ABSTRACT:

Montelukast sodium [1-[[1(R)-3-[(2-(7-chloro-2-quinolinyl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]-methyl cyclopropylacetic acid sodium salt (MK-476, Singulair) is a potent and selective antagonist of the cysteiny leukotriene (Cys-LT₁) receptor and is under investigation for the treatment of bronchial asthma. To assess the metabolism and excretion of montelukast, six healthy subjects received single oral doses of 102 mg of [¹⁴C]montelukast, and the urine and feces were collected. Most of the radioactivity was recovered in feces, with ≤0.2% appearing in urine. Based on these results and the reported modestly high oral bioavailability of montelukast, it could be concluded that a major part of the radioactivity was excreted via bile. A second clinical study was conducted to identify biliary metabolites of montelukast. The bile was aspirated using a modified procedure involving a nasogastric tube placed fluoroscopically near the ampulla of Vater, after an oral dose of 54.8 mg of [¹⁴C]montelukast. This technique appears to be a new application for drug metabolism studies. The study was conducted with fasted and nonfasted subjects, with the bile being aspirated continuously under suction over periods of 2-8 hr and 8-12 hr after the dose, respectively. Two hours before the end of the collection procedure, cholecystokinin carboxyl-terminal octapeptide was administered iv to stimulate gallbladder contraction. Plasma samples also were collected periodically over 10 hr.

Due to the nature of the collection procedure and the limited sampling time, recovery of radioactivity in bile was incomplete and varied from 3 to 20% of the dose. Radiochromatographic and LC-MS/MS analyses of bile showed the presence of one major and several minor metabolites, along with small amounts of unchanged parent drug. The minor metabolites were identified, by LC-MS/MS comparison with synthetic standards or by NMR, as acetylglucuronide (M1), sulfoxide (M2), 21-hydroxy (diastereomers of a benzyl alcohol, M5a and M5b), and 36-hydroxy (diastereomers of a methyl alcohol, M6a and M6b) analogs of montelukast. The major metabolite was characterized as a dicarboxylic acid (M4), a product of further oxidation of the hydroxy-methyl metabolite M6. Chiral LC-MS/MS analyses of M4 revealed that this diacid, like M5 and M6, was formed in both diastereomeric forms. The levels of metabolites in the systemic circulation were low in the fed as well as fasted subjects, with <2% of the circulating radioactivity being due to metabolites M5a, M5b, M6a, and M6b. Overall, this bile aspiration technique, which is less invasive than either T-tube drainage or fine-needle percutaneous puncture, provided a convenient and expedient means of identifying the biliary metabolites of montelukast, relatively free of contributions from colonic microflora.

Materials and Methods

Chemicals. Montelukast sodium and its [¹⁴C]-labeled analog were prepared at Merck Research Laboratories. The [¹⁴C] labels were incorporated in the methyl groups of the isopropylphenyl moiety (position 36), with a radiochemical purity of >99%. The final specific activities of radiolabeled montelukast

1 Abbreviations used are: Cys, cysteinyl; LT, leukotriene.

MTABOLIC PROFILES OF MONTELUKAST SODIUM (SINGULAIR), A POTENT CYSTEINYL LEUKOTRIENE, RECEPTOR ANTAGONIST, IN HUMAN PLASMA AND BILE


Departments of Drug Metabolism (S.K.B., X.X., R.D.A., R.R.M., B.H.A., G.A.D., M.C., J.D.R., J.H.L., T.A.B.) and Clinical Pharmacology (A.F., S.D.H., J.I.S., B.J.G.), Merck Research Laboratories; Department of Medicinal Chemistry, Merck Frosst Centre for Therapeutics (C.D.); Clinical Pharmacology Associates (K.C.L.); and Department of Medicine, UCSD Medical Center at San Diego (V.P., M.A.K., J.I.I.), University of California, San Diego

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used in clinical studies 1 and 2 were 2.2 and 0.82 μCi/mg, respectively. All other chemicals were of either analytical or HPLC grade.

Clinical Study 1. In an open-label, single-oral dose study, six healthy male subjects (age, 25–41 years; weight, 66–84 kg) each were administered 102 mg (83.8 μCi) of montelukast after an overnight fast. The dose was administered as five capsules taken with water. Normal food intake was resumed 4 hr after the dose. Blood samples were collected before the dose and at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 hr after the dose. Urine was collected over the periods of –2 to 0, 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, and 96–120 hr. Feces were collected up to 5 days after the dose. Blood was centrifuged immediately to separate plasma. All samples were kept frozen at −70°C until analysis. This study was conducted at Clinical Pharmacology Associates (Miami, FL). Approval of the Institutional Review Board was obtained, as was written informed consent from all subjects. All sample handling was performed under amber light conditions, because of the light sensitivity of the parent compound.

