DISPOSITION OF THE SELECTIVE CHOLESTEROL ABSORPTION INHIBITOR EZETIMIBE IN HEALTHY MALE SUBJECTS

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ABSTRACT:

Ezetimibe [SCH 58235; 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4-(S)-(4-hydroxyphenyl)-2-azetidinone], a selective cholesterol absorption inhibitor, is being developed for the treatment of primary hypercholesterolemia. The absorption, metabolism, and excretion of ezetimibe were characterized in eight healthy male volunteers in this single-center, single-dose, open-label study. Subjects received a single oral 20-mg dose of [14C]ezetimibe (100 μCi) with 200 ml of noncarbonated water after a 10-h fast. Concentrations of radioactivity and/or ezetimibe (conjugated and unconjugated) were determined in plasma, urine, and fecal samples. Ezetimibe was rapidly absorbed and extensively conjugated following oral administration. The main circulating metabolite in plasma was SCH 60663 [1-O-[4-[trans-2(S,3R)-1-(4-fluorophenyl)-4-oxo-3-(S)-hydroxy-3-(4-fluorophenyl)propyl]-2-azetidinyl]phenyl]-β-α-glucuronic acid], the glucuronide conjugate of ezetimibe. Plasma concentration-time profiles of unconjugated and conjugated drug exhibited multiple peaks, indicating entero-hepatic recycling. Approximately 78 and 11% of the administered [14C]ezetimibe dose were excreted in feces and urine, respectively, by 240 h after drug administration. Total recovery of radioactivity averaged 89% of the administered dose. The main excreted metabolite was the glucuronide conjugate of ezetimibe. The primary metabolite in urine (0- to 72-h composite) was also the glucuronide conjugate (about 9% of the administered dose). Significant amounts (69% of the dose) of ezetimibe were present in the feces, presumably as a result of SCH 60663 hydrolysis and/or unabsorbed drug. No adverse events were reported in this study. A single 20-mg capsule of [14C]ezetimibe was safe and well tolerated after oral administration. The pharmacokinetics of ezetimibe are consistent with extensive glucuronidation and enterohepatic recirculation. The primary metabolic pathway for ezetimibe is by glucuronidation of the 4-hydroxypropyl group.

Hypercholesterolemia is a significant risk factor for the development of coronary heart disease, which is a leading cause of mortality and morbidity in many countries (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 1993). Results from several landmark clinical trials have shown that 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (i.e., statins) effectively and significantly reduce elevated serum low-density lipoprotein cholesterol concentrations of dietary cholesterol in patients with hypercholesterolemia (van Heek et al., 1997, 2000). Such cholesterol absorption inhibitors prevent the absorption of cholesterol by inhibiting the passage of dietary and biliary cholesterol across the intestinal wall (van Heek et al., 2000). These drugs represent a new class of pharmaceutical agents that can be used to treat patients with hypercholesterolemia.

Ezetimibe was discovered through identification of the active biliary metabolites of SCH 484611 and extensive structure-activity relationship information (Rosenblum et al., 1998). Ezetimibe is an important advance over its predecessor, SCH 48461, because of its increased in vivo potency, lack of hepatic weight gain and cytochrome P450 enzyme induction, reduced systemic exposure, little if any

