Pharmacokinetics of Lamivudine and BCH-189 in Plasma and Cerebrospinal Fluid of Nonhuman Primates

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2'-Deoxy-3'-thiacytidine is a dideoxycytidine analog with a sulfur in place of the 3' carbon of the ribose. There are two enantiomeric forms of the compound, both of which inhibit human immunodeficiency virus type 1 and 2 replication in vitro. However, the (−) enantiomer (lamivudine) appears to be significantly less cytotoxic to uninfected lymphocytes than is the (+) enantiomer. Lamivudine has entered initial clinical trials, and the present study was designed to describe the pharmacokinetic behavior of this compound in both plasma and cerebrospinal fluid (CSF) of primates. Lamivudine was administered as an intravenous bolus dose of 20 mg/kg to five nonhuman primates, and plasma and CSF (ventricular and lumbar) were sampled at frequent intervals for 24 h after administration. Urine samples were also obtained from two animals. The same dose of the racemate (BCH-189) was administered to one animal. The drug was quantitated in CSF and plasma with a reverse-phase high-pressure liquid chromatography technique. Elimination of lamivudine from plasma was biexponential, with a mean alpha phase half-life of 5.4 min, a mean beta phase half-life of 84 min, and a total clearance of 6.1 liters/h. The total clearance of the same dose of BCH-189 in a single animal was 11.0 liters/h. In two animals from which urine was obtained for 12 h postadministration, 32 and 59% of the drug was recovered unchanged. The deamination product of lamivudine was not detected. The CSF/plasma ratio of lamivudine was significantly higher when the drug was measured in the lumbar CSF (mean, 0.41) than when it was measured in the ventricular CSF (mean, 0.079). The measured CSF/plasma ratio for ventricular CSF is equivalent to that of other dideoxycytidine analogs, confirming the importance of the nucleobase in determining the degree of CSF penetration. The difference in lamivudine exposure in ventricular and lumbar CSF suggests that there is a transport mechanism for efflux of cytidine analogs from ventricular CSF.

Materials and Methods

Drugs. Lamivudine and BCH-189 were provided by Glaxo Group Research and Development Ltd. (Middlesex, England). They were reconstituted in sterile water to a final concentration of 40 mg/ml and filtered through a 0.22-μm-pore-size filter prior to administration.

Monkeys. Adult male rhesus monkeys (Macaca mulatta) ranging in weight from 5.0 to 10.7 kg were used in these experiments. The animals were fed a National Institutes of Health open formula extruded nonhuman primate diet twice daily and group housed in accordance with the Guide for the Care and Use of Laboratory Animals (11). Blood samples were drawn through a catheter placed in either the femoral or the saphenous vein opposite the site of drug administration. Ventricular CSF samples were obtained from a chronically indwelling Pudenz catheter attached to a subcutaneously implanted Ommaya reservoir (10). Lumbar CSF samples were obtained from several animals by using an indwelling temporary lumbar catheter.

Experiments. The pharmacokinetics of lamivudine were studied in five animals following administration of an intravenous (i.v.) bolus dose of 20 mg/kg. Blood was collected in heparinized tubes containing 50 μl of 1 mM tetrahydrouridine (THU) to a final concentration of 40 mg/ml and filtered through a 0.22-μm-pore-size filter prior to administration.

FIG. 1. Structure of lamivudine ([−] enantiomer of 2'-deoxy-3'-thiacytidine).
before and at 5, 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after the dose was administered. Plasma was immediately separated by centrifugation. CSF samples were collected from an Ommaya reservoir or a lumbar catheter before and at 15 and 30 min and 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after drug administration. The reservoir was pumped four times before and after each sample collection to ensure adequate mixing with ventricular CSF. Urine was collected at 6-h intervals from 0 to 12 h from two animals following drug administration. All samples were frozen at −20°C until assayed.

The pharmacokinetics of BCH-189 were studied in a single animal following administration of a 20-mg/kg i.v. bolus dose. Blood and CSF sampling times were identical to those used for lamivudine. Blood and CSF samples were stored at −20°C until assayed.

