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Antiviral, Metabolic, and Pharmacokinetic Properties of the Isomeric Dideoxynucleoside 4(*S*)-(6-Amino-9H-Purin-9-yl)Tetrahydro-2(*S*)-Furanmethanol

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4(*S*)-(6-Amino-9H-purin-9-yl)tetrahydro-2(*S*)-furanmethanol (IsoddA) is the most antivirally active member of a novel class of optically active isomeric dideoxynucleosides in which the base has been transposed from the natural 1′ position to the 2′ position and the absolute configuration is (*S*,*S*). IsoddA was active against human immunodeficiency virus type 1 (HIV-1) (strain IIIB), HIV-2 (strain ZY), and HIV-1 clinical isolates. Combinations of the compound with zidovudine (3′-azido-3′-deoxythymidine), 2′,3′-dideoxyinosine, or 5-fluoro-2′-deoxy-3′-thiacytidine showed synergistic inhibition of HIV. A moderate reduction of activity was observed with clinical isolates resistant to zidovudine. An IsoddA-resistant virus (eightfold-increased 50% inhibitory concentration) was selected in vitro by repeated passage of HIV-1 (HXB2) in the presence of increasing concentrations of IsoddA. The reverse transcriptase-coding region of the mutant virus contained a single base change resulting in a change at codon 184 from Met to Val. IsoddA was also active against hepatitis B virus (HBV) in vitro; however, it lacked substantial selective activity in an in vivo HBV model. IsoddA was inefficiently phosphorylated in CEM cells; however, the half-life of the triphosphate was 9.4 h, and IsoddATP was a potent inhibitor of HIV-1 reverse transcriptase, with a K_i of 16 nM. The cytotoxicity 50% inhibitory concentrations of IsoddA were greater than 100 μM for CEM, MOLT-4, IM9, and the HepG2-derived HBV-infected 2.2.15 (subclone P5A) cell lines but were 12 and 11 μM for human granulocyte-macrophage (CFU-GM) and erythroid (BFU-E) progenitor cells, respectively. When given orally to rats and mice, the compound was very well absorbed and rapidly eliminated. However, there was no detectable brain penetration by IsoddA in rats. Catabolic metabolites were not detected, and this is consistent with the observed resistance of the compound to metabolic degradation by adenosine deaminase.

Many modified nucleosides have been found to have antiviral activity against human immunodeficiency virus (HIV). 2′,3′-Dideoxynucleosides have shown remarkable activity against HIV (6, 7, 10, 14, 26–29, 36, 39), and compounds of this class (zidovudine [3′-azido-3′-deoxythymidine] [AZT], 2′,3′-dideoxycytidine [ddC], 2′,3′-dideoxyinosine [ddI], and 3′-deoxy-2′,3′-didehydrothymidine) are currently in clinical use. All of these compounds have limitations as anti-HIV agents, including toxicity, development of resistant strains upon prolonged clinical use, and cross-resistance with other nucleoside analogs (11, 24, 34, 43). For this reason, the discovery of new and distinctly different nucleosides is of considerable importance.

We have designed a new class of dideoxynucleosides that possess anti-HIV activity, glycosidic bond stability, and enzymatic stability with respect to catabolic enzymes. The nucleobase has been transposed from the natural 1′ position to the 2′ position, while the *cis* relationship of the base and the $-CH_2OH$ group has been maintained. The most interesting of the resulting isomeric nucleosides with respect to antiviral activity was 4(*S*)-(6-amino-9H-purin-9-yl)tetrahydro-2(*S*)-furanmethanol (IsoddA) (191W91) (Fig. 1). The synthesis of this compound has been reported by us, and its absolute stereochemistry has been established by X-ray crystallography (4, 30). The enantiomer of this compound has been synthesized and discussed elsewhere (18). Isomeric dideoxynucleosides related to IsoddA and its enantiomer have also been synthesized but have not been found to possess significant antiviral activity (31, 38). In this paper we describe our evaluation of IsoddA as an anti-HIV and/or anti-hepatitis B virus (anti-HBV) agent.

