2'-β-FLUORO-2',3'-DIDEOXYADENOSINE, LODENOSINE, IN Rhesus Monkeys: Plasma and Cerebrospinal Fluid Pharmacokinetics and Urinary Disposition

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(Received February 17, 1999; accepted June 16, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:
2'-β-Fluoro-2',3'-dideoxyadenosine (F-ddA, lodenosine) is a nucleoside analog that was rationally designed as a more chemically and enzymatically stable anti-AIDS drug than its parent compound 2',3'-dideoxyadenosine or didanosine. Plasma and cerebrospinal fluid (CSF) pharmacokinetics of this compound and its major metabolite, 2'-β-fluoro-2',3'-dideoxyinosine (F-ddI), were studied in three rhesus monkeys after a single 20 mg/kg dose administered as an i.v. push. F-ddA exhibited a mean residence time of 0.17 h in plasma and its plasma concentration time profile appeared to be biexponential. The majority of plasma exposure was from F-ddI, with a mean parent drug area under the curve (AUC) to metabolite AUC ratio of 0.16. CSF levels were low, with a mean CSF AUC to plasma AUC ratio of 0.068, with approximately one-quarter of this exposure in CSF due to unchanged drug. Urinary excretion accounted for half of the drug administered with the majority recovered as the metabolite, F-ddI. In a separate experiment, one monkey received a 20 mg/kg i.v. dose of F-ddI. The total dideoxynucleoside plasma exposure was greater than it was after administration of F-ddA; however, the CSF AUC to plasma ratio was a factor of 4 lower (0.017). Thus, F-ddA central nervous system penetration is at least comparable to that of didanosine, indicating that this experimental drug has potential as an addition to currently approved AIDS therapies.

2'-β-Fluoro-2',3'-dideoxyadenosine (lodenosine, F-ddA) is an experimental anti-AIDS drug that is currently undergoing adult and pediatric Phase I clinical trials at the National Cancer Institute. This synthetic nucleoside was rationally designed to have improved chemical and enzymatic stability compared with 2',3'-dideoxyadenosine (ddA), its parent compound, or ddA’s major metabolite, 2',3'-dideoxyinosine (didanosine or ddI; Marquez et al., 1987). The addition of an electrophilic fluorine in the 2' position of the dideoxyribose ring (Fig. 1) yields a compound that is acid stable and has a greater than 90% reduction in adenosine deaminase (ADA)-catalyzed hydrolysis compared with ddA (Marquez et al., 1987). F-ddA pharmacokinetics has been studied previously in other animals, and the CNS pharmacology of a variety of nucleoside analogs (didanosine, zidovudine, lamivudine, zalcitabine, and stavudine) currently approved by the Food and Drug Administration to treat HIV infection (Food and Drug Administration, 1998).

F-ddA has two potential advantages over didanosine for treating AIDS. Its acid stability makes it a much better candidate for oral administration. Dog studies have demonstrated better bioavailability compared with didanosine (Soltz et al., 1989). Also, F-ddA is more lipophilic than didanosine (Barchi et al., 1991), which may increase its ability to cross the blood-brain barrier. Penetration into the central nervous system (CNS) is critical in the treatment of patients with AIDS-related dementia (Gallo et al., 1987).

F-ddA pharmacokinetics has been studied previously in other animals, and the CNS pharmacology of a variety of nucleoside analogs has been investigated in our nonhuman primate model, but F-ddA has not been studied in primates. In earlier studies, F-ddA, administered to rats as a 2-h i.v. infusion, was rapidly converted to 2'-β-fluoro-2',3'-dideoxyinosine (F-ddI) with a plasma-concentration time profile exhibiting biexponential elimination (Singhal et al., 1996). Other studies investigated the effects of halo-substitutions on the cerebrospinal fluid (CSF) penetration of 2',3'-dideoxyguanosine (ddG) in monkeys. Administration of these halo-substituted compounds produced higher CSF to plasma ratios of ddG exposure compared with ddG administration; however, the produgs themselves were present at very low concentrations in the CSF so the mechanism of the improved penetration is unclear (Hawkins et al., 1995).

The purpose of the present study was to determine the pharmacokinetics and urinary excretion of F-ddA and its major metabolite, F-ddI, in a nonhuman primate. In addition, the extent of penetration into the CNS was investigated using the ratio of CSF area under the
curve (AUC) to plasma AUC as a measure of the relative exposure to these compounds. The results from these experiments are compared and contrasted with those of other nucleoside analogs as well as data from various animal models.