Clinical Study 2. This open-label, two-part study involved six healthy subjects (five men and one woman; age, 30–52 years; weight, 66–84 kg) who received a single oral dose of 54.8 mg (122 μCi) of montelukast as two capsules with water. In part A, three subjects were given the drug between 7:00 and 8:00 a.m., after an overnight fast. The bile and gastric juices were collected by a modification of a method used to obtain bile samples for the diagnosis of cholecystitis or cholelithiasis (16, 17), under suction using a modified 16 French standard nasogastric tube (Andersen Products, NC). Through an opening adjacent to the gastric suction outlet, two strips of polyethylene tubing (PE 160) were passed into the lumen of the nasogastric tube. The polyethylene tubing was retrieved from the gastric end of the nasogastric tube and attached to a fenestrated metal weight for distal suction. The length of the polyethylene tubing outside the Andersen tube casing ensured placement of the metal weight in the duodenum when the nasogastric tube was positioned in the greater curvature of the antrum. The tube was passed orally and advanced by the volunteer, 1 hr after the dose. The position was checked under X-ray fluoroscopy. The radiopaque metal weight was positioned at the center of the vertical limb of the duodenal loop, near the ampulla of Vater, to collect bile, which placed the suction ports in the nasogastric tube in the gastric antrum, for juice suction. After reduction of any loops in the stomach, the tube was secured at the mouth, to prevent distal migration during the experiment. The gastric juices and the bile/duodenal fluids were collected, using continuous suction, from 2 to 8 hr after the dose. No medication was given concomitantly. In part B, three subjects were given the dose at 11:00 p.m., 5 hr after ingestion of a standardized high-fat meal. The next morning, an oro-gastro-duodenal tube was placed as in part A, and bile was collected from 8 to 12 hr after the dose. In both parts, cholecystokinin carboxyl-terminal octapeptide (20 ng/kg) was infused iv over 5 min, to stimulate gallbladder contraction and thereby enhance the bile flow, 2 hr before the end of the collection procedure. Plasma samples were collected before the dose and 1, 2, 3, 4, 5, 6, 7, 8, and 10 hr after the dose in parts A and B. Subjects received continuous iv fluid, but no food, during the course of bile collection. All samples were stored at −70°C in the dark until analysis. This study was conducted at San Diego Medical Center, University of California. The protocol was approved by the local Institutional Review Board, and participants provided written informed consent.

Radioactivity Assay. Aliquots of plasma (0.1 or 0.5 ml), bile (0.1 ml), and urine (2 ml) were mixed with 10–18 ml of Beckman Ready-Safe liquid scintillation cocktail and analyzed in a Beckman LS 5000CE or LS 5801 counter. Feces were homogenized with water, and 0.5-ml aliquots of the homogenates were air-dried and combusted using a Packard sample oxidizer (Packard Instruments, Downers Grove, IL). The resulting carbon dioxide was trapped and analyzed by liquid scintillation counting.

Quantitation of Montelukast in Plasma. Aliquots (10–200 μl) of plasma were mixed with 40 μl (200 ng) of an internal standard (an analog of montelukast in which the cyclopropyl group in the carboxylic acid side-chain is replaced by a gem-dimethylmethylene group), a volume (0–190 μl) of control plasma, and 400 μl of acetonitrile (18). The mixture was vortex-mixed and centrifuged, and the supernatant was analyzed on a Hewlett-Packard 1090 HPLC system using an Apex C18 column (4.6 × 50 mm) eluted isocratically with 62% acetonitrile in 0.05 M ammonium phosphate buffer, pH 3.5, flowing at 1.5 ml/min. The HPLC effluent was monitored with a Perkin-Elmer LC-240 fluorescence detector with the excitation wavelength set at 350 nm and emission at 400 nm. Peak height ratios were used to generate standard curves.