1 Abbreviations used are: SCH 48461, (3R,4S)-1,4-bis-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone; SCH 60663, 1-O-[4-[trans-2(S,3R)-1-(4-fluorophenyl)-4-oxo-3-(S)-hydroxy-3-(4-fluorophenyl)propyl]-2-azetidinyl]phenyl]-β-α-glucuronic acid; SCH 57871, (3R,4S)-1-(4-fluorophenyl)-3-[3-oxo-3-(4-fluorophenyl)propyl]-4-(4-hydroxyphenyl)-2-azetidinone; LSS, liquid scintillation spectroscopy; LOQ, lower limit of quantitation; SCH 58053, (1S,2R,7S)-7-(4-chlorophenyl)-2-(1-fluorophenyl)-7-hydroxy-3-R-4-hydroxyphenyl)-2-azaspiro[3.5]nonan-1-one (enantiomer A); SPE, solid-phase extraction; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; HPLC, high-pressure liquid chromatography; AUC, area under the plasma concentration-time curve.
potential for pharmacokinetic interactions, and better safety profile in clinical and nonclinical studies (e.g., no evidence of nephrotoxicity or hepatotoxicity) (Salisbury et al., 1995; Sybertz et al., 1995; Rosenblum et al., 1998; Davis et al., 2000; Kosoglu et al., 2000; Lipka et al., 2000; Zhu et al., 2000b). Following oral administration, ezetimibe is rapidly and extensively converted to ezetimibe glucuronide. Both ezetimibe and ezetimibe glucuronide are eliminated slowly; the terminal elimination half-life of ezetimibe has been reported as 20 to 30 h (Zhu et al., 2000a; Ezzet et al., 2001). Ezetimibe glucuronide (SCH 60663) has been found to be more potent than ezetimibe in inhibiting cholesterol absorption in an animal model (van Heek et al., 2000). Ezetimibe effectively reduces low-density lipoprotein cholesterol concentrations as a monotherapy (Lipka et al., 2000) and in combination with statins (Kosoglu et al., 2000). Ezetimibe also increases high-density lipoprotein cholesterol and may reduce elevated triglyceride concentrations (Lipka et al., 2000). This clinical study was designed to characterize the absorption, metabolism, and excretion of a single oral 20-mg dose of [14C]ezetimibe in healthy male volunteers.

Materials and Methods

Radioabeled Drug and Chemicals. [14C]Ezetimibe (Fig. 1) was synthesized by the radiochemistry group at the Schering-Plough Research Institute (Kenilworth, NJ). The mean ezetimibe content was 19.4 mg/capsule (range, 19.0–20.1 mg/capsule). The mean of the radioactivity determinations was 99.7 μCi/capsule. The glucuronide conjugate of ezetimibe SCH 60663 and the ketone metabolite of ezetimibe SCH 57871 were obtained from the Chemical Research Department of Schering-Plough. All reagents and solvents were obtained from commercial sources.

Subjects. Eight healthy, nonsmoking male volunteers between 24 and 37 years old (mean 32 years), whose body weight was within 15% of ideal and who met the eligibility criteria, participated in this study. Subjects provided written informed consent before the start of the study. Subjects were in good health, based on medical history, physical examination, a 12-lead electrocardiogram, and clinical laboratory test results (complete blood count, blood chemistries, serology, and urinalysis). Subjects who had an infectious disease within 4 weeks of the study, used prescription or over-the-counter drugs within 2 weeks of the study, or had a positive urine drug screen were excluded from participation. Subjects who had received an investigational drug within 90 days before the start of the study, had previously received ezetimibe, or had a significant history of drug or food allergy or intolerance to any component of ezetimibe, lactose, or eggs also were excluded.

Study Design. This phase I trial was an open-label, single-dose, 10.5-day, inpatient study of the absorption, metabolism, and excretion of [14C]ezetimibe. This study was approved by the Institutional Review Board (Peninsular Testing Corporation, Miami, FL) and conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. After eligibility screening, eight subjects received a single oral dose of 20 mg of [14C]ezetimibe (administered as a capsule) and were assessed over the next 10 days. All subjects were confined to the study site from 16 h before drug administration until the study procedures were completed (240 h postdose). After an overnight fast, each subject took one capsule containing 20 mg of [14C]ezetimibe with 200 ml of room-temperature, noncarbonated water in the morning. Subjects continued to fast until 4 h after study-drug administration. Meals were the same for all subjects and were provided at the same time each day during the confinement period. To promote daily bowel movements, breakfast included a high-fiber bran muffin or cereal. To assure adequate urine output, subjects were encouraged to drink water throughout the study period.

Sample Collection. Blood, urine, and fecal samples were collected over 10 days. Blood samples (10 ml) were collected into chilled, heparinized Vacutainers (BD Biosciences, Lincoln Park, NJ) at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h after ezetimibe administration for determination of plasma and blood radioactivity and plasma ezetimibe (total, conjugated, and unconjugated) concentrations. Each sample was gently mixed and stored on ice until centrifuged. Whole blood (2 ml) was placed in a polypropylene cryotube and frozen at −20°C or below until analysis for total radioactivity in blood. The remaining blood from each sample was centrifuged, and the resultant plasma was divided into two samples. Two milliliters of plasma were removed and transferred into a cryogenic-compatible tube and stored frozen at −20°C or below until analysis.