Materials and Methods

Chemicals and Reagents. F-ddA (NSC-613792, lodenosine) and F-ddI (NSC-616290) were supplied by the Pharmaceutical Resources Branch, National Cancer Institute (Bethesda, MD), and the ADA inhibitor, 2'-deoxycoformycin (NSC-218321) was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD). The internal standard, 2-chloroadenosine, was purchased from Sigma Chemical Co. (St. Louis, MO). HPLC grade methanol, acetonitrile, and water, as well as certified 1.00 N sodium hydroxide solution, were purchased from Fisher Scientific (Fairlawn, NJ). HPLC grade dimethyl sulfoxide (DMSO), used for preparation of stock solutions of analytes and internal standard, was procured from Aldrich Chemical Co. (Milwaukee, WI) and monobasic potassium phosphate used for buffer preparation was obtained from Mallinkrodt (St. Louis, MO). Phosphate-buffered saline (0.9% NaCl, pH 7.0) was purchased from Biofluids, Inc. (Rockville, MD).

Animals. Three adult male rhesus monkeys (Macaca mulatta) weighing from 4.5 to 10.3 kg were used for this study. Animals were fed National Institutes of Health Open Formula Extruded NonHuman Primate Diet ad libitum and were housed in groups in accordance with established guidelines (National Institutes of Health, 1996). The monkeys were dosed through either a saphenous catheter or jugular port. Blood was collected from a contralateral catheter or port. CSF was collected from a chronically indwelling s.c. Ommaya reservoir attached to a fourth ventricular Pudenz catheter (McCully et al., 1990). Urine was obtained by a catch collection sometime during the first 10 h after the experiment.

Experimental Design. F-ddA was dissolved in a small volume of DMSO by sonication and then sufficient saline solution was added to make the final dosing solution contain 25 to 33% DMSO. F-ddI was dissolved in normal saline. Both dosing solutions were filter-sterilized before administration to the animals. Three monkeys received F-ddA as a 20 mg/kg i.v. push that lasted 3 to 5 min. In addition, in a subsequent experiment, F-ddI was administered to one of the original animals as a 20 mg/kg 4-min i.v. push. Blood was collected at various times into heparinized Vacutainers (Becton Dickinson & Co., Franklin Lakes, NJ) and plasma was separated by centrifugation. CSF and urine were also collected. Plasma and urine had sufficient 2'-deoxycoformycin added to give a final concentration of 20 μM of the ADA inhibitor. Plasma, CSF, and urine samples were immediately frozen and stored until analysis. Monkeys were not sacrificed for tissue samples, but were returned to the monkey colony after the experiment was completed.

Analytical Methodology and Pharmacokinetic Analysis. Plasma concentrations of F-ddA and F-ddI were determined using a previously published method (Roth and Kelley, 1995). Plasma samples were treated the same, except an initial 0.25-ml aliquot was used. Urine samples were sonicated for 10 min after thawing and then diluted by a factor of 200 before processing.

Values for F-ddA plasma concentrations versus time were curvefit to a biexponential equation with 1/C p 2 weighting using the Prism program (GraphPad Software Inc., San Diego, CA). The F-ddI metabolite levels from the last several points for both F-ddA and F-ddI doses were fit to a monoexponential decay to determine the elimination half-life using the same program. Pharmacokinetic parameters were then calculated by standard methods. Plasma and CSF AUC were determined using the linear trapezoid rule. There was no extrapolation after the final time point for F-ddA in either plasma or CSF. For F-ddI levels, after either the F-ddA or F-ddI dose, the remainder of the AUC was extrapolated to infinity by adding the term 1.44 3 C 3 T 1/2 (b), in which C is the last measured concentration in plasma or CSF and T 1/2 (b) is the terminal phase half-life (Gibaldi and Perrier, 1982). These calculations were performed using a BASIC program written in our laboratory.

Results

Pharmacokinetics after F-ddA Dose. Plasma and CSF kinetics after a 20 mg/kg dose of F-ddA were determined in three male rhesus monkeys with Ommaya reservoirs. The plasma concentration versus time curves from these experiments are shown in Fig. 2. The F-ddA levels appeared to be described by a two-compartment model with a correlation coefficient from a fit to a biexponential decay better than...
0.999 in each case. However, the F-ddA elimination half-lives could not be accurately determined because this portion of the curve was defined by only a few points near the limit of quantitation. Rather than include a parameter with so great an uncertainty, a noncompartmental value, the mean residence time (MRT), is reported instead. A summary of pharmacokinetic values from the three monkeys is shown in Table 1. F-ddA was rapidly metabolized to F-ddI with a total body clearance ranging from 106 to 156 ml/min/kg and MRTs ranging from 0.12 to 0.21 h for the three monkeys. F-ddI accounted for the majority of plasma exposure and represented 84 to 89% of the total AUC on a molar basis. F-ddA and F-ddI were both detected in the CSF with F-ddA accounting for a much higher percentage of dideoxynucleoside exposure than in the plasma, ranging from 12 to 36% of total AUC. The mean combined CSF-to-plasma ratio of F-ddA and F-ddI based on F-ddA equivalents was 7%.

