Pharmacokinetics of the Protease Inhibitor KNI-272 in Plasma and Cerebrospinal Fluid in Nonhuman Primates after Intravenous Dosing and in Human Immunodeficiency Virus-Infected Children after Intravenous and Oral Dosing

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KNI-272 is a human immunodeficiency virus (HIV) protease inhibitor with potent activity in vitro. We studied the pharmacokinetics of KNI-272 in the plasma and cerebrospinal fluid (CSF) of a nonhuman primate model and after intravenous and oral administration to children with HIV infection. Plasma and CSF were sampled over 24 h after the administration of an intravenous dose of 50 mg of KNI-272 per kg of body weight (approximately 1,000 mg/m2) to three nonhuman primates. The pharmacokinetics of KNI-272 were also studied in 18 children (9 males and 9 females; median age, 9.4 years) enrolled in a phase I trial of four dose levels of KNI-272 (100, 200, 330, and 500 mg/m2 per dose given four times daily). The plasma concentration-time profile of KNI-272 in the nonhuman primate model was characterized by considerable interanimal variability and rapid elimination (clearance, 2.5 liters/h/kg; terminal half-life, 0.54 h). The level of drug exposure achieved in CSF, as measured by the area under the concentration-time curve, was only 1% of that achieved in plasma. The pharmacokinetics of KNI-272 in children were characterized by rapid elimination (clearance, 276 ml/min/m2; terminal half-life, 0.44 h), limited (12%) and apparently saturable bioavailability, and limited distribution (volume of distribution at steady state, 0.11 liter/kg). The concentrations in plasma were maintained above a concentration that is active in vitro for less than half of the 6-h dosing interval. There was no significant increase in CD4 cell counts or decrease in p24 antigen or HIV RNA levels. The pharmacokinetic profile of KNI-272 may limit the drug’s efficacy in vivo. It appears that KNI-272 will play a limited role in the treatment of HIV-infected children.

Protease inhibitors have become increasingly important as part of the therapeutic options against human immunodeficiency virus (HIV) infection. KNI-272 is a peptide-based antiretroviral agent that inhibits the catalytic activity of the HIV-1-specific aspartic protease (18) and that has potent activity in vitro against a wide spectrum of HIV type 1 (HIV-1) and HIV-2 strains (6, 13). In preclinical pharmacokinetic studies with rodents and dogs, concentrations in plasma that exceeded the 50% effective concentration (EC50; 0.1 μM) were achieved and were maintained for several hours without unacceptable toxicity (14, 15).

We evaluated the pharmacokinetics of KNI-272 after intravenous and oral administration to children who were treated in a phase I trial. We previously measured the levels of penetration of a number of antiretroviral drugs into cerebrospinal fluid (CSF) in a nonhuman primate model which has been predictive of the degree of penetration into the CSF of humans (1–3, 7, 10). In the present study we therefore evaluated the pharmacokinetics of KNI-272 in plasma and CSF of this well-established animal model.

MATERIALS AND METHODS

Nonhuman primates. (i) Animals. Three adult male rhesus monkeys (Macaca mulatta) weighing 8.1, 9.3, and 11.2 kg, respectively, were studied. The animals were group housed in accordance with the Guide for the Care and Use of Laboratory Animals (21) and received food and water ad libitum. Heparinized blood samples were drawn from a saphenous or femoral venous catheter (contralateral to the site of injection) prior to administration of the dose and 5, 15, and 30 min and 1, 2, 4, 6, 8, 12, and 24 h after administration of the dose. The plasma was immediately separated by centrifugation and was frozen at −70°C until it was assayed. For animals CH980 and CH957, CSF was collected prior to administration of the dose, 30 and 60 min after the beginning of the infusion, and 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 h after the end of the infusion from a chronically indwelling subcutaneous Ommaya reservoir attached to a 4th ventricular Pudenz catheter (20). For animal 608PR CSF samples were obtained from a newly placed temporary lumbar catheter.

(ii) Drug formulation and administration. The white powder of KNI-272 was mixed in a ratio of 1:60 (wt:wt) with hydroxypropyl-β-cyclodextrin (HPCD) (5a), reconstituted with 0.9% sodium chloride, and adjusted to a pH of 2.0 to 3.5 with hydrochloric acid. The final concentration of this solution was 2.8 mg/ml. Prior to intravenous injection, the drug solution was sterilized by filtration through a Millex-GV 0.22-m pore-size filter (Millipore Corporation, Bedford, Mass.). Animal 608PR received 405 mg (0.75 mmol/kg of body weight) over 5 min, animal CH980 received 471 mg (0.76 mmol/kg) over 68 min, and animal CH957 received 518 mg (0.69 mmol/kg) over 85 min. This dose corresponds to 1,000 mg/m2.