MATERIALS AND METHODS

Compounds. The syntheses of enantiomerically pure (*S*,*S*)-isodideoxyadenosine and (*S*,*S*)-isodideoxyinosine from D-xylose have been reported previously by Nair and coworkers (4, 30). [^{3}H]IsoddA (0.730 and 12.6 Ci/mmol) was prepared from IsoddA by Moravek Biochemicals (Brea, Calif.). IsoddA triphosphate (IsoddATP) was prepared by phosphorylation of IsoddA in triethylphosphate with phosphorus oxychloride followed by condensation of the initial product with tetrabutylammonium diphosphate. IsoddATP (as its triethylammonium salt) was purified by ion-exchange chromatography on DEAE-Sephadex followed by reversed-phase high-pressure liquid chromatography (HPLC) and was characterized by multinuclear nuclear magnetic resonance and UV data. Many commercially available reagents, enzymes, and cell lines were used in this investigation, and they are described in the discussions of the various methods below.

Antiviral assays. The anti-HIV activities of compounds were assessed with the human T-cell lymphotropic virus type 1-transformed cell line MT4 infected with HIV type 1 (HIV-1) (strain IIIB) or HIV-2 (strain ZY) as described previously (9). Experiments using IsoddA in combination with AZT, β-L-5-fluoro-2′-deoxy-3′-thiacytidine (β-L-FTC), or ddI were also performed by using the MT4 cell assay as described previously (12). Fractional inhibitory concentrations were calculated, and isobolograms were constructed at 50% inhibition.

The anti-HIV activities of compounds were also assessed with Ficoll-sepa-

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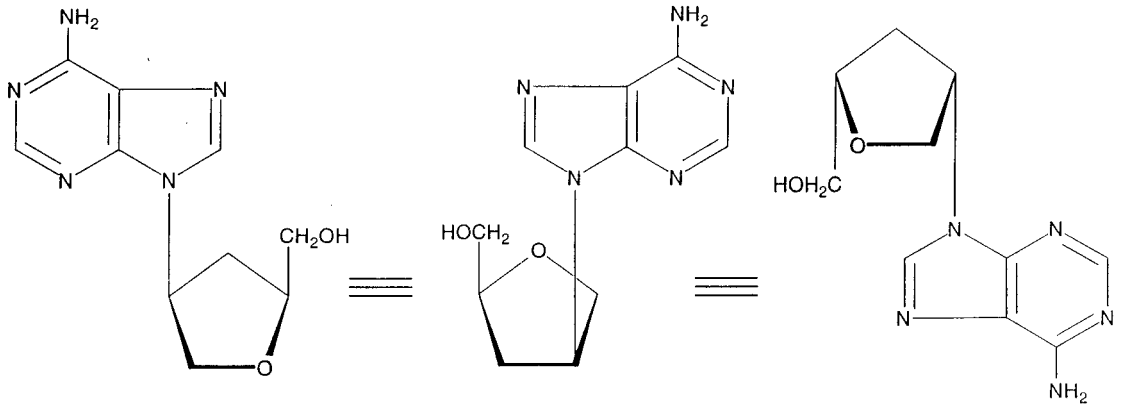

FIG. 1. Several representations of the structure of IsoddA.

rated, phytohemagglutinin-stimulated peripheral blood lymphocytes (PBLs) that were infected with HIV-1 (strain IIIB) or various AZT-sensitive or AZT-resistant HIV clinical isolates, as described previously (9).

The P5A passage of HBV-producing 2.2.15 cells (37) derived from transfected HepG2 cells was generously provided by B. Korba (Georgetown University). The in vitro anti-HBV activity of IsoddA was determined as previously described (19). The in vivo anti-HBV activity of IsoddA was determined in a murine chemotherapy model as previously described (8). In this system, mice are implanted subcutaneously with 2.2.15 cells 1 week prior to the initiation of treatment. After tumor formation was confirmed, IsoddA treatment was begun via the drinking water at the concentrations indicated in Results and was continued for 3 weeks. Each treatment group contained a minimum of five mice. Drug exposure was confirmed by measurement of water consumption. At the end of the treatment period, mice were sacrificed and the effects of treatment were evaluated as previously described (8).

