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

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(Received May 15, 2001; accepted December 26, 2001)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

Ezetimibe [SCH 58235; 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropoxy]-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-(2S,3R)]-1-[4-fluorophenyl]-4-oxo-3-[3(S)-hydroxy-3-(4-fluorophenyl)propoxy]-2-azetidinyl]phenyl]-β-D-glucuronic acid], the glucuronide conjugate of ezetimibe. Plasma concentration-time profiles of unconjugated and conjugated drug exhibited multiple peaks, indicating enterohepatic 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-hydroxyphenyl 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, improve cardiovascular outcome, and reduce the risk for coronary events (Scandinavian Simvastatin Survival Study Group, 1994; Shepherd et al., 1995; Sacks et al., 1996; Downs et al., 1998; LIPID Study Group, 1998). Despite the availability of statins and other lipid-lowering drugs, a significant number of patients with hypercholesterolemia do not achieve adequate cholesterol reduction, having blood cholesterol concentrations higher than desired (Marcelino and Feingold, 1996; EUROASPIRE Study Group, 1997; Feely et al., 2000; Pearson et al., 2000). Thus, treatment options with different mechanisms of action and improved safety profiles are needed to be used alone or coadministered with existing therapies in the treatment of patients with hypercholesterolemia.

Ezetimibe was discovered through identification of the active biliary metabolites of SCH 48461 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-(2S,3R)]-1-(4-fluorophenyl)-4-oxo-3-[3(S)-hydroxy-3-(4-fluorophenyl)propoxy]-2-azetidinyl]phenyl]-β-D-glucuronic acid; SCH 57871, (3R,4S)-1-(4-fluorophenyl)-3-[3-oxo-3-(4-fluorophenyl)propoxy]-4-(4-hydroxyphenyl)-2-azetidinone; LSS, liquid scintillation spectroscopy; LOQ, lower limit of quantitation; SCH 58053, (+)-7-(4-chlorophenyl)-2-(4-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

Radio-labeled 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.
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 blinder (Seward Medical, London, England). A Polytom homogenizer (Brinkman Instruments 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 samples were not analyzed. Selected fecal homogenate samples were shipped on dry ice to Schering-Plough Research Institute for metabolite 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 calibration curve ranges of 1.00 to 100 ng/ml and 5.02 to 502 ng/ml for unconjugated and total ezetimibe, respectively.

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. For the determination of total ezetimibe, 100 μl of internal standard working solution, 500 μl of sodium acetate (0.5 M, pH 5.0), and 50 μl of β-glucuronidase (100,000 units/ml) were added to each 200-μl aliquot of plasma. The samples were then incubated at 50°C for 60 min, followed by the addition of 500 μl of sodium borate solution (1.0 M). The samples were then extracted with 8.0 ml of 1-chlorobutane. After the addition of 1-chlorobutane, the samples for the determination of unconjugated or total ezetimibe were processed 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 spectrometer.

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 392.3 to 133.1, whereas those for the internal standard (SCH 58053) were 432.2 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 coefficients (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 metabolite 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 tC18 cartridges (Waters Corp., Milford, MA) were conditioned by 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-evaporated 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 (0–4, 4–8, 8–12, 12–24, 24–48, and 48–72 h) was pooled by volume (2%) for each subject. To prepare a 0–72 h composite across all subjects, aliquots (2% by volume) were removed from each of the 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 blinder (Tekmar Co., Cincinnati, OH). Fecal homogenates from each subject were then pooled (0.5% by weight) from each collection interval (0–24, 24–48, 48–72, and 72–96 h). To prepare the final 0– to 96-h composite across all subjects, aliquots (20% by weight) were removed from each of the individual 0– to 96-h pools and combined. The homogenized 0– to 96-h fecal composite (subjects 1–8) was processed by liquid-liquid extraction. Approximately 2 g of fecal homogenate was slowly (~10 min) loaded onto an Extrelut 3, wide-pore, diatomaceous earth column (EM Science, Cherry Hill, NJ). Ezetimibe and metabolites were eluted with 120 to 250 ml of isopropanol/ethyl acetate/dichloromethane (20:60:20, v/v/v). The solvent was vacuum evaporated at room temperature with a Büchi model RE111 Rotavapor, and the residue was dissolved in a minimum amount of methanol. After transferring to a 15-ml glass test tube, the methanol was evaporated under a gentle stream of nitrogen at 23°C using a Turbo-Vap LV evaporator. The final sample residue was dissolved in 150 to 200 μl of dimethyl sulfoxide, centrifuged (12,400g) for 15 min, and the supernatant transferred into a 2-ml autosampler vial equipped with a low-volume insert. An aliquot (30–70 μl) was injected for analysis. A mean of 96.33 ± 3.12% of the fecal radioactivity was recovered after this extraction procedure.

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 a 17 to 23% split of the column effluent, which was diverted and analyzed by a TQ-S700 or TQ-S7000 mass spectrometer (Thermo Finnigan MAT, San Jose, CA). The balance (77–83%) of the column effluent was directed to the radiometric detector.