HPLC-Radioactivity Analysis of Plasma and Bile. Aliquots of plasma (from clinical study 2) were pooled (1–10 hr) proportionally to time intervals to obtain a single sample representative of the entire time range (e.g. 100 μl of plasma sample from 1–10 hr pooled for AUC0–10 hr). The pooled plasma sample was deproteinized with acetonitrile. The solvent was evaporated under a stream of nitrogen at 37°C, followed by reconstitution with 90% acetonitrile in 1 mM ammonium acetate, pH 3.5. An aliquot of the reconstituted sample was analyzed with a Waters 990 HPLC system using a Beckman C18 column (4.6 × 250 mm), eluted at a flow rate of 1.1 ml/min, with a linear gradient from 35 to 45% acetonitrile in 1 mM ammonium acetate, pH 3.5, in 5 min, 45 to 55% acetonitrile in 35 min, 55 to 87% acetonitrile in 20 min, and then 87 to 95% acetonitrile in 0.3 min. The HPLC effluent was mixed with Packard Flow-Scint III cocktail, flowing at 2.2 ml/min, and was analyzed with a Ramona 5 radioactivity detector (Raytest) (system A). Plasma samples from study 1 were pooled across time points for individual subjects and were analyzed similarly. Bile samples were analyzed directly after centrifugation, using system A.

LC-MS/MS Analysis of Plasma and Bile. Bile (samples mostly highly enriched with radioactivity from two subjects in each part of study 2) and processed plasma samples (studies 1 and 2) were analyzed using system A but using a Hewlett-Packard 1050 HPLC system coupled to a SCIEX III mass spectrometer (Perkin-Elmer, CT), employing a turbo-ion spray interface operating under positive-ion mode. The HPLC effluent was mixed with 90% acetonitrile and 1% trifluoroacetic acid in water, at 50–100 μl/min, before entry into the mass spectrometer (system B). The 21- and 36-hydroxy analogs produced similar values for ion abundance per unit weight of standards.

In addition studies, metabolites were isolated from plasma and bile using the HPLC conditions described for system A and were then analyzed off-line by mass spectrometry. One of the metabolites (36-hydroxy analog) also was analyzed using a Chiral-AGP column (Chrom-Tech, 4 × 100 mm) eluted with a 12-min linear gradient from 20 to 40% acetonitrile in 1 mM ammonium acetate, pH 4.5, at a flow rate of 0.8 ml/min. The remainder of the conditions were the same as those described in system B (system C). Under these conditions, the two diastereomers of the 36-hydroxy analog eluted at about 8.5 and 10.1 min.

Identification of Metabolites. Metabolites M1 and M2 and the diastereo-meric 21-hydroxy (M5a and M5b) and 36-hydroxy (M6a and M6b) analogs were identified by LC-MS/MS comparison with authentic standards (prepared at Merck Frosst, Montreal, Canada). Metabolite M3 was identified by both mass spectrometry and NMR spectroscopy. The proton NMR spectra were recorded on a Varian Unity-500 instrument using CD3OD as solvent and tetramethylsilane as an internal reference. Metabolite M4, isolated from bile using system A, was repurified on a Zorbax Eclipse C8 column (4.6 × 250 mm), eluted with a 15-min linear gradient from 28% acetonitrile and 28% methanol in water to 47% acetonitrile and 47% methanol in water (system D). The retention time of M4 was ~15 min. Isolated M4 was treated with a solution of anhydrous methanoic HCl for 2 hr, and the products were analyzed by MS/MS. M4 was further characterized by comparison with a synthetic standard using mass spectrometry and NMR spectroscopy. To separate the diastereomers of M4, a new method was developed, involving LC-MS/MS using an Astec Cyclobond I column (4.6 × 250 mm) eluted with a 20-min linear gradient from 30% to 62% acetonitrile in 1 mM ammonium acetate, pH 3.3, at a flow rate of 1 ml/min. The mass spectrometric conditions were as described for system B (system E). The two diastereomers of M4 eluted at 15.1 and 16.7 min.

Results

Study 1. The mean plasma concentration-time curves for total radioactivity and unchanged montelukast after a single oral dose of 102 mg [14C]montelukast are shown in fig. 1. Generally, levels of total radioactivity were slightly higher than the drug concentration at all time points, with mean ± SD AUCliner values of 25.2 ± 4.7 μg/hr/ml and 31.2 ± 4.9 μg-equiv/length/ml (determined using the LAGRAN program) (20), Cmax values of 3.61 ± 0.86 μg/ml and 3.93 ± 0.67 μg-equiv/ml, and Tmax values of 3.5 ± 1.8 and 3.7 ± 1.5 hr for montelukast and total radioactivity, respectively. The
AUC$_{0-\infty}$ geometric mean ratio (drug/$[^{14}C]$) was 0.80, indicating that the systemic exposure due to metabolites was low. The urinary recovery of radioactivity was 0.12 ± 0.04% of the dose over 120 hr, the majority of which was eliminated in the first 24 hr. The fecal recovery of radioactivity over the same time interval was 86.3 ± 3.6%, with a major portion being excreted on days 2 and 3 of the study. Radiochromatographic analysis of pooled plasma showed a small peak at the retention time of metabolites M5/M6 and the major peak due to the parent compound. The metabolite peak was isolated for analysis by LC-MS/MS, which demonstrated the presence of similar levels of M5 and M6.