Sample analysis. Concentrations of lamivudine and BCH-189 were measured in plasma, urine, and CSF by using a recently described reverse-phase high-performance liquid chromatography assay (7). Aliquots of plasma or CSF were mixed with a perchloric acid solution (4%, wt/vol) containing 20 μg of Carbovir, the internal standard, per ml. Samples were then vortexed and centrifuged for approximately 3 min in a microcentrifuge. An aliquot (0.02 ml) of the resulting supernatant was injected onto the analytical column. Chromatography was performed with a BDS Hypersil column (15 by 0.46 cm) eluted at a flow rate of 1 ml/min with a mobile phase of methanol (15%) in ammonium acetate (0.1 M) acidified by addition of glacial acetic acid (0.1%). The eluant was monitored by UV detection at a wavelength of 270 nm. Quantification was done on the basis of the internal standard ratio by using a calibration line constructed from the analysis of plasma spiked with known amounts of lamivudine. Calibration lines were linear over a range of 0.4 to 440 μM (0.1 to 100 μg/ml). The sensitivity was 0.4 μM, at which concentration the intrabatch coefficient of variation was about 17%. The coefficient of variation was less than 4% at concentrations greater than 8 μM. Aliquots of urine were injected onto a BDS Hypersil column (25 by 0.46 cm) maintained at a temperature of 36°C. This was eluted at a flow rate of 1 ml/min with methanol (10%) and acetic acid (0.1%) in 0.1 M ammonium acetate. The eluant was monitored by UV detection at a wavelength of 270 nm. Calibration lines were linear over a range of 4.4 to 440 μM. The sensitivity of the method was 1.0 μg/ml, at which concentration the intrabatch coefficient of variation was about 1%.

Pharmacokinetic analysis. Plasma concentration versus time data from the individual lamivudine i.v. bolus experiments were fitted to a two-compartment open model consisting of central and peripheral compartments with first-order elimination from the central compartment (14). The model parameters were estimated with a weighted (1/concentration2) fit of the data to the differential equations describing the drug concentrations in the central and peripheral compartments by using MLAB (8). The equation describing the concentration in the central compartment is $\frac{dC_C}{dt} = k_{0e} - V_p k_{pc} (C_C - C_p)$, and the equation describing the concentration in the peripheral compartment is $\frac{dC_p}{dt} = k_{pc} (C_C - C_p)$, where $C_C$ and $C_p$ are the drug concentrations in the central and peripheral compartments at time $t$, $k_e$ is the drug infusion rate, $V_p$ is the volume of the central compartment, $k_{0e}$ is the elimination rate constant, and $k_{pc}$ and $k_{pc}$ are the rate constants describing drug transfer between the central and peripheral compartments. Other pharmacokinetic parameters (clearance, volume of distribution at steady state, and half-lives) were derived from the estimates of the model parameters by using standard techniques (6).

The areas under the concentration-time curves (AUCs) for CSF were derived by the linear trapezoidal method (6). The fraction of drug penetrating the CSF was calculated from the ratio of the AUCs (to the last time point) for CSF and plasma after bolus dose administration.

RESULTS

Plasma pharmacokinetics. The disappearance of lamivudine from plasma following administration of an i.v. bolus dose was rapid, with a mean alpha phase half-life of 5.4 min (range, 4.1 to 6.1 min) and a mean beta phase half-life of 84 min (range, 60 to 102 min) (Fig. 2). The mean total body clearance (CLTb) was 6.2 liters/h (range, 4.7 to 7.8 liters/h). The pharmacokinetic parameters for the i.v. bolus doses are listed in Table 1.

In the animal that received an i.v. bolus dose of BCH-189, the pharmacokinetics were assessed.

