Disposition of Atovaquone in Humans

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Atovaquone is an antiprotozoal compound with good in vitro stability against metabolic inactivation. Previous human studies which did not involve radiolabelling had not accounted for a substantial proportion of the dose. The possible metabolism of atovaquone in men was examined in a radiolabelling study involving four healthy male volunteers. Radioactivity was eliminated almost exclusively via the feces. All radioactivity in plasma, urine, and feces was accounted for by atovaquone, with no evidence of metabolites. Radiolabelled atovaquone was administered to a patient with an indwelling biliary tube after surgery. Biliary radioactivity was approximately 10- to 40-fold higher than that in plasma and was accounted for by atovaquone. Atovaquone is not significantly metabolized in humans but is excreted into bile against a high concentration gradient.

Atovaquone is a naphthoquinone with potent antiprotozoal activity against Plasmodium, Pneumocystis, and Toxoplasma species (3–5). It is licensed in several countries for the treatment of Pneumocystis carinii pneumonia in patients intolerant to cotrimoxazole. Its pharmacokinetics are characterized by incomplete, variable, and food-dependent absorption (6, 7) and a plasma elimination half-life (t1/2) of 2 to 3 days (7). Previous analogs in the developmental series of antiprotozoal naphthoquinones were rapidly metabolized in vitro by human liver microsomes and in humans, but atovaquone was not metabolized in vitro. We have administered radiolabelled atovaquone to healthy volunteers, as well as to one patient with an external biliary diversion, to account for its disposition.

MATERIALS AND METHODS

Clinical. (i) Study 1. In this study, four healthy male volunteers each received a single 750-mg dose of atovaquone containing 3.7 MBq (100 μCi) of [14C] atovaquone. The objectives were to quantitatively account for the administered dose in excreta and to search for possible metabolites in blood, plasma, and excreta. The estimated absorbed radiation dose equivalent was 1.4 mSv, and approval from the Administration of Radioactive Substances Advisory Committee and an independent ethics committee. Up to four patients with biliary cannulae were to be included, but only three were enrolled.

(ii) Study 2. The objective of this study was to search for metabolites of atovaquone in human bile and feces samples. Atovaquone was administered at a dose and in a formulation similar to that used in study 1. Approval was obtained from the Administration of Radioactive Substances Advisory Committee and an independent ethics committee. Up to four patients with biliary cannulae were to be included; if they were taking drugs known to affect hepatic metabolism, biliary composition, or enterohepatic recirculation or if plasma aspartate or alanine aminotransferase or alkaline phosphatase levels were greater than twice the normal upper limit. The drug suspension was given after two slices of toast with 28 g of butter had been consumed to increase absorption. Blood samples were taken before dosing and at 1, 2, 3, 4, 6, 8, 24, 48, and 72 h after dosing, and all feces were collected through 72 h postdosing. Aliquots of bile were taken at the same time points, but the volumes were small (5 ml) so as not to interrupt the flow of bile into the gut. Complete recovery was not attempted as the objective was qualitative rather than quantitative analysis.

Laboratory. (i) Radiometric analysis. Triplicate portions of plasma, chylomicrons, bile, and urine were weighed into tared scintillation vials. Liquid scintillant (Liquiscint) was added in 10-ml volumes, and radioactivity was quantified by liquid scintillation counting (Beckman LS5000CE and LS6801). Homogenates of feces, lyzed whole blood, and vitrums samples were weighed into tared combustion-tacomes with pads. After being dried, they were combusted in a Canberra Packard Sample Oxidizer (B306 or D306) before performance of liquid scintillation counting. Fecal collections containing <10% of the administered dose were extracted with acidic and basic acetonitrile and methanol. The residues (containing 100% of the radioactivity) were solubilized and analyzed by high-performance liquid chromatography (HPLC) on a 12.5-μm diameter Spherisorb 50DS1 column with a mobile phase of acetonitrile-water-phosphoric acid (650:350:5) at a flow rate of 2.5 mL/min. A UV detector (set at 254 nm) and a radiometric detector (Ratayt Ramona SLS; Lachôge, Sheffield, United Kingdom) were connected in series to the outflow. The extracts were also examined by thermospray mass spectrometry for structural confirmation. Bile samples collected at 1 and 2 h were analyzed by two HPLC systems using a 25-μm diameter Spherisorb 50DS1 column and mobile phases of acetonitrile-water-phosphoric acid (650:350:5 and 400:600:5) with flow and detection conditions similar to those used for feces. The limit of quantification was 0.11 μg equivalents per g of plasma or bile.

