ABSTRACT:

Montelukast sodium [1-\{(1(R))-3-(2-(7-chloro-2-quinolinyl)-1(E)-ethenyl)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 acyl glucuronide (M1), sulfoxide (M2), 21-hydroxy (diastereomers of a methyl 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.

The Cys-LTs¹ (LT₄, LTD₄, and LTE₄) have been shown to mediate airway obstruction evoked by factors such as allergens and exercise, which trigger asthmatic reactions including mucus production, decreased mucociliary clearance, changes in vascular permeability, and smooth muscle contraction (broncho- and/or vasoconstriction) (1, 2). In human tissues, activation of the Cys-LT₁ receptors appears to mediate these effects of Cys-LTs. Recent studies with several LT receptor antagonists have demonstrated their ability to block LTD₄ and exercise-induced bronchoconstriction in asthmatic subjects. The compounds studied included zafirlukast (ICI-204,219, Accolate), SKF-104353, MK-571, MK-679, ONO-1078, BAY x7195, and montelukast (MK-476, Singular) [1-\{(1(R))-3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)phenyl\}-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]-methyl)cyclopropylacetic acid sodium salt] (3–9). In addition, zileuton (Zyflo), a LT synthesis inhibitor, has been shown to be effective in reducing asthma symptoms (10). Montelukast is a specific and potent Cys-LT₁ receptor antagonist (11) that has been shown to block LTD₄-induced bronchoconstriction in patients with mild asthma (12) and to improve baseline pulmonary function in patients with chronic asthma (13, 14). In human subjects, montelukast is well absorbed, with oral bioavailability in the range of 58–66% (15). The present studies were conducted to characterize the metabolism and excretion of montelukast in human subjects, with bile collection by a technique that may represent a new application for drug metabolism studies.

Materials and Methods

Chlorinates. 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.

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

**Clinical Study 1.** In an open-label, single-orl 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, 5, 6, 8, 12, 24, 36, 48, 72, 96, and 120 hr after the dose. Urine was collected over the periods of 0–2, 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 cholcyestis or cholestathiasis (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 concurrently. 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-gastroduodenal 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, 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. 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 8.8 and 10.1 min.

**Identification of Metabolites.** Metabolites M1 and M2 and the diastereomers 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 CD3 OD 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 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 8.8 and 10.1 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 AUCh max values of 25.2 ± 4.7 μg/ml and 31.2 ± 4.9 μg-equivalents/ml (determined using the LAGRAN program) (20), Cmax values of 3.61 ± 0.86 μg/ml and 3.93 ± 0.67 μg-equivalents/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 acyl 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–S bond), 422 (loss of water from m/z 440), 292 (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 M5 and M6 were identical to those of the corresponding authentic standards (21, 22).

M3 was isolated for identification by $^1$H-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**

**Recovery of radioactivity in bile after oral administration of $^{14}$C-montelukast**

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
<th>Subject 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–4</td>
<td>1.07</td>
<td>1.21</td>
<td>1.72</td>
<td>1.62</td>
<td>2.42</td>
<td>0.56</td>
</tr>
<tr>
<td>4–6</td>
<td>3.52</td>
<td>7.98</td>
<td>0.11</td>
<td>6.43</td>
<td>8.58</td>
<td>2.53</td>
</tr>
<tr>
<td>6–8</td>
<td>3.65</td>
<td>10.46</td>
<td>3.82</td>
<td>8.05</td>
<td>11.00</td>
<td>3.09</td>
</tr>
<tr>
<td>Total</td>
<td>8.24</td>
<td>19.65</td>
<td>5.65</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of samples per time period varied from 3 to 14.
subject 4; bottom trace center (C35).

determined to be present as mixtures of the epimers at the new chiral
M2 could not be obtained for this purpose. Both M4 and M6 were
analysis by chiral LC-MS/MS. Unfortunately, sufficient quantities of
exist as a pair of diastereomers, these metabolites were isolated for
LC-MS/MS and NMR. Because each of M2, M4, and M6 also could
Merck Frosst, it was found to be identical to the biliary metabolite by
putative dicarboxylic acid metabolite M4 was later synthesized at
M6 with human liver microsomes, which yielded M4. When the
tially to a carboxylic acid. This was later confirmed by incubation of
isolated M4, a dimethyl derivative was obtained, indicating the pres-
from modifications in the phenylisopropanol moiety of montelukast,
of hydroxylation is at C25 rather than C26.

FIG. 3. Representative HPLC-radioactivity profiles for human bile after an
oral dose of 54.8 mg of [14C]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.

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 pres-
ence 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 sequential-
ly 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 [14C]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 (38–66%), plasma clear-
ance (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 ultrason-
ically guided percutaneous, sub- or tranhepatic fine-needle puncture
of gallbladder (25, 26) or T-tube drainage (27–29) in patients under-
going surgical exploration of the biliary tract. Both of these pro-
dedures have their merits but, due to the involvement of surgery, each
may cause complications in some cases, e.g. bile leakage, hemor-
rhage, 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 suc-
cion of bile (along with some pancreatic/duodenal fluids). Another
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 deter-
mination of biliary excretion is not possible. In these studies, non-
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 recom-
mended 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 Tmax (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 estab-
lished 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 varia-
tions 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 sub-
jects, 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 bioavailabil-
ity, 

\[ F = \frac{f_{abs} \cdot (1 - f_{d}) \cdot (1 - f_{k})}{f_{abs}} \]

where \( f_{abs} \) is the fraction of the drug absorbed from the gastrointestinal tract and \( f_{d} \) and \( f_{k} \) 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

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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 (21$^S$- and 21$^R$-, M5a and M5b, respectively), and the 36-hydroxy analog (M6a and M6b) (fig. 4). Metabolite M6 was more prevalent than M5 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.

Acknowledgments. We thank the following members of the Department of Drug Metabolism, Merck Research Laboratories: Drs. Haiyung Cheng, Michael R. Dobrinska, Anthony Y. H. Lu, and K. Chiu Kwan for their invaluable suggestions, Dr. Margaret R. Davis for a mass spectral analysis; and Dr. Dennis C. Dean, Tina M. Marks, Dr. Allen N. Jones, and Dr. David G. Melillo for [14C]montelukast. Also, we thank Marc Labelle of the Medicinal Chemistry Department at Merck Frosst for a gift of metabolite standards.

References

![Fig. 4. Metabolic pathways of montelukast in human subjects.](https://example.com/fig4.png)


