interval specified; and *EF*, the ejection fraction. Therefore, $\frac{X_{Bile}^{120\text{-}150\,\mathrm{min}}}{EF}$ estimates the total amount of Tc-99m mebrofenin that would have been collected from the duodenal region if the *EF* = 1. This corrected amount, expressed as a percentage of the administered dose, represents the recovery of Tc-99m mebrofenin *excreted* from the gallbladder during the study, and assumes that no additional Tc-99m mebrofenin remains to be excreted into the duodenum from 150 to 180 minutes. Therefore this value can therefore provide an estimate of recovery efficiency and performance of the oroenteric tube.

Pharmacokinetic Data Analysis

Blood activity-time profiles were analyzed by noncompartmental analysis using WinNonlin (Version 4.1, Pharsight Corp, Mountain View, CA). The frequency of the blood sampling at the beginning of the experiment and the slow clearance of radioactivity from the injection site allowed the characterization of Tc-99m mebrofenin distribution into the systemic circulation. Therefore, the kinetics of administration were modeled as an IV infusion of duration T_{max} to account for this observation. The area under the blood concentration-time curve (AUC) was calculated using the linear trapezoidal rule, and where appropriate, extrapolated to infinity using the slope obtained from linear regression of the last 5 to 6 time points. Total blood clearance of Tc-99m mebrofenin was determined as the ratio of the administered dose and the AUC extrapolated through infinite time. Biliary clearance was determined as the ratio of the cumulative amount of Tc-99m mebrofenin recovered in bile from 0 to 180 minutes (not corrected for EF) and the AUC from 0 to 180 minutes.

RESULTS

Proper positioning of the oroenteric tube required between 0.5 and 2 hours. However, 1 subject failed to pass the tube through the pylorus and into the duodenum in the allocated time and, therefore, did not complete the study. Correct positioning of the oroenteric tube was confirmed by fluoroscopic radiography. A representative image of the tube located in the correct anatomical position is shown in Figure 2. The marker at the pyloric sphincter and the tungsten tip weights just beyond the Ligament of Treitz (immediately preceded by the 2 balloon attachment rings) are clearly visible.

As expected, rapid and extensive biliary excretion of Tc-99m mebrofenin was observed. As evident in animated Figure 3 (Panels A, B, C, and D), after IV administration Tc-99m mebrofenin was rapidly taken up into the liver, secreted into the canalicular space, and collected in the common bile duct and gallbladder. Despite the consistency in the rate and extent of Tc-99m mebrofenin accumulation in the gallbladder, each subject exhibited a different pattern of bile secretion into the intestine, presumably due to differing spontaneous contraction patterns of the gallbladder.[19] These differing release patterns of Tc-99m mebrofenin in bile are evident in animated Figure 3 and in the profiles shown in Figure 4. Subject 1 did not expel Tc-99m mebrofenin from the gallbladder until administration of cholecystokinin-8 at 120 minutes, whereas subjects 2 and 4 exhibited partial spontaneous contraction of the gallbladder prior to cholecystokinin-8. Of interest, subject 3 immediately ejected bile and Tc-99m mebrofenin into the intestine before accumulation in the gallbladder and did not respond to cholecystokinin-8 as quickly or to the same extent as the other subjects.

The gamma camera images obtained during the study allow a visual, subjective assessment of tube performance and indicate that the biliary and duodenal secretions were efficiently and entirely removed via the duodenal aspiration ports. Specifically, Tc-99m mebrofenin and bile accumulated in a small segment of the duodenum and were not visible in more distal regions of the intestinal tract (Figure 3, Panels B, C, and D). At times, Tc-99m mebrofenin was recovered as quickly as it was expelled from the gallbladder, resulting in little accu-