(ii) Unlabelled-specimen analysis. Atovaquone in plasma was assayed by HPLC as described previously (7). The limit of quantification was 0.05 μg/mL, and the precision and bias at 0.5 and 5.0 μg/mL were 11.7 and 2.8%, and 7.7% and 1.0%, respectively.

(iii) Data analysis. Noncompartmental analysis (Siphar, Simed, France) was used to determine the maximum concentration (Cmax, time to maximum concentration (tmax), area under the concentration-time curve (AUC) from time zero to infinity (AUC0-inf), and values for plasma and whole-blood radioactivities and plasma atovaquone. Cmax and tmax values were direct observations from the experimental data. The elimination rate constant, k, was determined as the slope of the log concentration-time curve. In study 1, for atovaquone in plasma, 9 to 10 points were used to calculate the slope; for plasma radioactivity, between 6 and 8 points were used, and for whole-blood radioactivity, 5 points were used. Mean percentages (± standard deviations [SDs]) of extrapolated AUC values were 2.8% ± 2.5%, 18% ± 7%, and 40% ± 13% for plasma atovaquone, plasma radioactivity, and whole-blood radioactivity, respectively. In study 2, 8 points were used to estimate plasma k values, with 40% of the AUC value being extrapolated, and 3 points were used to estimate the bile k1 values, with 29% of AUC value being extrapolated. AUC values in plasma and bile were calculated by the trapezoidal rule until the last quantifiable concentration, with the remainder being extrapolated as using the equation last measured concentration (Clast)/k1. Apparent clearance (clearance/bioavailability [CL/F]) was calculated as dose/AUC. Biliary clearance of atovaquone was estimated with the equation (typical bile flow rate × AUCplasma)/AUCduodenal where AUCplasma and AUCduodenal represent the values for AUC of the total radioactivity in bile and plasma, respectively. The apparent volume of distribution in the terminal phase (Vz/F) was calculated as apparent clearance/k2.

RESULTS

Study I. Four healthy men (mean age, 33 years [range, 29 to 35]) with hematocrit values between 42 and 46% participated in this study. One volunteer reported an episode of migraine.

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without aura (he had a history of infrequent previous episodes) on the day of dosing, but otherwise no adverse events were reported. The total recovery for three volunteers was between 95 and 113% of the dose, but for the fourth it was 70%, suggesting that a sample had been missed. Urinary excretion of radioactivity was negligible (<0.6%). Radiochromatography of feces resulted in one peak which coeluted with atovaquone and was confirmed to be atovaquone by mass spectrometry. Pharmacokinetic parameters of total radioactivity and unchanged atovaquone are shown in Table 1. Mean concentration-time profiles for atovaquone in plasma and total radioactivity in plasma and whole blood are shown in Fig. 1. The profiles for total radioactivity in plasma and for atovaquone by a specific assay are superimposable, confirming the absence of significant quantities of circulating metabolites. The whole-blood levels of activity were approximately half those of plasma, suggesting that atovaquone does not partition into erythrocytes.

Study 2. Two patients entered the study, but one had to be dropped from the study because his bile samples could not be aspirated from the tube. Hence, data are available for only one patient, a man 61 years of age. Pharmacokinetic parameters of total radioactivity and unchanged atovaquone are shown in Table 1. The peak level of radioactivity in plasma was 1.16 µg equivalents of atovaquone and occurred at 3 h; the \( t_{1/2} \) was

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### Table 1. Pharmacokinetic parameters of total radioactivity and atovaquone