**Pharmacokinetics after F-ddI Dose.** Plasma and CSF kinetics after a 20 mg/kg dose of F-ddI were also determined in one of the original rhesus monkeys used for the F-ddA experiment. The plasma time versus concentration curve fit well to a biexponential decay as can be seen in Fig. 3B. The elimination half-lives for F-ddI from plasma and CSF were similar to that obtained after the F-ddA dose. However, the CSF/plasma AUC ratio was much lower at only 1.7% instead of 6.0% for the same monkey after F-ddA administration (Table 2).

**Fig. 2.** Plasma concentration versus time profiles for F-ddA (●) and F-ddI (■) in three rhesus monkeys after 20 mg/kg i.v. push of F-ddA.

Solid lines are computer fits of F-ddA data to biexponential decay and dashed lines are fits of final F-ddI points to a monoexponential decay.

**Fig. 3.** Plasma and CSF concentration versus time profiles in monkey 3 after 20 mg/kg i.v. push of F-ddA (A) or F-ddI (B).

F-ddA in plasma (●), F-ddI in plasma (■), F-ddA in CSF (□), and F-ddI in CSF (□). Solid lines have been computer fit and dashed lines are point to point.

**Urinary Disposition.** Urine was collected for each of the monkeys after the F-ddA dose for several hours and analyzed for both F-ddA and F-ddI. About half of the total dose was accounted for by urinary excretion and the majority of the dose was excreted as F-ddI. A summary of the data appears in Table 3.

**Discussion**

F-ddA pharmacokinetics in monkeys is similar to that of its non-fluorinated predecessors, ddA and didanosine, with the important exception of its much greater stability. A previous study in which rhesus monkeys were dosed with ddA showed such rapid disappear-

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**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma*a</th>
<th>CSF*a</th>
</tr>
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<tbody>
<tr>
<td>F-ddA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (µg/ml·h)</td>
<td>2.64 ± 0.45</td>
<td>0.30 ± 0.12</td>
</tr>
<tr>
<td>ClTB (ml/min/kg)</td>
<td>126 ± 26</td>
<td>0.62 ± 0.08</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.172 ± 0.045</td>
<td>15.2 ± 5.8</td>
</tr>
<tr>
<td>C max (µg/ml)</td>
<td>17.3 ± 2.1</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>F-ddI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (µg/ml·h)</td>
<td>16.7 ± 1.5</td>
<td>1.0 ± 0.45</td>
</tr>
<tr>
<td>T 1/2 elim (h)</td>
<td>0.94 ± 0.20</td>
<td>0.95 ± 0.28b</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.14 ± 0.25</td>
<td>1.07 ± 0.28</td>
</tr>
<tr>
<td>C max (µg/ml)</td>
<td>17.3 ± 2.1</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>Combined (F-ddA &amp; F-ddI) AUC:F-ddA/F-ddI ratio</td>
<td>0.16</td>
<td>0.30</td>
</tr>
<tr>
<td>AUC:CSF/plasma ratioc</td>
<td>0.068 ± 0.023</td>
<td></td>
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*a Mean value ± S.D. (n = 3).

b Apparent elimination constant (intercompartmental transfer rate unknown).

c Total AUC from compound and metabolite in F-ddA equivalents.
Plasma and CSF pharmacokinetics in rhesus monkey 3 F-ddA versus F-ddI

Interspecies scale-up has been used successfully for two designing initial dosage regimens in human clinical trials (Obach et al., 1995). Gained importance as interspecies scaling has been shown useful in degradation is not a major factor in the plasma clearance of didanosine is comparable with the results for F-ddI. This implies that the PNP (Ravasco et al., 1992; Odinecs et al., 1996) or rhesus monkeys 1990). Several studies of i.v. didanosine in pigtailed macaques F-ddATP, intracellularly (Hitchcock et al., 1990; Masood et al., 1995). By contrast, the MRT for F-ddA ranged from 1.5 to 2.0 h and for F-ddI from 1.8 to 2.9 h. These results are similar to the data presented here for rhesus monkeys. In addition, results from rat studies have demonstrated that animals treated with 120-min i.v. infusions of either ddI, F-ddI, or F-ddA demonstrated much more rapid clearance of ddI relative to F-ddA or F-ddI, as would be expected. However, there was a much longer terminal half-life for F-ddA in rats than in dogs or monkeys (Singhal et al., 1997). It is unclear why the rat data differ from the other two animals, but earlier experiments had indicated that monkeys are a good model for didanosine in humans (Ravasco et al., 1992). It is interesting to note that the deamination kinetics of F-ddA were determined in fresh rat and monkey plasma with a resulting half-life for each of 4 to 5 h (Roth and Kelley, 1995). This indicates that the difference in vivo results is probably not due to a simple difference in the plasma ADA levels.