Additional 20-ml samples of blood were collected at 0.5, 5, 12, and 24 h postdose for metabolite profiling; after centrifugation, the resultant plasma was frozen and stored at −20°C or below until analysis.

Urine samples were collected just before drug administration (0 h) and then in block collections at 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, and 216 to 240 h after dosing. After recording the volume, each freshly voided urine sample was acidified with glacial acetic acid (0.1% by volume). Urine samples collected during the block collection period were pooled and refrigerated. At the end of the block collection period, the total volume of each block collection (i.e., sum of individual voids) was recorded. Two 5.0-ml aliquots of each well mixed block collection were transferred into appropriate cryotubes. The remaining urine was stored frozen (−20°C or below).

Fecal samples were collected before drug administration; all bowel movements were collected up to 240 h after dosing. For each subject, the total weight of feces collected and the time of each defecation relative to the time of drug administration were recorded on the label. Fecal samples were collected at each defecation and stored in separate containers. The fecal samples and wipes were frozen immediately and stored at −20°C or below until assayed for radioactivity.

Sample Analysis. Measurement of radioactivity. All samples (blood, plasma, urine, and feces) were assayed for total radioactivity using liquid scintillation spectroscopy (LSS) at Phoenix International Life Sciences (Montreal, Canada). The radioactivity associated with all samples was determined in a Packard Tri-Carb 1900 TR liquid scintillation counter (Packard Canberra Instruments, Mississauga, ON, Canada). All samples were counted for 20 min or to 2% (r). Quench corrections were made by the external standard method. Radioactivity was reported as below the lower limit of quantitation (LLOQ) if less than twice background disintegrations per minute were detected and was assigned a value of zero. The LOQs of radioactivity in plasma and blood were 13 and 58 ng of equivalent/g, respectively.

The concentration of radioactivity in duplicate aliquots of urine (500 μl) and plasma (200 μl) was determined by LSS directly in 10 ml of Hionic Fluor scintillation fluid (Packard BioScience, Meriden, CT). Duplicate aliquots of whole blood (50 μl) were solubilized in 1 ml of a cocktail of Soluene-350 tissue solubilizer (Packard BioScience) and isopropanol (BDH, Toronto, ON, Canada) (1:1, v/v) for 4 h at 52°C and then overnight at approximately 4°C. The mixtures were then colorized by the addition of 500 μl of hydrogen peroxide at room temperature for 15 min, followed by a 30-min incubation at 52°C. Radioactivity content was determined by LSS after the addition of 15 ml of Hionic Fluor.
Before analyses, feces were thawed, weighed, and homogenized in water at
a weight-to-volume ratio of 1:1 to 1:2 using a Stomacher lab blender (Seward
Ltd., Mississauga, ON, Canada) was used in place of a Stomacher when fecal
homogenate sample size was <100 g but ≥40 g. Manual homogenization was
used when fecal homogenate sample size was <40 g. The homogenate
was weighed, and duplicate aliquots (180 mg) were removed and solubilized for 4 h
at approximately 52°C and then overnight at approximately 4°C with 1 ml of
Soluene-350 tissue solubilizer. Before determination of radioactivity in 10 ml
of Hionic Fluor, samples were decolorized by addition of 500 µl of isopropanol
and 200 µl of 30% hydrogen peroxide, followed by incubation at room
temperature for 15 min and at 52°C for 30 min. Fecal samples were analyzed
individually. The wipes were not analyzed. Selected fecal homogenate samples
were shipped on dry ice to Schering-Plough Research Institute for metabolic
profiling.