**Study 2.** After a lower, single oral dose of $[^{14}C]$montelukast (54.8 mg), the plasma concentration-time curves for radioactivity and unchanged montelukast were similar to each other in part A, as well as in part B. A plot of the plasma concentration- and plasma radioactivity-time curves from part A is shown in fig. 2. The radiochromatographic profile of extracted 0- to 10-hr pooled plasma showed the presence of ≈2% of radioactivity eluting in the region where 21- and 36-hydroxy metabolite (M5/M6) standards eluted. The 21-hydroxy metabolite existed in two diastereomeric forms, M5a and M5b, which were separable using system A or B. LC-MS/MS analyses (system B) revealed that the 36-hydroxy metabolite was more prevalent than the 21-hydroxy analog. Because the two diastereomeric standards of the 36-hydroxy analog coeluted in systems A and B, the peak corresponding to the 36-hydroxy analog was isolated by conventional HPLC and then analyzed by chiral LC-MS/MS using system C, which showed that this metabolite also existed as a pair of diastereomers (M6a and M6b).

The recovery of radioactivity in bile samples from different subjects is listed in table 1. Bile was collected in multiple tubes (3–14 samples per collection period). The aspirated fluid volumes varied from about 35 ml to 361 ml per collection period. Hence, only the total radioactivity per time period is listed. As shown, the combined recovery of radioactivity ranged from 3 to 20% of the dose in parts A and B of the study. Metabolic profiles of bile samples containing the highest levels of radioactivity from different subjects and time points were found to be qualitatively similar to each other. Fig. 3 displays three representative radiochromatograms. Overall, one major and several minor metabolites were observed, of which the parent compound was a minor component. LC-MS/MS analyses of these samples demonstrated the presence of an acetyl glucuronide (M1), a sulfoxide (M2), a phenol (M3), a dicarboxylic acid (generated by further oxidation of metabolite M6) (M4), and 21-hydroxy (M5a and M5b) and 36-hydroxy (M6) analogs of montelukast. M1 and M2 were present in only trace quantities. Characteristic ions in the mass spectrum of montelukast were at m/z 586 (M+H)$^+$, 440 (cleavage of the C19–C20 bond), 422 (loss of water from m/z 440), 298 (cleavage of the C20–C21 bond from m/z 422), 278 (cleavage of the C19–C20 bond from m/z 422), and 131 (cleavage of the C20–C21 bond with the loss of a water molecule). Based on this fragmentation pattern, it is possible to infer the identities of metabolites by their mass spectral characteristics. Diagnostic ions in the mass spectra of the metabolites were as follows: M1, m/z 762 (M+H)$^+$, 586, 440, 422, 292, 278; M2, m/z 602 (M+H)$^+$, 440, 422, 292, 278; M3, m/z 602 (M+H)$^+$, 456, 438, 292, 278, 147; M4, m/z 616 (M+H)$^+$, 486, 470, 452, 292, 278; M5, m/z 602 (M+H)$^+$, 147; M6, m/z 602 (M+H)$^+$, 456, 438, 420, 292, 278. The retention times and the mass spectra of M1, M2, M5, and M6 were identical to those of the corresponding authentic standards (21, 22).

M3 was isolated for identification by 1H-NMR spectroscopy. The NMR spectrum showed three novel resonances, at 6.96 (d, $J = 2.6$ Hz), 6.88 (d, $J = 2.6$ Hz), and 6.59 (dd, $J = 2.6, 8.3$ Hz) ppm, with a splitting pattern diagnostic of a 1,2,4-trisubstituted benzene ring, indicating hydroxylation at either the C25 or C26 position of montelukast. These two possibilities were distinguished by means of a nuclear Overhauser effect experiment in which the narrow (meta-coupled) doublet at 6.88 ppm was enhanced upon irradiation of the