Because the brain is a sanctuary for HIV and AIDS dementia is a devastating manifestation of HIV infection (Gallo et al., 1987), CNS penetration is an important consideration for anti-AIDS drugs. Although lipophilicity and plasma protein binding are generally predictive of CNS entry, a study in pyrimidine dideoxynucleosides indicated that these factors were not sufficient to explain the differences among several members of this group. It was suggested that an unknown carrier-mediated process was a major factor (Collins et al., 1988). Because entry into the CNS has been poor for other dideoxy-nucleosides, with studies on didanosine in rhesus monkeys yielding only a 4.8% CSF-plasma ratio (Hawkins et al., 1995), it was predicted the more lipophilic F-ddA would lead to improved penetration of the blood-brain barrier. In fact, previous investigations in rats had demonstrated improved CNS delivery of total dideoxynucleosides when animals were dosed with F-ddA compared with didanosine. AUC brain-plasma ratios increased from 3.8% for ddI to 12.1% for F-ddA and AUC CSF-plasma ratios increased from 1.4 to 9.1%, respectively (Singhal et al., 1997). However, the AUC CSF-plasma ratio for F-ddA in monkeys was similar to that of didanosine at 6.8 versus 4.8%. Perhaps this is due to an unknown carrier-mediated process similar to the case of the pyrimidine nucleosides. Also, in vitro studies using bovine brain tissue suggest that the levels of ADA and PNP in the case of the pyrimidine nucleosides. Perhaps this is due to an unknown carrier-mediated process similar to the case of the pyrimidine nucleosides. Also, in vitro studies using bovine brain tissue suggest that the levels of ADA and PNP in several species of this group. It was suggested that an unknown carrier-mediated process was a major factor (Collins et al., 1988). Because entry into the CNS has been poor for other dideoxy-nucleosides, with studies on didanosine in rhesus monkeys yielding only a 4.8% CSF-plasma ratio (Hawkins et al., 1995), it was predicted the more lipophilic F-ddA would lead to improved penetration of the blood-brain barrier. In fact, previous investigations in rats had demonstrated improved CNS delivery of total dideoxynucleosides when animals were dosed with F-ddA compared with didanosine. AUC brain-plasma ratios increased from 3.8% for ddI to 12.1% for F-ddA and AUC CSF-plasma ratios increased from 1.4 to 9.1%, respectively (Singhal et al., 1997). However, the AUC CSF-plasma ratio for F-ddA in monkeys was similar to that of didanosine at 6.8 versus 4.8%. Perhaps this is due to an unknown carrier-mediated process similar to the case of the pyrimidine nucleosides. Also, in vitro studies using bovine brain tissue suggest that the levels of ADA and PNP in the case of the pyrimidine nucleosides. Perhaps this is due to an unknown carrier-mediated process similar to the case of the pyrimidine nucleosides. Also, in vitro studies using bovine brain tissue suggest that the levels of ADA and PNP in the case of the pyrimidine nucleosides. Perhaps this is due to an unknown carrier-mediated process similar to the case of the pyrimidine nucleosides. Also, in vitro studies using bovine brain tissue suggest that the levels of ADA and PNP in the case of the pyrimidine nucleosides. Perhaps this is due to an unknown carrier-mediated process similar to the case of the pyrimidine nucleosides. Also, in vitro studies using bovine brain tissue suggest that the levels of ADA and PNP in the case of the pyrimidine nucleosides. Perhaps this is due to an unknown carrier-mediated process similar to the case of the pyrimidine nucleosides. Also, in vitro studies using bovine brain tissue suggest that the levels of ADA and PNP in the case of the pyrimidine nucleosides.
tially valuable component as a substitute in multiple drug therapy. In addition, due to its acid stability, F-ddA may be administered orally without the use of antacids or buffers and still retain good bioavailability. Therefore, given that its penetration into the CNS in this monkey model is at least comparable with that of didanosine, F-ddA remains as a potential candidate for addition to the current arsenal of therapeutic agents in the treatment of AIDS.

Acknowledgments. We thank Dr. Harry Ford for his many helpful comments and discussions of this work.

References


Food and Drug Administration (1998) Antiretroviral HIV drug approvals and pediatric labeling comments and discussions of this work.