Ezetimibe plasma assay. Plasma samples were assayed for unconjugated and
total (unconjugated plus the glucuronide conjugate) ezetimibe concentrations
using validated liquid chromatographic-tandem mass spectrometric (LC-
MS/MS) methods at Phoenix International Life Sciences (Saint Laurent, QC,
Canada). The analytical methods had an LOQ of 1.00 and 5.02 ng/ml and
inter assay coefficient of variation for plasma samples was 10%. The analyses
were performed in quadruplicate. Mass spectrometric detection was performed in the positive ion
dominant mode, and the mass spectrometric performance liquid chromatograph equipped with a Sciex API III mass spec-
trometer. A conventional volume collection procedure. The chromatographic system used
for the analysis of unconjugated ezetimibe, 100 µl of internal standard
(SCH 58053) working solution (513 ng/ml in water), and 1.0 ml of water were
added to a 200-µl aliquot of each sample before extraction with 8.0 ml of 1-chlorobutane. After the addition of 1-chlorobutane, the samples for the determination of unconjugated or total ezetimibe were extracted similarly. Each tube was shaken for 15 min at room temperature
before centrifugation at 491 g for 10 min at 20°C. The organic layer was
isolated and evaporated to dryness using a Turbo-Vap LV evaporator (Zymark
Corporation, Hopkinton, MA), reconstituted in 500 µl of methanol, and again
evaporated to dryness. The residues were then reconstituted in 50 µl of methanol
before analysis using a Hewlett-Packard 1090 Series II high-performance liquid chromatograph equipped with a Sciex API III mass spec-
trometer.

The chromatographic conditions included a chromatographic column (Spherisorb-ODS2, 10-cm × 0.46-cm × 10-µm particle size; Chromato-
graphic Sciences Co., St-Laurent, QC, Canada) and an isotropic mobile phase
composed of 90% methanol/10% 0.025 M ammonium acetate (1.5 ml/min
flow rate). Mass spectrometric detection was performed in the positive ion
mode, and the m/z transitions monitored for ezetimibe were 392 to 133.1, whereas those for the internal standard (SCH 58053) were 432 to 216.1.
A least-squares linear regression equation with a 1/concentration weighting was
used to construct each calibration curve (i.e., y = mx + b, where y is the peak
area ratio of ezetimibe versus internal standard, x is the concentration of
ezetimibe, m is the slope, and b is the intercept). The concentration of ezetimibe for the determination of unconjugated or total ezetimibe in each human plasma sample was determined by inverse-
prediction from the calibration curve equation. Sample concentrations were
automatically calculated using PhIRSt (Montreal, Canada), a software system
developed and validated to regress and report analytical data. For all acceptable
runs, percent bias and percent CV for calibration standards were within 15%
(20% at the LOQ) of nominal. Additionally, for all quality control samples
percent bias and percent CV were within 20% of nominal. Correlation coeffi-
cients (r) were greater than or equal to 0.994 for the determination of unconjugated ezetimibe and 0.991 for the determination of total (unconjugated
plus conjugated) ezetimibe. Ezetimibe and conjugated ezetimibe were shown
to be stable in plasma for at least 6 months when stored at −20°C or below.

Plasma sample preparation. Plasma radioactivity was too low for metabo-
lite profiling of individual subjects at each collection time. Therefore, at
selected time points (0.5, 5, 12, and 24 h postdose), 5 ml of plasma from each
subject was pooled (~40 ml), assayed for radioactivity by LSS, and then
processed as follows. Solid-phase extraction (SPE; 10 g) trace cartridges
(Waters Corp., Milford, MA) were conditioned with washing with a 20-ml
aliquot of 0.1% trifluoroacetic acid in methanol, 40 ml of methanol, and then
a 40-ml aliquot of water. Each aliquot (8 ml) of pooled plasma was loaded onto
a separate conditioned SPE cartridge. Each cartridge was washed with 40 ml
of water, and the effluent collected in a 50-ml graduated cylinder. Triplicate
aliquots (500 µl) of the water wash were each mixed with 10 ml of scintillation
fluid and assayed for radioactivity. Drug-derived material absorbed onto each
SPE cartridge was then eluted using 0.1% (trifluoroacetic acid) in methanol (40
ml) and recovered radioactivity, determined as described above for the water
wash. A mean (S.D.) of 96.23 ± 6.97% of the plasma radioactivity was
extracted and profiled. Solvent from each plasma extract was vacuum-evapo-
rated at room temperature with a model RE111 Rotavapor (Büchi Labortech-
nik AG, Flawil, Switzerland), the residue dissolved in a minimal amount of
methanol, and then transferred to a 15-ml centrifuge tube. Before LC-MS
analyses, each methanolic extract was dried under a gentle stream of nitrogen
at 23°C using a Turbo-Vap LV evaporator. Each residue was dissolved in an
appropriate amount (ca. 100 µl of dimethyl sulfoxide. Typically, greater than
half (ca. 65 µl) of the sample was injected for analysis.