Pediatric phase I trial. The pediatric phase I trial and pharmacokinetic study of KNI-272 were approved by the National Cancer Institute’s Institutional Review Board, and written informed consent was obtained from the parent or legal guardian of each child.

(i) Study population. Between November 1994 and November 1995, 21 children were enrolled in the pediatric phase I trial of KNI-272. Pharmacokinetic studies were performed with 18 children (9 males and 9 females; median age, 9.4 years; age range, 2.7 to 16.8 years). Thirteen children had acquired HIV infection perinatally, and the other five children in the pharmacokinetic studies acquired it from the transfusion of blood products or clotting factors. All children had...
previously been treated with dideoxynucleosides, but none had received a pro- tease inhibitor. All children were in stable condition and free of acute infections.

Patients were required to have a total leukocyte count of >1,500 cells/mm³ and a neutrophil count of >750 cells/mm³, a hemoglobin level of >8 g/dl, and a platelet count of >75,000/mm³, a serum creatinine level of <2 mg/dl, and values for liver function tests <2.5 times the upper limit of normal prior to study entry.

(ii) Study design, drug formulation, and drug administration. This 12-week pediatric phase I trial of KNI-272 was an open-label, dose-escalation study. The clinical results of this trial will be reported separately. Pharmacokinetic sampling was performed with separate cohorts of patients treated with each of the four dose levels: 100 mg/m² per dose (n = 5), 200 mg/m² per dose (n = 6), 330 mg/m² per dose (n = 3), and 500 mg/m² per dose (n = 5) (dose levels 1 to 4, respectively). For the two lower dose levels, a single intravenous dose of KNI-272 was admin- istered on the first day of treatment to evaluate the pharmacokinetics of the drug. KNI-272 was subsequently administered orally four times a day.

KNI-272 was manufactured by Pharmaceutical and Biotechnology Research Laboratories, Nikko Kogyo Co. (Saitama, Japan), and was distributed by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md.). The intravenous formulation was supplied in vials containing 50 mg of hylpoliphilic powder with 3,000 mg of HPCD, a complexing agent approved for investigational standards prepared in untreated plasma were extracted with Bond-Elut C 18, and the residue was reconstituted in 200 l of HPLC-grade methanol (Fisher Scien
tific Company, Pittsburgh, Pa.) followed by 6 ml of deionized water. The samples or standards (1 ml) were loaded onto the column, the columns were then washed with 2 ml of deionized water, and the drug was eluted from the column with 2.5 ml aliquots of methanol. The eluant was evaporated to dryness under a stream of nitrogen, and the residue was reconstituted in 200 µl of the mobile phase (see below). Prior to injection onto the HPLC column the samples were clarified by centrifugation through Ultrafree-MC 0.45-µm pore-size filter units (Millipore Corporation). The CSF samples were directly injected onto the HPLC column.

Chromatographic analysis was performed on a Waters HPLC system consist- ing of a WISP model 715 Ultra injector, a model 600E solvent-delivery system, and a model 490 programmable multiwavelength UV detector. The mobile phase consisted of 65% methanol, 35% deionized water, and 0.01% triethylamine (vol/vol/vol) at an isocratic flow rate of 1.4 ml/min through a Brownlee 5µm, OD-GU guard column and a C18 Steel Nova-PAK 4µm phenyl column (3.9 by 150 mm; Waters Associates). The eluant was monitored at a wavelength of 230 nm. Under these conditions, the retention time for KNI-272 was 8 min. The standard curve was linear in the range investigated (0.1 to 10 µmol/liter), and recovery, verified by comparison with an aqueous standard, was >90%.

Pharmacokinetic calculations. A two-compartment open model was fitted to the plasma KNI-272 concentration-time data after administration of an intravenous dose to nonhuman primates and pediatric patients who were treated at the 200-mg/m² dose level by using MLAB nonlinear curve fitting software (Civilized Software, Bethesda, Md.) (17). The following differential equations were used to describe the concentration in the central compartment (C C) and the amount of drug in the peripheral (C p) compartment at time t:

\[
d C_C \over dt = -k_0 C_C - k_{cp} C_C + k_{pc} C_P \over V_C
\]

where \( k_0 \) is the rate of drug infusion, \( k_{cp} \) is the elimination rate constant, \( k_{pc} \) and \( k_{cp} \) are the rate constants describing the transfer between the central and peripheral compartments, and \( V_C \) is the volume of the central compartment. The data were weighted by using the built-in MLAB weighting function EWT, which computes a weight vector from estimates of reciprocal variance values. The fitted model parameters were then used to calculate clearance (CL = CL = \( V_C \cdot k_0 \)) and the volume of distribution at steady state (\( V_{ss} = V_C \cdot [k_{pc} + k_{cp}] \)). Half-lives were derived from the rate constants described previously (9).