**Table 1**

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Recovery of radioactivity in bile after oral administration of $[^{14}C]$montelukast</th>
<th>Part A. Fasted Subjects</th>
<th>Part B. Nonfasted Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject 1</td>
<td>Subject 2</td>
<td>Subject 3</td>
</tr>
<tr>
<td>2–4</td>
<td>1.07</td>
<td>1.21</td>
<td>1.72</td>
</tr>
<tr>
<td>4–6</td>
<td>3.52</td>
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<td>0.11</td>
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<td>6–8</td>
<td>3.65</td>
<td>10.46</td>
<td>3.82</td>
</tr>
<tr>
<td>8–10</td>
<td>1.62</td>
<td>2.42</td>
<td>0.56</td>
</tr>
<tr>
<td>10–12</td>
<td>6.43</td>
<td>8.58</td>
<td>2.53</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
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</tbody>
</table>

Number of samples per time period varied from 3 to 14.
gem-dimethyl protons at 1.48 ppm, clearly indicating that the position of hydroxylation is at C25 rather than C26.

The major biliary metabolite M4 (molecular weight of 615) resulted from modifications in the phenylisopropanol moiety of montelukast, based on mass spectral evaluation. Upon methyl esterification of the isolated M4, a dimethyl derivative was obtained, indicating the presence of two carboxylic acid groups in the molecule. The key feature in the NMR spectrum of M4 was the absence of the characteristic gem-dimethyl peak at 1.5 ppm and the appearance of a new signal at 1.78 ppm (relative area, 3 H). From these data it could be inferred that one of the gem-methyl groups at position 35 was oxidized sequentially to a carboxylic acid. This was later confirmed by incubation of M6 with human liver microsomes, which yielded M4. When the putative dicarboxylic acid metabolite M4 was later synthesized at Merck Frosst, it was found to be identical to the biliary metabolite by LC-MS/MS and NMR. Because each of M2, M4, and M6 also could exist as a pair of diastereomers, these metabolites were isolated for analysis by chiral LC-MS/MS. Unfortunately, sufficient quantities of M2 could not be obtained for this purpose. Both M4 and M6 were determined to be present as mixtures of the epimers at the new chiral center (C35).

Discussion

After an oral dose of $[^{14}C]$montelukast was administered to six healthy subjects, 86% of the radioactivity was excreted in the feces and <0.2% in the urine over a period of 120 hr. Plasma analysis showed that the AUC for total radioactivity was slightly higher than that for unchanged montelukast, reflecting the presence of low levels of metabolites in the systemic circulation. Radiochromatographic and LC-MS/MS analyses demonstrated that the plasma contained the 21-hydroxy (M5) and 36-hydroxy (M6) metabolites. Radioactivity in the feces could be largely due to biliary and intestinal secretion of metabolites and the parent compound, as well as nonabsorbed dose. Based on the reported oral bioavailability (58–66%), plasma clearance (46.5 ml/min), and half-life (2.7–5.5 hr) of montelukast in healthy subjects (15) (Merck Research Laboratories, data on file), it is likely that the drug was well absorbed and underwent hepatic/gut metabolism and biliary excretion. Because drug-related metabolites present in feces may not represent true biliary metabolites, due to the possible involvement of colo-rectal microbes (23, 24) that are known to catalyze mainly hydrolyses and reductions of compounds, the second study was designed to investigate the contents of bile directly.

Bile collection generally is performed surgically, e.g., via ultrasonically guided percutaneous, sub- or transhepatic fine-needle puncture of gallbladder (25, 26) or T-tube drainage (27–29) in patients undergoing surgical exploration of the biliary tract. Both of these procedures have their merits but, due to the involvement of surgery, each may cause complications in some cases, e.g., bile leakage, hemorrhage, right upper quadrant pain, or infection (16, 26, 30–32). For research purposes, the success rate for finding volunteers is extremely low. A procedure that is less invasive and more amenable to studies in healthy subjects was used in the present study; it involved bile aspiration through a modified nasogastric tube (16, 17). The tube was placed fluoroscopically near the duodenal ampulla of Vater for suction of bile (along with some pancreatic/duodenal fluids). Another tube or port was placed in the stomach to remove gastric juices and avoid contamination of the bile. The procedure as described also has some constraints, in that the bile collection time is limited by the comfort and tolerability levels of the subjects and quantitative determination of biliary excretion is not possible. In these studies, no intubation-related adverse events were encountered. In the present study, early bile samples were obtained from the fasted subjects (part A) and later bile samples from subjects receiving drug as recommended for clinical treatment of asthma (i.e., dosing at bedtime) (part B). Complete collection of bile was not expected. For part A, the bile collections were performed around the $T_{max}$ (3.7 hr) observed in study 1. Thus, the 2–8-hr bile collection would provide information on metabolites excreted in the early phase of drug elimination. The transit time for the aqueous solution of the drug through the stomach and duodenum is predicted to be <2 hr (33); thus, the bile collected would be free of parent compound that had not been absorbed. In part B, bile was collected 8–12 hr after the dose, to complete the profile established in part A and to take advantage of the possible enrichment of drug and metabolites in bile that had become concentrated overnight due to resting of the gallbladder (34). No temporal or diurnal variations in the pharmacokinetics were observed in previous studies (Merck Research Laboratories, data on file).