TABLE 1. Pharmacokinetic parameters in two-compartment open model

<table>
<thead>
<tr>
<th>Drug and monkey</th>
<th>$V_c$ (liters/kg)</th>
<th>$V_{ss}$ (liters/kg)</th>
<th>$k_{0e}$ (h⁻¹)</th>
<th>$k_{pc}$ (h⁻¹)</th>
<th>$k_{pc}$ (h⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Lamivudine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8635</td>
<td>0.361</td>
<td>1.155</td>
<td>1.744</td>
<td>3.820</td>
<td>1.737</td>
</tr>
<tr>
<td>CH843</td>
<td>0.279</td>
<td>0.996</td>
<td>3.351</td>
<td>5.248</td>
<td>2.043</td>
</tr>
<tr>
<td>85Z</td>
<td>0.210</td>
<td>0.920</td>
<td>4.122</td>
<td>5.177</td>
<td>1.532</td>
</tr>
<tr>
<td>X854</td>
<td>0.415</td>
<td>1.438</td>
<td>1.757</td>
<td>3.971</td>
<td>1.608</td>
</tr>
<tr>
<td>AJ</td>
<td>0.300</td>
<td>1.262</td>
<td>2.620</td>
<td>3.910</td>
<td>1.195</td>
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<tr>
<td>Mean ± SD</td>
<td>0.313 ± 0.078</td>
<td>1.158 ± 0.210</td>
<td>2.719 ± 1.031</td>
<td>4.425 ± 0.721</td>
<td>1.623 ± 0.309</td>
</tr>
<tr>
<td>BCH-189, CH843</td>
<td>0.590</td>
<td>1.829</td>
<td>3.719</td>
<td>4.645</td>
<td>2.214</td>
</tr>
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</table>

*Abbreviations: $V_c$, apparent volume of central compartment; $V_{ss}$, apparent volume of distribution at steady state; $k_{0e}$, elimination rate constant; $k_{pc}$, rate constant for drug transit from central compartment to peripheral compartment; $k_{pc}$, rate constant for drug transfer from peripheral compartment to central compartment.
the disappearance from plasma was biexponential with a terminal half-life of 49 min and a total body clearance of 11.0 liters/h (Fig. 3).

Urines samples were obtained from the first two animals for 12 h following drug administration. In these two animals, 59 and 32% of the administered 3TC dose was recovered unchanged in the urine. The lamivudine renal clearance values in these animals were 3.3 and 1.5 liters/h, respectively.

CSF pharmacokinetics. The plasma and CSF concentration-time profiles for lamivudine are shown in Fig. 4. The mean (± standard deviation) CSF/plasma AUC ratios (Table 2) for lamivudine were 0.079 ± 0.047 with ventricular CSF and 0.41 ± 0.23 with lumbar CSF. In the single animal that received an i.v. bolus of the racemate, BCH-189, the concentrations in ventricular CSF were 0.70 and 0.52 μM at 45 min and 1.3 h, respectively. In comparison, the lamivudine concentrations in ventricular CSF were 1.79 μM at 45 min, 1.62 μM at 1 h, and 0.44 μM at 2.1 h.

Toxicity. The i.v. bolus doses of both lamivudine and BCH-189 were well tolerated by all of the animals. One animal had one episode of emesis following administration of lamivudine. No other acute or chronic toxicity was observed.

**DISCUSSION**

The plasma and CSF pharmacokinetics of lamivudine and BCH-189 were studied in a nonhuman primate model which has previously been predictive of human CSF penetration by drugs (13). The mean CL\(_{TB}\) of lamivudine in nonhuman primates was very similar to that reported in humans (16). The CL\(_{TB}\) was significantly greater than the glomerular filtration rate (15). The mean CL\(_{TB}\) (0.78 liters/h/kg) was more than twofold higher than the mean renal clearance (0.34 liters/h/kg) in the two animals from which urine samples were obtained. A similar phenomenon was also observed in human pharmacokinetic studies in which the mean CL\(_{TB}\) of lamivudine was 1.5 times the mean renal clearance (16).