In vitro selection of IsoddA-resistant HIV-1. Selection of HIV-1 resistant to IsoddA was achieved by the in vitro passage strategy of Larder et al. (23). Briefly, 1 ml of cell-free culture medium from each virus harvest was mixed with 1 ml of MT4 cells (2×10^6 cells per ml, <0.1 PFU per cell) in 90% RPMI–10% fetal bovine serum–20 μg of gentamicin per ml–4 μg of Polybrene per ml, and virus was allowed to adsorb for 2 h. The cells and virus were then diluted 10-fold in the same medium minus Polybrene and cultured in the presence or absence of IsoddA until an HIV-induced cytopathic effect was evident, usually after 6 to 8 days. The concentration of IsoddA was held constant or increased twofold at each passage, and the highest concentration showing a complete cytopathic effect was used for the next passage. Cell-free virus was stored in aliquots, frozen at −70°C, for subsequent passage and determinations of 50% inhibitory concentrations (IC_{50}s). Sensitivity assays were performed in a monolayer assay for HIV-1 infection with $CD4^+$ HeLa cells expressing the *Escherichia coli lacZ* gene controlled by the HIV-1 long terminal repeat (HeLa-β-gal) (20).

Nucleotide sequence analysis of RT by sequencing of PCR products. HIV-1 (HXB2) derived from the molecular clone pHXB2-D (13) was used in these studies. IsoddA-resistant HIV-1 was selected for as described above, except that the initial passage of virus was at 60 μM drug and the drug concentration was increased to 240 μM by passage 4. Cell-free virus supernatants were prepared at passage 4 for sensitivity testing by plaque reduction in HT4LacZ-1 cells as previously described (22). The IsoddA sensitivity of an FTC- and 2′-deoxy-3′-thiacytidine (3TC)-resistant mutant (M-184→V [M184V]) constructed by site-directed mutagenesis of the reverse transcriptase (RT)-coding region in pHXB2-D (13) was also determined. DNA was extracted from HIV-infected MT-4 cells at each passage, and 795 bp of the RT-coding region (amino acid 1 to 265) was amplified by PCR. The product was sequenced from PCR products, using an ABI automated sequencer, between codons 40 and 225 by using specific oligonucleotide primers as described previously (21).

Cell culture cytotoxicity assays. The MOLT-4 and CCRF CEM T-cell lymphoblastic leukemia cell lines and the IM9 B-cell leukemia cell line were obtained from the American Type Culture Collection. The cells were maintained in exponential growth in RPMI 1640 supplemented with 10% fetal bovine serum and 1μg of gentamicin per ml at 37°C in a 5% CO_2 humidified atmosphere. For growth inhibition assays (33), cells (4×10^4 cells per ml in 96-well plates) were incubated for 96 h in the presence of various concentrations of IsoddA. Inhibition of growth was determined by measuring cellular DNA with the propidium iodide assay (3).

Human bone marrow progenitor cell growth assays. The assay of inhibition of human bone marrow progenitor colony formation was performed according to a previously published method (12). Values for IsoddA and AZT were determined in three separate experiments using cells from three different donors. Values were reported as means ± standard error.

Phosphorylation studies. CCRF CEM cells were maintained as described above. Log-phase cells were seeded into duplicate or triplicate T-75 flasks at approximately 2×10^5 cells per ml (total, 5×10^6 cells). Twenty-four hours later, [^{3}H]IsoddA was added at a final concentration of 0.1, 1.0, or 10 μM, and the cultures were incubated for up to 24 h. For samples used in the investigation of the half-life of IsoddATP, the cells were washed twice with 5 ml of phosphate-buffered saline at 37°C and then incubated in medium containing no drug for an additional 6 to 25 h. Cell extracts and samples of the media were prepared for HPLC analysis as described previously (32).

The medium ultrafiltrates and cellular extracts were analyzed by reversed-phase HPLC on a Partisphere octadecylsilica column (4.7 by 235 mm; Whatman, Maidstone, England). Samples were eluted at a flow rate of 1 ml/min in 50 mM ammonium acetate (pH 5.4) with a linear gradient from 0 to 36% acetonitrile over 30 min. Fractions of the column effluent were collected at 1-min intervals, and the radioactivity was quantitated by liquid scintillation counting in 5 ml of Ready Safe (Beckman, Fullerton, Calif.). The cellular extracts were further analyzed by ion-exchange HPLC as described previously (32).

DNA polymerase assays. [8^{-3}H(N)]dATP (18.8 Ci/mmol) was from Dupont-New England Nuclear. DNA polymerases α, β, and γ were purified and characterized as described previously (25). P. A. Furman and P. Ray of Burroughs Wellcome Co. supplied RT from the HXB2 strain of HIV-1. Inhibition constants for IsoddATP with all of the polymerases, with activated calf thymus DNA as the nucleic acid substrate, were determined as described previously (25).