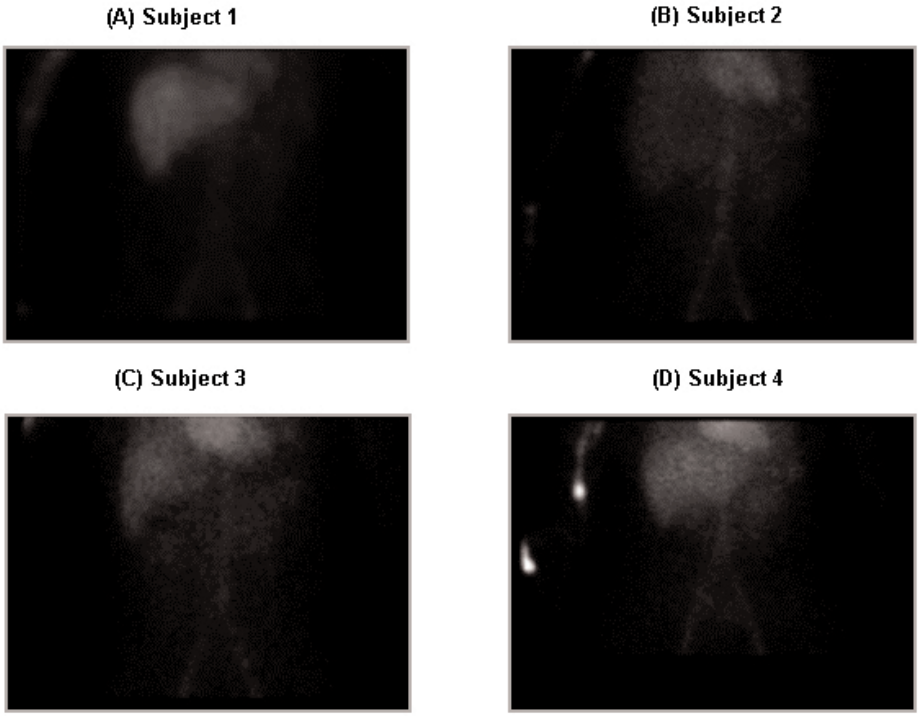

Figure 3. Animated gamma scintigraphic images of Tc-99m mebrofenin disposition (from 0 to 180 minutes). Clicking on each panel will initiate animation: A, subject 1; B, subject 2; C, subject 3; D, subject 4. Hepatic uptake of Tc-99m mebrofenin was rapid, followed by excretion into the canalicular space and accumulation in the gallbladder. Gallbladder contraction, facilitated by an IV infusion of cholecystokinin-8 at 120 minutes, resulted in expulsion of bile containing Tc-99m mebrofenin from the gallbladder into the duodenum and subsequent aspiration via the oroenteric tube. (Animation available online only.)