Urine sample preparation. Urine from each collection interval (24 h).
Fecal homogenates from each subject were then pooled (0.5% by weight) from each
collection interval (0-, 24 h) and the fecal (0- to 72-h pools from each subject.
Aliquots (8 ml) from the composite (subjects 1–8) and individual (subjects 1,
2, 4, and 6) pooled urine samples were extracted by SPE, as described above
for plasma. On average, 93.34 ± 6.30% of the sample radioactivity was
recovered.

Fecal sample preparation. Feces were thawed and mixed thoroughly on a
model 3500 Stomacher lab blender (Tekmar Co., Cincinnati, OH). Fecal
homogenates from each subject were then pooled (0.5% by weight) from each
collection interval (0-, 24 h) and the fecal (0- to 72-h pools from each subject.
Aliquots (8 ml) from the composite (subjects 1–8) and individual (subjects 1,
2, 4, and 6) pooled urine samples were extracted by SPE, as described above
for plasma. On average, 93.34 ± 6.30% of the sample radioactivity was
recovered.

Column Recovery. HPLC column recovery experiments were conducted with extracts from each matrix (plasma, urine, and feces). The mobile phase
split ratio to the radiometric and mass spectral detectors was established using
a conventional volume collection procedure. The chromatographic system used
was a 17 to 23% split of the column effluent, which was diverted and analyzed by
a TSK-700 or TSK-7000 mass spectrometer (Teshio Finnigan MAT, San
Jose, CA). The balance (77–83%) of the column effluent was directed to the
radioactivity detector.

Recovery of radioactivity off the HPLC column from a 0- to 72-h urine
extract was determined by collecting the radioactive effluent during the HPLC
run and comparing the recovered radioactivity with the amount directly mea-
sured in an equivalent volume of the injected sample. This approach was
unsuccessful for the 0.5-, 5-, and 12-h plasma extracts because of difficulty in
dissolving all the extract residue and the low amount of radioactivity recov-
ered. The following procedure was used to better estimate column recovery
from the remaining plasma (24 h) and the fecal (0–96 h) sample. The first step
 involved spiking a portion of the column effluent and the scintillation fluid
from a sham run with an equivalent volume of sample to obtain a 100%
thoretical value. To accomplish this, the HPLC gradient method (50 min) was
initiated without injection of a sample. The scintillation fluid and the mobile
element eluting from the radiometric detector were collected in a 250-ml
graduated cylinder. A 10-µl aliquot from the 24-h plasma extract was added to 20

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Results

Absorption and Excretion. Ezetimibe was rapidly absorbed and conjugated after oral administration; maximum plasma concentrations of conjugated ezetimibe occurred an average of 2 to 3 h after study drug administration (Fig. 2). Thereafter, ezetimibe and conjugated ezetimibe concentrations rapidly declined and then increased, exhibiting multiple peaks consistent with enterohepatic recycling. Subsequently, plasma concentrations declined slowly, with concentrations quantifiable up to 48 h after the dose. Mean concentrations of ezetimibe remained relatively constant from about 0.5 to 36 h after the dose of ezetimibe, although fluctuations occurred, with concentrations ranging from 1.02 to 3.66 ng/ml. Plasma concentration-time profiles of conjugated and unconjugated ezetimibe were quantifiable until 24 to 48 h after the dose. Due to multiple peaks in these profiles, it was not possible to reliably estimate the terminal phase rate constant by regression analysis; therefore, terminal phase half-life (t\textsubscript{1/2}) was not estimated. Table 1 summarizes the mean pharmacokinetic parameters, and individual AUC and C\textsubscript{max} values are shown in Fig. 3. The pharmacokinetic parameters of ezetimibe exhibited moderate variability. Coefficients of variation for C\textsubscript{max} and AUC ranged from 30 to 56% (Table 1; Fig. 3). Exposure (AUC) to ezetimibe represented approximately 10% of the exposure to total (unconjugated plus conjugated) ezetimibe. The systemic exposure (AUC) to total ezetimibe was approximately 93% of the systemic exposure to plasma radioactivity (Table 1). Plasma concentration-time profiles of total ezetimibe were similar to those of the drug-derived radioactivity in plasma (Fig. 4).