The area under the concentration-time curve (AUC) in CSF and after the administration of an oral dose was calculated by the linear trapezoidal rule (9). The fraction of drug penetrating into the CSF was derived from the ratio of the AUC in CSF to the AUC in plasma. The fraction of the oral dose absorbed (\( f_d \)) was estimated from the ratio of the AUCs after administration of oral and intravenous doses of 200 mg/kg.

RESULTS

Nonhuman primates. The first animal (animal 608PR) received KNI-272 intravenously over 5 min and experienced an acute adverse reaction 3 min into drug administration manifested by facial erythema, prolonged capillary refill, hypothermia, and a mild drop in blood pressure which was treated by the administration of intravenous fluids. The animal’s blood pressure and temperature normalized after 1 h, but the animal remained lethargic and had intermittent chills for several hours. No other infectious or metabolic etiology was identified.

When the vehicle (HPCD) was injected alone as a 5-min bolus, no reaction occurred, and further infusions of KNI-272 were administered over at least 1 h and were well tolerated.

The two-compartment model adequately described the disposition of plasma KNI-272 concentrations. Table 1 lists the pharmacokinetic parameters for the three animals. The elimination of KNI-272 from plasma was rapid, with a mean CL of 2.5 liters/kg/h (approximately 800 ml/min/m²) and a mean ter-

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### TABLE 1. Pharmacokinetic parameters for KNI-272 in the nonhuman primate model

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Length of infusion (min)</th>
<th>( V_C ) (liter/kg)</th>
<th>( k_{cp} ) (h⁻¹)</th>
<th>( k_{pc} ) (h⁻¹)</th>
<th>( k_{el} ) (h⁻¹)</th>
<th>AUC (µM·h)</th>
<th>CL (liters/kg)</th>
<th>( V_{CSF} ) (liters/kg)</th>
<th>( t_{1/2a} ) (h)</th>
<th>( t_{1/2b} ) (h)</th>
<th>AUC (µM·h)</th>
<th>CSF/P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>608PR</td>
<td>5</td>
<td>0.72</td>
<td>0.14</td>
<td>0.18</td>
<td>0.2</td>
<td>0.059</td>
<td>0.09</td>
<td>0.07</td>
<td>0.08</td>
<td>0.05</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>CH980</td>
<td>68</td>
<td>0.31</td>
<td>1.31</td>
<td>3.2</td>
<td>0.068</td>
<td>0.085</td>
<td>0.79</td>
<td>0.14</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>CH957</td>
<td>85</td>
<td>0.25</td>
<td>2.5</td>
<td>0.9</td>
<td>0.24</td>
<td>0.09</td>
<td>0.08</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
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</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.43</td>
<td>5.0</td>
<td>3.9</td>
<td>3.2</td>
<td>0.90</td>
<td>0.47</td>
<td>0.90</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.25</td>
<td>2.1</td>
<td>0.9</td>
<td>1.0</td>
<td>0.10</td>
<td>0.43</td>
<td>0.90</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
</tr>
</tbody>
</table>

* KNI-272 was administered intravenously at 50 mg/kg; \( t_{1/2a} \) and \( t_{1/2b} \) distribution and elimination half-lives, respectively; CSF/P, ratio of AUC in CSF to AUC in plasma; the other abbreviations are defined in the text.
minal half-life of 0.54 h. There was considerable variability in the disposition of KNI-272 in plasma, as evidenced by the fivefold range in CL and nearly fourfold range in terminal half-life. The penetration of KNI-272 into the CSF was extremely limited. The level of drug exposure in CSF (AUC) was only 1% of that in plasma. The peak concentrations of KNI-272 in CSF ranged from 0.09 to 0.38 μM.

**Pediatric phase I trial.** After the 1-h intravenous infusion of KNI-272, the peak (end of infusion) concentration in plasma was 11 ± 8 μM at the 100-mg/m² dose level and 19 ± 5 μM at the 200-mg/m² dose level. The concentration in plasma declined rapidly to <0.1 μM by a median of 3 h after the start of the infusion for the 100-mg/m² dose level and 4 h for the 200-mg/m² dose level. The two-compartment pharmacokinetic model was fit to the concentrations in patients for five patients who received an intravenous dose of 200 mg/m². At the 100-mg/m² dose level, there were too few measurable concentrations in plasma for most patients for pharmacokinetic modeling. The pharmacokinetic parameters derived from administration of the intravenous dose are listed in Table 2. KNI-272 was rapidly eliminated in children. The mean CL was 276 ml/min/m², and the mean CL by a median of 3 h after the start of the infusion for the 100-mg/m² dose level and 4 h for the 200-mg/m² dose level. The two-compartment pharmacokinetic model was fit to the concentrations in patients for five patients who received an intravenous dose of 200 mg/m². At the 100-mg/m² dose level, there were too few measurable concentrations in plasma for most patients for pharmacokinetic modeling. The pharmacokinetic parameters derived from administration of the intravenous dose are listed in Table 2. KNI-272 was rapidly eliminated in children. The mean CL was 276 ml/min/m², and the terminal half-life was 0.44 h. Drug distribution was also limited. The mean $V_m$ was 0.064 liter/kg, and the mean $V_{SS}$ was 0.11 liter/kg.