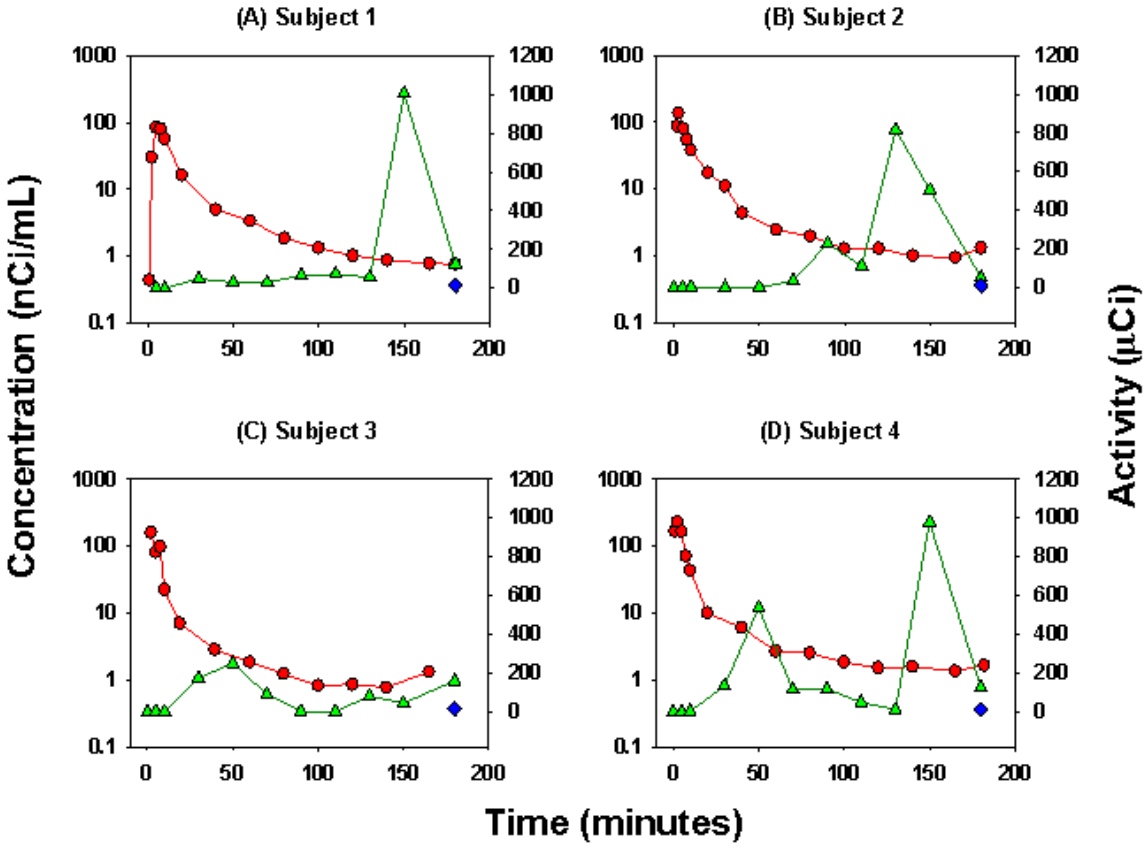

Figure 4. Tc-99m mebrofenin blood concentration (red circles) and amount in bile (green triangles) versus time profiles, and the amount in urine at 180 minutes (blue diamonds): A, subject 1; B, subject 2; C, subject 3; D, subject 4. Cholecystokinin-8 was administered at 120 minutes as a 30 minute IV infusion to facilitate contraction of the gallbladder.

Table 1. Summary of Tc-99m Mebrofenin Dose, Recovery, Gallbladder Ejection Fraction, and Pharmacokinetic Parameters for Subjects 1, 2, 3, and 4*

	Subject 1	Subject 2	Subject 3	Subject 4	Mean	SD
Theoretical dose (μCi)	2188	2498	2500	2497	2421	155
Administered dose (μCi)	†	2072	2282	2476	2277	202
Biliary recovery (% of dose‡)	65.5	84.3	35.2	83.5	67.1	23.0
Urinary recovery (% of dose‡)	0.7	0.5	0.8	0.5	0.6	0.2
Total recovery (% of dose‡)	66.2	84.8	36.0	84.0	67.8	22.8
Ejection fraction	0.82	0.86	0.03	0.86	0.64	0.4
Recovery of *excreted* dose (% of dose‡)	70.9	89.1	91.9	84.9	84.2	9.3
$AUC_{0-\infty}$ (nCi.min/mL)	1466	1613	1422	2305	1701	410
Cl_{total} (mL/min/kg)	16.4	16.2	19.9	16.7	17.3	1.7
AUC_{0-180} (nCi.min/mL)	1371	1462	1279	2054	1542	350
$Cl_{biliary}$ (mL/min/kg)	11.5	15.0	7.8	15.6	12.5	3.6

*AUC indicates area under the blood concentration-time curve, $Cl_{total} = Dose/AUC_{0-\infty}$, $Cl_{biliary} = (Mass\ in\ bile)_{0-180}/AUC_{0-180}$

†Not determined, all calculations based on theoretical dose.

‡Administered dose, where available.