The primary route of elimination of drug-derived radioactivity after oral administration of [14C]ezetimibe was via the feces (0–240 h), which contained a mean of 77.7 ± 8.33% (range, 62.6 to 87.4%) of the administered dose (Fig. 5). A mean of 11.3 ± 4.70% of the dose was excreted in the urine. On average, most of the fecal radioactivity was recovered within 24 to 72 h after the dose, whereas most of the urinary radioactivity was recovered within 24 h after dosing. At 240 h after the dose, the total recovery of radioactivity averaged 89.0 ± 5.65% of the administered dose (range, 78.6 to 94.9%).

Metabolism. The results of the metabolite profiling and identification using LC-MS, LC-MS/MS, and radiometric detection demonstrated that the major metabolic pathway for ezetimibe in humans was via glucuronidation of the 4-hydroxyphenyl group.

Plasma. The major metabolite observed in plasma was ezetimibe glucuronide (SCH 60663), which eluted at approximately 8 min. Ezetimibe (R\textsubscript{t} ≈ 39 min) was also observed in the 0.5-, 5-, and 12-h plasma samples but not in the 24-h sample (Table 2).

Urine. LC-MS-detected metabolites and their relative contribution to the percentage of dose are summarized in Table 3 for subjects 1, 2, 4, and 6 and for the pooled sample from subjects 1 to 8. As with plasma, the predominant metabolite in urine was SCH 60663, the glucuronide conjugate of ezetimibe, which accounted for approximately 9% of the dose. The structure of ezetimibe glucuronide was confirmed by LC/MS analysis (Fig. 6), which shows the selected ion chromatogram (Fig. 6, top panel) and LC/MS spectrum (Fig. 6, bottom panel) of the peak eluting at 7.0 min following analysis of an extract from pooled 0- to 72-h urine from subjects 1 to 8. Most radiometric profiles of 0- to 72-h urine also revealed the presence of a minor metabolite, which was identified as the 4-hydroxyphenyl glucuronide conjugate of the ketone analog (SCH 57871) of ezetimibe. The structure of this minor metabolite, which accounted for approximately 0.9% of the dose, was assigned based on the selected ion chromatogram (Fig. 7, top panel) at m/z 582 and LC/MS spectrum (Fig. 7, bottom panel) of the peak eluting at ~11.5 min.

Feces. Unconjugated ezetimibe, which exhibited a retention time of 41 min, accounted for approximately 96% of the chromatographic radioactivity. The selected ion chromatogram and LC/MS spectrum (Fig. 8) confirmed that this major peak had a molecular ion (m/z 408) consistent with ezetimibe and that migrated with a retention time consistent with the ezetimibe standard. The fecal profile also revealed
the presence of a minor ezetimibe metabolite (SCH 57871), which resulted from the oxidation of the benzylic hydroxyl group to a ketone. The structure of this metabolite was confirmed by its selected ion chromatogram and LC/MS spectrum (Fig. 9), which were consistent with those of an authentic standard.

**Safety.** No adverse events, clinically significant abnormalities, or changes in the routine clinical laboratory safety tests (complete blood count, blood chemistries, and urinalysis) were reported during this study. No clinically significant changes were noted on physical examinations, and no deaths or serious adverse events occurred.

**Discussion**

Administration of [14C]ezetimibe capsules as a single oral 20-mg dose was safe and well tolerated by all eight subjects. After oral administration, ezetimibe was rapidly absorbed and extensively metabolized via glucuronide conjugation; exposure (AUC) to ezetimibe represented only about 10% of the exposure to total ezetimibe. These results are consistent with those from other studies (Zhu et al., 1999, 2000a).

Plasma concentration-versus-time profiles of ezetimibe, conjugated

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Unconjugated</th>
<th>Conjugated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;m&lt;/sub&gt;</td>
<td>ng/ml</td>
<td>5.21 (52)</td>
<td>61.2 (51)</td>
<td>64.2 (51)</td>
</tr>
<tr>
<td>T&lt;sub&gt;m&lt;/sub&gt;</td>
<td>h</td>
<td>9.88 (114)</td>
<td>2.31 (66)</td>
<td>2.31 (66)</td>
</tr>
<tr>
<td>AUC(tf)</td>
<td>ng · h/ml</td>
<td>86.4 (56)</td>
<td>636 (33)</td>
<td>726 (30)</td>
</tr>
<tr>
<td>Tf</td>
<td>h</td>
<td>33 (26)</td>
<td>39 (22)</td>
<td>39 (22)</td>
</tr>
</tbody>
</table>

N.C., not calculated.