The oral absorption of KNI-272 administered with Ora-Plus in children was limited and variable. In the five patients treated with the 200-mg/m² dose level, the mean absolute bioavailability was 12% (range, 3.5 to 25%; Table 2). The median time to the peak concentration after the administration of the oral dose was 0.5 h (range, 0.5 to 1.5 h). The maximum concentration of drug in plasma and AUC for patients treated with all four dose levels (Table 3) did not appear to increase in proportion to the increase in dose, suggesting that the absorption of KNI-272 was saturable in children. There was a trend toward higher AUCs after 12 weeks of continuous oral therapy compared with the initial measurement taken on either day 2 (100- and 200-mg/m² dose levels) or week 4 (330- and 500-mg/m² dose levels) of treatment, but this difference was not statistically significant ($P = 0.065$) for the 12 patients studied both initially and at week 12.

Three patients receiving dose level 2 developed an increase in hepatic transaminase levels that were between three and seven times the upper limit of normal. There was no apparent relationship between the pharmacokinetic parameters and the presence or severity of hepatotoxicity. No other dose-limiting toxicities were noted during this 12-week trial. There was no significant increase in the percentage or absolute numbers of CD4 cells and no significant decrease in serum p24 antigen or plasma HIV RNA levels.

**DISCUSSION**

Although the HIV protease inhibitor KNI-272 is active against HIV in vitro, its pharmacokinetic profile in vivo may limit its efficacy in treating patients with HIV disease. KNI-272 appeared to be rapidly eliminated from nonhuman primates and HIV-infected children, and as a result, the concentrations of KNI-272 in plasma are maintained above the EC₅₀ for less than half of the 6-h dosing interval used. The bioavailability of KNI-272 was also limited and appeared to be saturable over the dosage range studied in our phase I trial. Therefore, simply increasing the oral dose of KNI-272 is not likely to overcome the limited duration of exposure to concentrations exceeding the EC₅₀. The pharmacokinetic data suggest that a more frequent dose administration schedule may be required to provide continuous exposure to therapeutic concentrations of KNI-272.

KNI-272 is extensively protein bound (>98%) (12). In preclinical studies, the EC₅₀ increases from 0.004 μM under standard in vitro conditions which include 15% fetal calf serum to 0.1 μM in the presence of 80% fetal calf serum. This extensive protein binding of KNI-272 indicates that higher concentrations will be required in vivo to achieve antiviral effects similar to those demonstrated in vitro. The extensive protein binding may also account for its limited volume of distribution in our studies and contributes to its limited ability to penetrate across...
the blood-CSF barrier. Involvement of the central nervous system is an important and often devastating aspect of HIV infection, especially in children (4, 5). The limited penetration of KNI-272 into the CSF suggests that KNI-272, used as a single agent, may not be useful for treating HIV encephalopathy.

Four protease inhibitors (saquinavir, indinavir, ritonavir, and nelfinavir) are currently approved for use in the treatment of HIV-infected adults; ritonavir and nelfinavir are also approved for use in children (8, 16, 19, 22, 23). Limited absorption and difficulties in developing a liquid formulation have hampered the initial development of this class of agents, particularly for use in children. The rate of elimination of KNI-272 is more rapid than those of other HIV protease inhibitors, such as ritonavir, which has a terminal half-life of over 3 h (8). The bioavailability of KNI-272 is higher than that of saquinavir (4%) but lower than the relative bioavailability of indinavir sulfate (about 60%) (22, 24). Only limited data for pediatric subjects are available for ritonavir and indinavir, but the pharmacokinetic parameters are comparable in children and adults.

KNI-272 was rapidly absorbed in children. Peak concentrations were achieved 30 to 60 min after administration of the oral dose in children, and this is comparable to the time to the peak concentration in adults (11). However, the extent of absorption appeared to be lower in our group of pediatric patients (12%) than in adults (30%) (11).

In summary, the pharmacokinetics of KNI-272 in children are characterized by limited bioavailability, limited distribution (including poor penetration into the central nervous system), and rapid elimination, suggesting that the role of KNI-272 in the treatment of HIV-infected children may be limited.

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