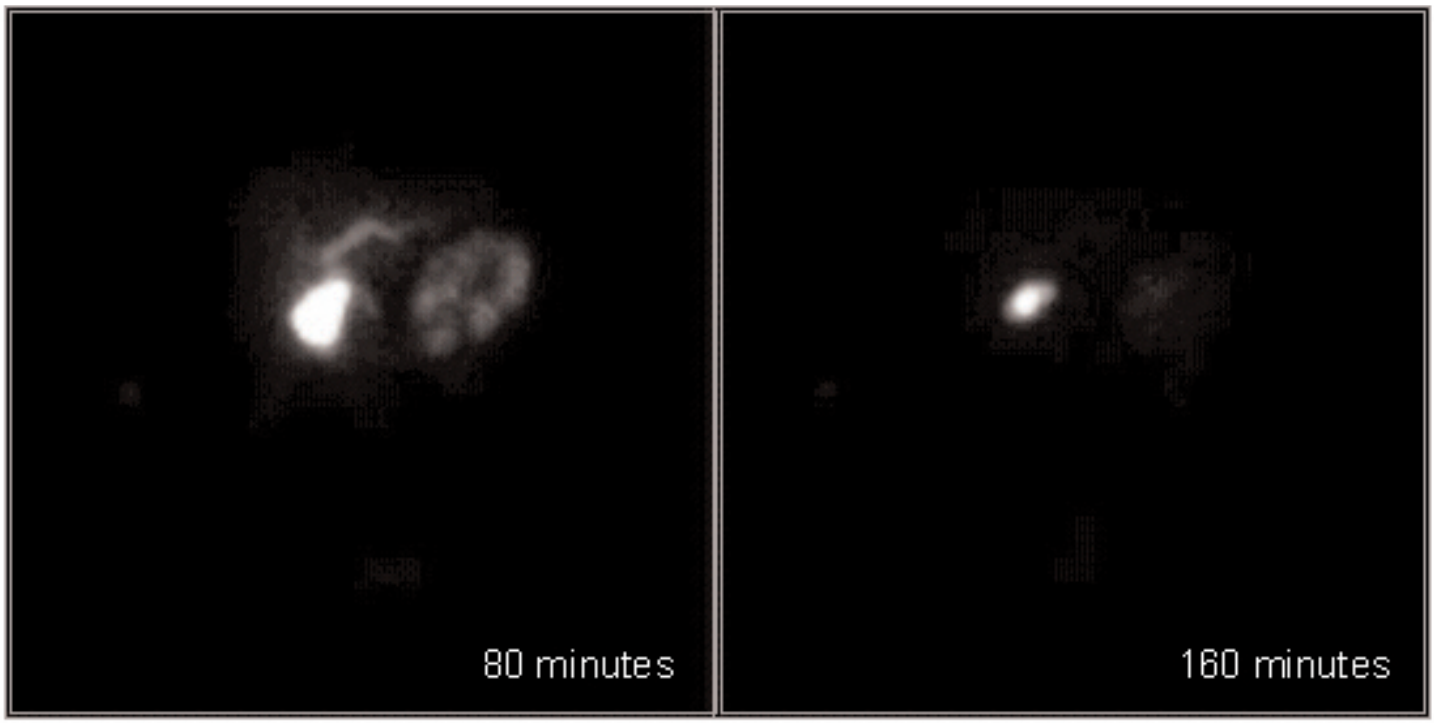

Figure 5. Static gamma camera images of subject 2 at 80 and 160 minutes after administration of Tc-99m mebrofenin. Tc-99 mebrofenin accumulation in the intestine after 80 minutes was aspirated efficiently via the oroenteric tube as limited radioactivity was present in the intestine by 160 minutes.

mulation of radioactivity in the duodenal region (Figure 3, Panel D). In subject 1, the scintigraphic images also allowed visualization of a small amount of Tc-99m mebrofenin that leaked beyond the occlusive balloon after cholecystokinin-8 administration (Figure 3, Panel A). This observation corresponded with a lower recovery of Tc-99m mebrofenin in subject 1 (Table 1). Static gamma camera images of subject 2 pre-cholecystokinin-8 and post-cholecystokinin-8 (Figure 5) clearly illustrate the efficiency of the oroenteric tube in removing Tc-99m mebrofenin and bile from the duodenum.

At the conclusion of the sampling period (180 minutes post-dose), residual radioactivity was evident in the gallbladder, consistent with incomplete emptying of the gallbladder after contraction.[18,20] Using planar images obtained before and after cholecystokinin-8 administration, the gallbladder ejection fraction was determined (Table 1). With the exception of subject 3, remarkably large and consistent ejection fractions were obtained. The extent of Tc-99m mebrofenin recovered in aspirated bile exhibited high intersubject variability (35%-84% of the administered dose); however, once corrected for the gall-

bladder ejection fraction, the recovery of *excreted* Tc-99m mebrofenin was consistently high (84.2% ± 9.3%, Table 1).

The blood concentration-time profiles, bile amount-time profiles, and the amount in urine at 180 minutes for subjects 1, 2, 3, and 4 are shown in Figure 4, Panels A, B, C, and D, respectively. The blood profiles are similar in all subjects; C_{max} was followed by a rapid distributional phase and polyexponential decay. A terminal monoexponential decay phase was not clearly evident after 3 hours of sampling. Table 1 summarizes the pharmacokinetic parameters obtained. Systemic exposure and total body clearance were comparable in all subjects, and biliary clearance ranged from 7.8 to 15.6 mL/min/kg (Table 1). The fraction of total clearance that can be attributed to biliary excretion is numerically equivalent to the biliary recovery (expressed as percentage of dose) of Tc-99m mebrofenin reported in Table 1, averaging 78% in subjects that responded to cholecystokinin-8 (subjects 1, 2, and 4). The variability in biliary clearance was due primarily to incomplete excretion of Tc-99m mebrofenin from the gallbladder during the collection period. The total amount of Tc-99m mebrofenin recovered in urine 3 hours postdose was very low (0.6% ± 0.2%).