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject(s)</th>
<th>Entity measured</th>
<th>( C_{\text{max}} ) (µg/ml)</th>
<th>( t_{\text{max}} ) (h)</th>
<th>AUC( 0-\infty ) (h · µg/ml)</th>
<th>( t_{1/2} ) (h)</th>
<th>CL/F (ml/min)</th>
<th>V/F (liters)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Healthy volunteers</td>
<td>Whole-blood radioactivity</td>
<td>2.43 ± 0.5</td>
<td>2 (2–4)</td>
<td>226 ± 52</td>
<td>86 ± 10.0</td>
<td>55.1 ± 12.2</td>
<td>412 ± 111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma total radioactivity</td>
<td>4.52 ± 0.93</td>
<td>3 (1.5–4)</td>
<td>479 ± 95</td>
<td>99.5 ± 23.7</td>
<td>26.9 ± 5.4</td>
<td>230 ± 71</td>
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<tr>
<td></td>
<td></td>
<td>Plasma atovaquone</td>
<td>5.30 ± 1.59</td>
<td>2 (2–4)</td>
<td>430 ± 103</td>
<td>77 ± 23</td>
<td>30.4 ± 7.3</td>
<td>203 ± 81</td>
</tr>
<tr>
<td>2</td>
<td>Biliary diversion patient</td>
<td>Plasma total radioactivity</td>
<td>1.27</td>
<td>4</td>
<td>76</td>
<td>52.1</td>
<td>164</td>
<td>741</td>
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<tr>
<td></td>
<td></td>
<td>Bile total radioactivity</td>
<td>51.2</td>
<td>4</td>
<td>1,292</td>
<td>51.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Study 1 entries are means ± SDs; study 2 entries are individual values.

*b Values shown are medians (values in parentheses are ranges).

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**Fig. 1.** Mean (± SD) concentration-time curves for plasma atovaquone (obtained by HPLC) (○), total radioactivity in plasma (◇), and total radioactivity in whole blood (■) for healthy volunteers who received radiolabelled atovaquone. The inset shows the profiles over the first 24 h.
Of atovaquone and occurred at 4 h; the t½ of protein binding contribute to its long metabolism in humans. This metabolic stability and high degree confirm the preclinical indication that atovaquone is not metabolized in humans. As it was also thought that atovaquone, unlike previous analogs in the developmental series of antiprotozoal naphthoquinones, would not be metabolized in humans. It was suspected that atovaquone, unlike previous analogs in the gastrointestinal milieu with regard to diet and the presence of bile (7), it is likely that the lower AUC value reflects the different diet, altered bile flow, and recent surgery of the patient.

For the subjects’ welfare, the minimum radioactive dose of atovaquone which would enable quantification of radioactivity in samples was used. This resulted in a higher quantification limit for radioactivity than for unchanged atovaquone by HPLC, with some resulting imprecision in the pharmacokinetic parameters based on radioactivity. However, the main objectives of seeking metabolites and determining routes of elimination were satisfactorily achieved, and the use of a higher radioactive dose would not have been warranted.

At all time points for which there were corresponding samples, the biliary concentration of atovaquone were substantially higher than that in plasma, with the ratio varying between approximately 10- and 40-fold. Thus, atovaquone is excreted into the bile against a concentration gradient, which is marked considering the very high degree of protein binding of atovaquone. The biliary clearance estimate of between 7 and 14 ml/min, although only a coarse approximation, accounts for a significant proportion of the total clearance of atovaquone of 10.3 ml/min (6), suggesting that biliary excretion is the major route of elimination of atovaquone. However, this comparison ignores any possible reduction in effective biliary clearance due to enterohepatic recirculation, which is not calculable from these two studies. However, as no other significant route of elimination has been identified, it remains likely that biliary excretion is the major method of atovaquone clearance.

The plasma AUC value for the one patient in study 2 was approximately sixfold lower than that in the healthy subjects. As the absorption of atovaquone is highly dependent on the gastrointestinal milieu with regard to diet and the presence of bile (7), it is likely that the lower AUC value reflects the different diet, altered bile flow, and recent surgery of the patient.

DISCUSSION

At all time points for which there were corresponding samples, the biliary concentration of atovaquone were substantially higher than that in plasma, with the ratio varying between approximately 10- and 40-fold. Thus, atovaquone is excreted into the bile against a concentration gradient, which is marked considering the very high degree of protein binding of atovaquone. The biliary clearance estimate of between 7 and 14 ml/min, although only a coarse approximation, accounts for a significant proportion of the total clearance of atovaquone of 10.3 ml/min (6), suggesting that biliary excretion is the major route of elimination of atovaquone. However, this comparison ignores any possible reduction in effective biliary clearance due to enterohepatic recirculation, which is not calculable from these two studies. However, as no other significant route of elimination has been identified, it remains likely that biliary excretion is the major method of atovaquone clearance.

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REFERENCES