Recovery of radioactivity of 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 measured 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 recovered. 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% theoretical yield. To accomplish this, the HPLC gradient method (50 min) was initiated without injection of a sample. The scintillation fluid and the mobile phase 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
Ezetimibe was rapidly absorbed and of the collected volume and thoroughly mixed before removing three 5-ml aliquots for radioactivity analysis by LSS. The mean value for these determinations adjusted for the volumes used in the spiking experiment and actual run was used to calculate 100% of the theoretical injected radioactivity ($R_{000%}$).

In the profiling experiment, an aliquot (40 µl) of plasma extract was injected into the HPLC. The column effluent and scintillation fluid from this injection was collected in a 250-ml graduated cylinder and radioactivity in each of three 5-ml aliquots was measured by LSS. The mean radioactivity value, was collected in a 250-ml graduated cylinder and radioactivity in each of three injected 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%).

**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 half-life ($t_{1/2}$) was not estimated.

**Pharmacokinetic Analyses.** Individual and mean plasma unconjugated ezetimibe, conjugated ezetimibe, total ezetimibe (unconjugated plus conjugated), and radioactivity concentrations above the respective assay LOQ were analyzed using model-independent methods (Gibaldi and Perrier, 1982). The maximum plasma concentration ($C_{\text{max}}$) and the time of maximum concentration ($T_{\text{max}}$) were the observed values. The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. Summary statistics, including means, standard deviations, and coefficients of variation, were provided for the plasma radioactivity and ezetimibe concentration data at each time point. Pharmacokinetic parameters were summarized using means, standard deviations, and coefficients of variation. Ninety-percent confidence intervals were calculated for the AUC and $C_{\text{max}}$. Pharmacokinetic parameters of blood radioactivity were not determined since only three samples had radioactivity concentrations that were above the LOQ (58 ng equivalent/ml).

**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 (Rt ~ 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>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Unconjugated</th>
<th>Conjugated</th>
<th>Total</th>
<th>Unit</th>
<th>Plasma</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max</td>
<td>ng/ml</td>
<td>5.21 (52)</td>
<td>61.2 (51)</td>
<td>64.2 (51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_max</td>
<td>h</td>
<td>9.88 (114)</td>
<td>2.31 (66)</td>
<td>2.31 (66)</td>
<td></td>
<td></td>
<td></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>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tf</td>
<td>h</td>
<td>33 (26)</td>
<td>39 (22)</td>
<td>39 (22)</td>
<td></td>
<td></td>
<td></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 1.10 days 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>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>0.5 h</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>SCH 60663(^b)</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>SCH 57871</td>
<td></td>
<td>N.D.</td>
</tr>
<tr>
<td>SCH 57871-glucuronide</td>
<td></td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not detected.

\(^a\) Subjects 1 to 8.

\(^b\) Ezetimibe glucuronide.

TABLE 3

| Metabolite percent of dose is the percentage of dose excreted during the collection interval (0–72 h) x the percentage of peak area contribution to total radioactivity in chromatogram.

<table>
<thead>
<tr>
<th>Rt</th>
<th>Identity</th>
<th>m/z of [M+H]+</th>
<th>Percent of Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7–12.2</td>
<td>SCH60663</td>
<td>584</td>
<td>12.4</td>
</tr>
<tr>
<td>14.9</td>
<td>SCH57871glucuronide</td>
<td>582</td>
<td>1.6</td>
</tr>
<tr>
<td>2.7–7.2</td>
<td>SCH60663</td>
<td>584</td>
<td>5.6</td>
</tr>
<tr>
<td>11.5</td>
<td>SCH57871glucuronide</td>
<td>582</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>SCH60663</td>
<td>584</td>
<td>10.2</td>
</tr>
<tr>
<td>2.9–9.5</td>
<td>SCH60663</td>
<td>584</td>
<td>6.3</td>
</tr>
<tr>
<td>11.4</td>
<td>SCH57871glucuronide</td>
<td>582</td>
<td>0.5</td>
</tr>
<tr>
<td>Subjects 1–8</td>
<td>7.1–8.0</td>
<td>584</td>
<td>9.0</td>
</tr>
<tr>
<td>Subjects 1–8</td>
<td>11.4</td>
<td>582</td>
<td>0.9</td>
</tr>
</tbody>
</table>

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 0–72 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 0–72 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 0–96 h human feces (subjects 1–8) after oral administration of 20 mg of [14C]ezetimibe.
administration of [14C]ezetimibe (5 mg/kg) to dogs. Bilary radioactivity was eliminated primarily and its glucuronide, was detected in urine and feces. These data are oxidative metabolite (4.1% of the dose), consistent with SCH 57871 of the drug-derived radioactivity recovered from 0- to 96-h fecal 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 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 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 unab sorbed drug. Radiometric 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., 1999; van Heek et al., 2000) and consists primarily of glucuronide conjugation of the 4-hydroxyphenyl group. Following intraduodenal dosing of [1H]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.

Acknowledgments. We thank Dr. Paul McNamara and the Radiochemistry Department of Schering-Plough for providing radiolabeled ezetimibe and Ning Wu for providing analytical support.

References