The penetration of ventricular CSF by lamivudine is similar to that reported for other ddC analogs, confirming the importance of the nucleobase in determining the degree of CSF penetration by dideoxypyrimidine analogs (4). The mean ratio of lamivudine concentrations in CSF and plasma was significantly higher when the drug was measured in the lumbar CSF (mean, 0.41) than when it was measured in the ventricular CSF (mean, 0.079). We have observed a similar phenomenon following i.v. administration of the cytidine analog cyclopentenyl cytosine. After administration of a 100-mg/m\(^2\) i.v. bolus dose of cyclopentyl cytosine, the CSF/plasma ratio with lumbar CSF was 0.119 (n = 1) versus 0.056 (n = 2) with ventricular CSF (2). A difference of similar magnitude was observed between the lumbar and ventricular CSF exposure to cyclopentenyl uridine, the deamination product of cyclopentenyl cytosine. One possible explanation for the difference in drug exposure between ventricular CSF and lumbar CSF is an active efflux mechanism for the cytidine analogs that is localized to the ventricles. We have observed that following a 24-h continuous ventricular CSF infusion of the cytidine analog cytarabine arabinoside, steady-state concentrations of the drug were not detectable in the lumbar CSF, despite cytarabine arabinoside concentrations in the ventricular CSF in the micromolar range (1a). This suggests that the drug is transported out of the ventricular CSF before it circulates through the CSF to the lumbar sac. It is unlikely that differences in cytidine deaminase levels between the ventricular CSF and the lumbar CSF could account for this phenomenon, since lamivudine does not appear to be a substrate for cytidine deaminase. Following i.v. drug administration, a deaminated metabolite of lamivudine was not detected in plasma, CSF, or urine.

**TABLE 2.** Pharmacokinetic parameters following administration of a 20-mg/kg i.v. bolus of lamivudine or BCH-189 to nonhuman primatesa

<table>
<thead>
<tr>
<th>Drug and monkey</th>
<th>Wt (kg)</th>
<th>CL(_{TB}) (liters/h)</th>
<th>(V_{ss}) (liters)</th>
<th>AUC(_{ss}) (μM · h)</th>
<th>AUC(_{CSF}) (μM · h)</th>
<th>AUC(_{VCSF}) (μM · h)</th>
<th>LCSF/P ratio</th>
<th>VCSF/P ratio</th>
<th>LCSF/P (t_{1/2a})</th>
<th>LCSF/P (t_{1/2b})</th>
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<tr>
<td>Lamivudine</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>8635</td>
<td>8.8</td>
<td>5.4</td>
<td>10.2</td>
<td>138</td>
<td>20.0</td>
<td>0.14</td>
<td>6.1</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH843</td>
<td>5.1</td>
<td>4.7</td>
<td>5.1</td>
<td>93</td>
<td>2.9</td>
<td>0.031</td>
<td>4.2</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85Z</td>
<td>6.5</td>
<td>5.6</td>
<td>6.0</td>
<td>101</td>
<td>4.9</td>
<td>0.049</td>
<td>4.1</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X854</td>
<td>10.7</td>
<td>7.8</td>
<td>15.3</td>
<td>119</td>
<td>13.0</td>
<td>0.109</td>
<td>6.0</td>
<td>102</td>
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<td></td>
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<tr>
<td>AJ</td>
<td>9.0</td>
<td>7.1</td>
<td>11.5</td>
<td>111</td>
<td>14.2</td>
<td>0.56</td>
<td>5.7</td>
<td>97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.1 ± 1.3</td>
<td>9.6 ± 4.2</td>
<td>112 ± 17</td>
<td>48.6 ± 24.8</td>
<td>8.8 ± 5.7</td>
<td>0.41 ± 0.23</td>
<td>0.079 ± 0.047</td>
<td>5.4 ± 1.2</td>
<td>84 ± 19</td>
<td></td>
</tr>
<tr>
<td>BCH-189, CH843</td>
<td>5.1</td>
<td>11.0</td>
<td>9.2</td>
<td>39</td>
<td></td>
<td></td>
<td>4.3</td>
<td>49</td>
<td></td>
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</table>

\(^a\) Abbreviations: \(V_{ss}\), volume of distribution at steady state; P, plasma; LCSF, lumbar CSF; VCSF, ventricular CSF; CSF/P, ratio of CSF AUC to plasma AUC; \(t_{1/2a}\), alpha phase half-life; \(t_{1/2b}\), beta phase half-life.
Since human immunodeficiency virus encephalopathy has potential severe consequences that may be partially reversible by agents that penetrate the CSF, drug distribution throughout the neuroaxis following systemic administration is important. The differences in lumbar and ventricular exposures to lamivudine observed in this study suggest that CSF sampling from a ventricular access device may not be predictive of overall central nervous system drug exposure.

REFERENCES