DISCUSSION

This study used a customized oroenteric tube and tailored clinical protocol to validate successfully an experimental procedure that allowed efficient collection of biliary secretions from healthy human volunteers. In a previous pilot study, only modest outcomes were obtained: gallbladder ejection fractions after cholecystokinin-8 administration were abnormally low, and the oroenteric tube used failed to completely collect biliary secretions. It was hypothesized that the lack of response to cholecystokinin-8 in the pilot study was a result of the prolonged fasting period required by the protocol (the intubation procedure took place the night before the study), while the incomplete biliary recovery was due to the design of the tube and the absence of an occlusive balloon. The clinical protocol designed in the present study sought to maintain a more physiologically normal gallbladder refilling state, thereby maximizing the response to cholecystokinin-8. With the exception of subject 3, subjects responded to the infusion of cholecystokinin-8 in a manner typical for healthy volunteers, with gallbladder ejection fractions greater than 0.8.[17] To optimize the filling status of the gallbladder during the study, subjects received a high-fat evening meal and snack ~15 and 9 hours, respectively, prior to Tc-99m mebrofenin administration. This proved to be a successful method to ensure refilling of the gallbladder the following day, as evidenced by the rapid accumulation of Tc-99m mebrofenin in the gallbladder. These results suggested that the gallbladder was not overfilled, a state often associated with reduced bile flow, poor accumulation of cholecystographic agents in the gallbladder, and in some cases, significant spontaneous contraction of the gallbladder in interdigestive states.[21]

The oroenteric tube design used in the current study, and the presence of an occlusive balloon, allowed for more complete recovery of biliary secretions; a high percentage of the administered dose was collected in bile in subjects 1, 2, and 4 (Table 1). Unfortunately, only the theoretical dose was determined for subject 1, and a small amount of bile containing Tc-99m mebrofenin leaked past the occlusive balloon (Figure 3), thus a lower than expected recovery of Tc-99m mebrofenin was determined for this subject (Table 1).

The calculated ejection fraction theoretically represents the fraction of gallbladder content that is expelled into the intestine following infusion of cholecystokinin-8. Radioactivity remaining in the gallbladder after contraction, therefore, represents the amount of Tc-99m mebrofenin irreversibly removed from the systemic circulation that has not yet been excreted into the intestine. By correcting the amount of Tc-99m mebrofenin collected in the bile during the infusion of cholecystokinin-8 by the ejection fraction, and adding this value to the biliary recovery from 0 to 120 minutes, the subsequent recovery of Tc-99m mebrofenin represents the proportion of the *excreted* dose that was collected by the oroenteric tube. The recovery of *excreted* Tc-99m mebrofenin was high and exhibited less variability, ranging from 70.9% to 91.9% of the administered dose across all subjects. These results are consistent with the extent of biliary excretion of Tc-99m mebrofenin in humans (98% in 24 hours).[22] The correction for incomplete gallbladder ejection takes into account a poor or incomplete response to cholecystokinin-8 (as observed for subject 3) and demonstrates the efficiency of the oroenteric tube to collect *excreted* Tc-99m mebrofenin from the duodenal region.

The Tc-99m mebrofenin blood concentration-time profiles exhibited polyexponential decay (Figure 4), and a noncompartmental approach was employed to obtain primary pharmacokinetic parameters (Table 1). The total systemic clearance of Tc-99m mebrofenin (17.3 ± 1.7 mL/min/kg) approximated liver blood flow in humans (21 mL/min/kg)[23] and was in agreement with previously published animal and human data.[24] The calculated biliary clearance for subjects 1, 2, and 4 was 14.0 ± 2.2 mL/min/kg, consistent with the high biliary excretion of Tc-99m mebrofenin, and represented over 66% of the total body clearance observed in these subjects. The poor ejection fraction observed in subject 3 resulted in a lower recovery of Tc-99m mebrofenin, and subsequently, a lower biliary clearance (7.8 mL/min/kg), representing only 35% of the total body clearance for this subject. It is clear that if bile could be collected for a longer time period, eventually all but a fraction of the dose would be recovered in bile. However, owing to practical constraints, it is not possible to keep the balloon inflated for more than 3 to 4 hours. Therefore, quantitative assessment of the gallbladder ejection fraction is vital

to the correct interpretation of the biliary clearance value obtained in time-constrained studies such as those described here. Similarly, although samples were collected only for 180 minutes, the cumulative amount of Tc-99m mebrofenin excreted in the urine (0.6% ± 0.2%) was consistent with that reported in the literature (2% over 24 hours).[22]

In conclusion, the oroenteric tube and clinical protocol developed in this study represent a useful method to investigate the biliary excretion of the probe drug Tc-99m mebrofenin. The custom-made oroenteric tube fitted with an occlusive balloon allowed extremely efficient recovery of biliary secretions from the duodenum. The approach described in this study uses a combination of gamma scintigraphy and a carefully designed clinical protocol to overcome variability in biliary excretion and gallbladder responsiveness to cholecystokinin-8 observed in previous studies.[10,16] A similar experimental design may be used to investigate the biliary excretion of a variety of endogenous compounds and xenobiotics (including investigational drugs) and to calculate biliary clearance, a pharmacokinetic parameter that previously has been difficult to estimate or predict in healthy human subjects.

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