Adenosine deaminase assay. The kinetic constants for IsoddA were determined from initial velocity measurements by using calf intestinal adenosine deaminase as previously described (41). The change in the extinction coefficient for the conversion of IsoddA to IsoddI was determined to be 4.7 mM^{-1} cm^{-1} at 240 nm. The product was identified by its UV spectrum.

Pharmacokinetics of IsoddA. The plasma pharmacokinetics (PK), urinary recovery (UR), and brain penetration (BP) of IsoddA were studied in male CD rats weighing 275 g (PK and UR studies) or 85 g (BP studies) (Charles River Breeding Laboratories, Wilmington, Mass.). The UR of the drug was also determined with male CD-1 mice (Charles River) weighing 40 g. The animals were housed in individual metabolic cages (Nalge Co., Rochester, N.Y., and Lab Products Inc., Rockville, Md.), which separated urine from feces. Standard laboratory food and water were provided ad libitum, except in PK studies, in which there was fasting 12 h prior to dosing and for a period of 4 h following dosing. The Burroughs Wellcome Co animal management program is accredited by the American Association for the Accreditation of Laboratory Animal Care and meets National Institutes of Health standards (31a).

IsoddA, dissolved in deionized water (5 to 10 mg/ml), was administered orally to rats at a dose of 25 mg/kg. There were three rats in the PK-UR study and three rats per time point in the BP study. In the mouse UR studies, three groups of mice (n = three per group) were each given 25 mg of IsoddA per kg intravenously, and another three groups were each given the same dose orally (1.5 mg of drug per ml dissolved in 0.9% saline).

Serial blood samples were obtained from rats through an external jugular cannula (45) at 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, and 24 h after drug administration. Plasma samples were prepared by centrifugation and were then ultrafiltered with the Centrifree micropartition system (Amicon, Danvers, Mass.) to remove protein. Rat and mouse urine was collected during the 0- to 24- and 24- to 48-h intervals postdose into tubes containing sodium azide. After each cage was rinsed, volumes were recorded and samples were filtered to remove particulates. For BP studies, CO_2-anesthetized rats were decapitated at 0.25, 0.5, 1, 3, and 5 h postdose. Blood was collected from the cervical wound for preparation of plasma, and the entire brain was immediately excised and frozen on solid CO_2. The brains were homogenized in cold deionized water (5 ml/g) and centrifuged ($1{,}900 \times g$, 15 min), and the supernatants were ultrafiltered. The plasma protein

binding of 50 μM IsoddA was estimated from the ratio of drug concentrations in the ultrafiltrates from samples of drug plus plasma to those from samples of drug plus protein-free plasma.

Urine and ultrafiltrates of plasma and brain were analyzed by reversed-phase HPLC on a Microsorb C_{18} column (4.6 mm [inner diameter] by 25 cm; Rainin Instrument Co., Woburn, Mass.). The UV absorbance of the column eluate was monitored at 260 and 275 nm. Samples were eluted at a flow rate of 1 ml/min in 50 mM ammonium acetate–0.1% triethylamine (pH 5.4) with a linear gradient of 0 to 36% acetonitrile over 30 min. Compounds were judged to be identical to authentic standards on the basis of HPLC retention times and UV spectra. The lower limit of quantitation was 0.5 μM.

Estimates of the elimination half-life and the area under the plasma concentration-time curve were obtained by standard noncompartmental pharmacokinetic analyses. The mean and standard deviation were calculated with SAS version 6.06 (SAS Institute, Cary, N.C.).

RESULTS

Anti-HIV activity in an acute-infection assay with MT4 cells and PBLs. The mean IC_{50} of IsoddA against HIV-1 in MT-4 cells was 26 ± 1 μM (n = 4). The IC_{50} against HIV-2 was 3.4 ± 0.9 μM. In PBLs, the IC_{50} against HIV-1 was 0.7 ± 0.2 μM. The activity against clinical isolates of HIV (three AZT-sensitive and three AZT-resistant isolates) was also measured. The IC_{50}s of IsoddA with AZT-sensitive isolates were 13 ± 2, 7 ± 3, and 1.3 ± 0.4 μM (mean IC_{50}, 7.1 μM), whereas the IC_{50}s with AZT-resistant isolates were 40 ± 30, 31 ± 8, and 10 ± 2 μM (mean IC_{50}, 27 μM). In comparison, the IC_{50}s of AZT with AZT-sensitive isolates averaged 0.2 μM, while the resistent isolates gave AZT IC_{50}s of greater than 10 μM. Combinations of IsoddA with AZT, ddI, or β-L-FTC were synergistic (Fig. 2).