*Mean pharmacokinetic parameters of radioactivity in blood were not calculated since only three samples had levels of radioactivity above the LOQ (58 ng equiv/ml).*
Ezetimibe, and total ezetimibe exhibited multiple peaks (Fig. 2) suggestive of enterohepatic recycling. As is evident in Fig. 2, multiple secondary peaks in the concentration-time profiles were observed at 4 to 6 and 10 to 12 h. These corresponded to the approximate time of meals. Similar results from a population pharmacokinetic assessment of ezetimibe were observed suggesting that ezetimibe and/or its glucuronide is released with bile into the lumen of the gastrointestinal tract as a result of gallbladder emptying with food (Ezzet et al., 2001). Food intake stimulates the emptying of the gallbladder; thus, enterohepatic recycling occurs every 4 h, consistent with physiology (Fuchs et al., 1998). Since the intestinal wall seems to be the site of action for ezetimibe, it has been suggested that the combination of glucuronidation and enterohepatic recirculation, whereby the drug is repeatedly delivered back to the site of action, may enhance the activity of the compound (van Heek et al., 2000). A reliable half-life for ezetimibe could not be reliably estimated by regression analysis; however, the half-life of drug-derived urinary radioactivity was estimated to be ~24 h based on the cumulative amount of radiocarbon excreted in the urine, mainly as ezetimibe glucuronide. Similar effective plasma half-life values were observed for ezetimibe glucuronide in humans (Zhu et al., 2000a). On average, about 10% of the total ezetimibe

### TABLE 2

<table>
<thead>
<tr>
<th>Characterized Metabolites</th>
<th>Percentage of Profiled Radioactivity</th>
<th>Percentage of Dose</th>
</tr>
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<tr>
<td></td>
<td>Plasma 0.5 h 12 h 24 h</td>
<td>Urine 0–72 h 0–96 h</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>4.2 13.7 21.5 0</td>
<td>68.6 N.D.</td>
</tr>
<tr>
<td>SCH 60663</td>
<td>90 86.3 78.5 100</td>
<td>9.0 N.D.</td>
</tr>
<tr>
<td>SCH 57871</td>
<td>N.D. N.D. N.D. N.D.</td>
<td>N.D. 3.2</td>
</tr>
<tr>
<td>SCH 57871-glucuronide</td>
<td>N.D. N.D. N.D. N.D.</td>
<td>0.90 N.D.</td>
</tr>
</tbody>
</table>

N.D., not detected.

* Subjects 1 to 8.

1 Ezetimibe glucuronide.

### TABLE 3

<table>
<thead>
<tr>
<th>Identity</th>
<th>m/z of [M-H]-</th>
<th>Percent of Dose</th>
</tr>
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<tbody>
<tr>
<td>SCH60663</td>
<td>584</td>
<td>12.4</td>
</tr>
<tr>
<td>SCH57871-glucuronide</td>
<td>582</td>
<td>1.6</td>
</tr>
<tr>
<td>SCH60663</td>
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<td>5.6</td>
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<td>SCH57871-glucuronide</td>
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</tr>
<tr>
<td>SCH60663</td>
<td>584</td>
<td>0.9</td>
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**Fig. 6.** Selected ion chromatogram at m/z 584 (top panel) and LC/MS spectrum (bottom panel) of a peak eluting at 7 min following analysis of an extract from pooled 0- to 72-h human urine (subjects 1–8) after oral administration of 20 mg of [14C]ezetimibe.

**Fig. 7.** Selected ion chromatogram at m/z 582 (top panel) and LC/MS spectrum (bottom panel) of a peak eluting at 11.5 min following analysis of an extract from pooled 0- to 72-h human urine (subjects 1–8) after oral administration of 20 mg of [14C]ezetimibe.

**Fig. 8.** Selected ion chromatogram at m/z 408 (top panel) and LC/MS spectrum (bottom panel) of a peak eluting at 41.29 min following analysis of an extract from pooled 0- to 96-h human feces (subjects 1–8) after oral administration of 20 mg of [14C]ezetimibe.