Due to the nature of the bile aspiration procedure and the limited duration of sampling (4–6 hr periods), the recovery of radioactivity in the bile was low in both parts of the study. Nonetheless, sufficient bile was collected to permit a qualitative assessment of biliary metabolites. Bile was collected continuously, resulting in multiple tubes per time period. The combined recovery of radioactivity from individual subjects, shown in table 1, ranged from 3 to 20% of the dose. The fraction of an oral dose of montelukast that is bioavailable ($F$) is reported to be in the range of $0.58–0.66$ (15). Using the equation for bioavailability, $F = \frac{f_{abs}}{f_{h}}(1 - f_{z})$, where $f_{abs}$ is the fraction of the drug absorbed from the gastrointestinal tract and $f_{h}$ and $f_{z}$ are the fractions of drug eliminated by the gut and liver, respectively, during the first passage of the drug (35), assuming minimal first-pass effects in the

![Fig. 3](image-url). Representative HPLC-radioactivity profiles for human bile after an oral dose of 54.8 mg of $[^{14}C]$montelukast.

Top trace, 2–4-hr bile from subject 1; middle trace, 8–10-hr bile from subject 4; bottom trace, 10–12-hr bile from subject 5.
gut and the liver, \( f_{\text{abs}} \) would be at least 0.58. Also, because study 1 showed that the dosed radioactivity was eliminated almost exclusively via the feces, a minimum of 58% of the dose may be eliminated through bile over the 5-day period. For one subject (subject 2), approximately 20% of the dose was recovered in bile over a period of 6 hr, indicating that it is possible to capture a reasonable proportion of the drug and metabolites excreted into bile by this technique.

In both parts A and B, metabolic profiles in bile from individuals at different time points were qualitatively similar to each other (fig. 3). Thus, the early metabolites (part A) were similar to later metabolites (part B), with one major and several minor metabolites and little unchanged montelukast being detected in both parts, demonstrating extensive metabolism of montelukast. The significant minor metabolites were identified as an acyl glucuronide (M1), a sulfoxide (M2), a phenol (M3), both diastereomers of the 21-hydroxy analog (21S- and 21R-, M5a and M5b, respectively), and the 36-hydroxy analog (M6a and M6b) (fig. 4). Metabolite M6 was more prevalent than M3 in both the plasma and bile, with M6a being more abundant than M6b. In bile M5a was more prominent than M5b, but the converse was true in plasma. The 21- and 36-hydroxylations were catalyzed by CYP3A4 and CYP2C9, respectively, in human liver microsomes (36).

The mean plasma concentration-time curves for montelukast and total radioactivity in study 2 were nearly identical to each other, implying that the metabolite concentration in plasma was low, with \(<2\%\) of the radioactivity being due to metabolites M5a, M5b, M6a, and M6b only.

In conclusion, the studies demonstrated that biliary excretion is the predominant pathway for the elimination of montelukast and its metabolites. Urinary excretion is insignificant, and thus dose adjustments in renally compromised patients would not be necessary. Bile collected by this modified, less invasive aspiration technique, involving oro-gastroduodenal intubation, provided definitive information on biliary metabolites in healthy subjects, relatively free of any confounding influence of colonic microflora. This qualitative technique appears to be a new application for drug metabolism studies and may serve as a model for future drug development activities. Metabolism of montelukast is extensive, and the early- and late-phase metabolic profiles in human subjects are similar. The systemic exposure to metabolites was low, with only diastereomeric mixtures of 21- and 36-hydroxy analogs of montelukast being detectable in plasma.

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References


**Fig. 4.** Metabolic pathways of montelukast in human subjects. HP, plasma; HB, bile. M4, M5, and M6 existed as diastereomeric pairs.