In vitro selection of HIV-1 resistant to IsoddA. HIV-1 derived from the molecular clone HXB2 was passaged in MT4 cells in the presence of IsoddA to select for resistant virus. Passage began at 8 μM IsoddA, and the drug concentration was increased twofold or held constant at subsequent passages. After eight passages, HIV-1 was able to replicate in the presence of 128 μM IsoddA. The IC_{50} of IsoddA with passaged virus, determined by the HeLa MAGI assay, was approximately eightfold higher than that with the wild-type HXB2.

Nucleotide sequence analysis of resistant virus. Genetic analysis of RT was carried out by sequencing PCR products. DNA was extracted from HIV-infected MT4 cells after each passage in the presence of IsoddA, and 795 bp of the RT-coding region (amino acids 1 to 265) was sequenced. After four passages, the IC_{50} of IsoddA was elevated approximately fourfold to 38.7 and 46.4 μM (duplicate determinations). A single base change in codon 184, from ATG to GTG, that predicted an amino acid change from Met to Val (M184V) was observed at passage 4. Consistent with this finding, the IC_{50} of IsoddA with the M184V mutant virus clone (44) was 46 and 33 μM (duplicate determinations).

In vitro anti-HBV activity of IsoddA. The activity of IsoddA against HBV was assessed with HBV-producing 2.2.15 cells (P5A passage). Evaluations of cell growth inhibition and anti-HBV activity were made by comparisons with untreated-cell controls. Results from two separate experiments were acquired. The IC_{50}s for cell growth inhibition (cytotoxicity) were 123 and 172 μM, whereas HBV inhibition IC_{50}s were 3.7 and 3.1 μM. Thus, IsoddA was a selective inhibitor of HBV in this in vitro system.

In vivo anti-HBV activity of IsoddA. The ability of orally administered IsoddA to inhibit HBV replication in mice was tested in two experiments. IsoddA, administered at a dose of 1 mg/ml in the drinking water (approximately 180 mg/kg/day), reduced the quantity of circulating HBV to 25% (± 20%) of the quantity observed in untreated mice. However, the treated mice had reduced hematocrits and appeared lethargic, suggesting some toxicity at this dose. When administered at 0.2 mg/ml in the drinking water, IsoddA did not significantly reduce circulating levels of HBV. Thus, IsoddA was a minimally selective anti-HBV agent in this in vivo chemotherapy model.