DISPOSITION OF EZETIMIBE IN HUMANS
administration of [14C]ezetimibe (5 mg/kg) to dogs. Bilary radioactivity was present mainly as conjugated ezetimibe in both species. This is consistent with the plasma metabolite profiles. These results indicate that ezetimibe glucuronide is the primary metabolite and partitioning of drug-derived radioactivity into red blood cells is negligible.

Elimination of drug-derived radioactivity was primarily via the feces (78%), indicating low absorption and biliary secretion of absorbed drug and/or metabolites. The elimination of radioactivity into the feces was gradual and consistent with enterohepatic recirculation and/or delayed fecal elimination of drug-derived radioactivity due to intestinal transit. Drug-derived radioactivity also was excreted in the urine (11%). On average, about 89% of the administered dose was excreted in urine and feces by 240 h after dose administration. Most of the drug-derived radioactivity recovered from 0- to 96-h fecal samples was associated with ezetimibe (68.6% of the dose), which probably resulted from hydrolysis of ezetimibe glucuronide during intestinal transit or incomplete absorption of ezetimibe. A minor oxidative metabolite (4.1% of the dose), consistent with SCH 57871 and its glucuronide, was detected in urine and feces. These data are consistent with studies performed in rats and dogs (Courtney et al., 2001) in which drug-derived radioactivity was eliminated primarily via the biliary route (~50% and ~85%, respectively) following oral administration of [14C]ezetimibe (1 mg/kg) to rats and intravenous administration of [14C]ezetimibe (5 mg/kg) to dogs. Biliary radioactivity was present mainly as conjugated ezetimibe in both species.

A schematic pathway for the biotransformation of ezetimibe in humans, which is via glucuronidation of the 4-hydroxyphenyl group, is shown in Fig. 1. The primary metabolite identified in human plasma was the glucuronide conjugate of ezetimibe (SCH 60663) (Table 2). A relatively small amount of ezetimibe was also detected by metabolite profiling. At 0.5 h after dosing, SCH 60663 contributed to approximately 90% of the total plasma radioactivity, whereas unconjugated (i.e., unchanged) drug accounted for less than 5%. At 24 h, only the glucuronide conjugate (SCH 60663) of ezetimibe was found in human plasma using metabolite profiling. The predominant metabolite in the pooled (0- to 72-h) human urine was ezetimibe glucuronide (SCH 60663); renal clearance accounted for 9.0% of the dose. A minor peak (m/z 582), whose identity was consistent with the glucuronide conjugate of a ketone (SCH 57871) metabolite of ezetimibe, represented less than 1% of the dose. Most of the drug-derived radioactivity (96% of the fecal radioactivity or 68.6% of the dose) recovered from 0- to 96-h feces was associated with ezetimibe. The presence of unconjugated ezetimibe in feces was most likely the result of hydrolysis of ezetimibe glucuronide in the intestine and/or unabsorbed drug. Radioactive and LC-MS profiling of a pooled (0- to 96-h) fecal sample showed that the ketone metabolite (SCH 57871) accounted for no more than 3.2% of the dose. More than 99% of the total radioactivity excreted in the urine (0- to 72-h) and feces (0- to 96-h) was characterized by LC-MS.

The major route of metabolism of ezetimibe in humans is qualitatively similar to that observed in animals (Zbaida et al., 1998; Iannucci et al., 2001; van Heek et al., 2000) and consists primarily of glucuronide conjugation of the 4-hydroxyphenyl group. Following intraduodenal dosing of [3H]ezetimibe in the rat, the only metabolite present in bile, the major route of elimination was ezetimibe glucuronide (van Heek et al., 2000). The only apparent difference in the metabolism of ezetimibe between animals and humans seems to be in the extent of glucuronide conjugation; ezetimibe seems more highly conjugated in animals than in humans.

In summary, ezetimibe was rapidly absorbed and extensively conjugated after oral administration. The pharmacokinetic, excretion, and metabolite profiles indicate extensive glucuronide conjugation and enterohepatic recirculation. The main metabolite identified in human plasma and urine was SCH 60663, the glucuronide conjugate of ezetimibe. As a new, potent, selective cholesterol absorption inhibitor, ezetimibe may present a new approach to the treatment of hypercholesterolemia.

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References