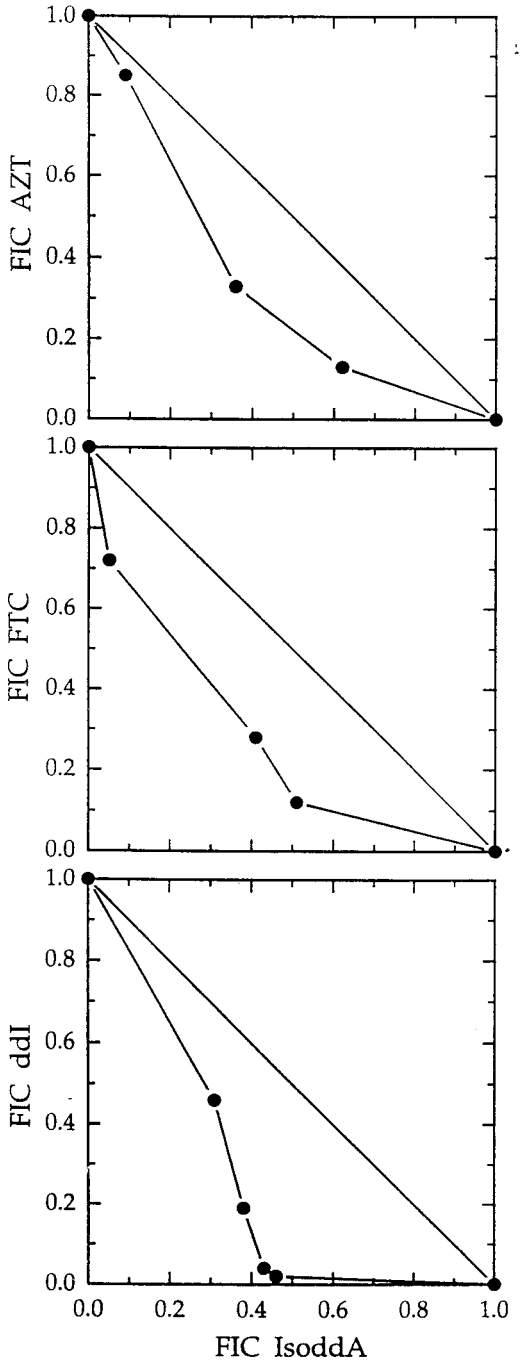

FIG. 2. Isobolograms for combinations of IsoddA with AZT, FTC, and ddI. Experiments were performed as described in Materials and Methods. FIC, fractional inhibitory concentration.

In vitro cytotoxicity. IsoddA at 100 μM did not significantly inhibit growth of the human leukemic cell lines CEM, MOLT-4, and IM9. In contrast, IsoddA did potently inhibit the growth of human bone marrow progenitor cells. IC_{50}s of IsoddA for CFU-granulocyte-macrophage (CFU-GM) and

TABLE 1. Intracellular metabolites of [^{3}H]IsoddA in CEM cells[a]

IsoddA concn[b] (μM)	Incubation time (h)	Amt of intracellular metabolite (fmol/10^6 cells)					
		ADP	IsoddADP	GDP	ATP	IsoddATP	GTP
1.0[c]	3	0.9	0.1	0.2	1.0	0.2	0.2
1.0[c]	24	5.7	0.4	0.3	2.6	0.4	0.5
10.0[d]	24	10	6	8	23	4	7

[a] Experiments were performed as described in Materials and Methods.
[b] The intracellular levels of unchanged IsoddA were 86% of the total intracelluular tritium level.
[c] The specific radioactivity of [^{3}H]IsoddA was 12.6 Ci/mmol.
[d] The specific radioactivity of [^{3}H]IsoddA was 0.73 Ci/mmol.

burst-forming unit-erythroid (BFU-E) cells were 12 ± 8 and 11 ± 7 μM, respectively. By comparison, the IC_{50}s of AZT were 7 ± 1 and 0.4 ± 0.2 μM, respectively.

In vitro anabolism of IsoddA. IsoddATP was measurable in cells incubated with 1 or 10 μM [^{3}H]IsoddA. The intracellular concentration of each metabolite is shown in Table 1. The results demonstrate that the intracellular concentration of IsoddATP depended on the concentration of IsoddA added to the cells and on the time of incubation. The concentrations of [^{3}H]IsoddA in the medium and inside the cells were approximately equal, indicating that the low level of phosphorylation was not due to inefficient transport (data not shown). Because of limitations in the supply of the [^{3}H]IsoddA, only three time points could be used to determine the half-lives of IsoddADP and IsoddATP. The values were 11.5 and 9.4 h, respectively. After 24 h of incubation with 10 μM IsoddA, 10.5% of the intracellular radioactivity and 0.6% of the radioactivity in the medium appeared to be IsoddI (on the basis of the UV spectrum and the retention time on reversed-phase HPLC). The concentration of IsoddI in the stock solution of [^{3}H]IsoddA was 0.1%.

Figure 3 shows the HPLC elution profile of intracellular metabolites in CEM cells following incubation with 1.0 μM [^{3}H]IsoddA. A radiolabeled peak coeluted with the UV peak for IsoddATP. A tritium-labeled metabolite eluting prior to IsoddATP coeluted with ATP. Because a standard sample of IsoddITP was not available, we were unable to conclusively determine whether this metabolite was ATP or IsoddITP. This metabolite was tentatively identified as ATP. A third radiolabeled metabolite coeluted with GTP. ATP and GTP would be expected to be produced in these relative proportions if a trace of adenine or hypoxanthine was released from IsoddA or IsoddI, respectively, and incorporated by the purine salvage pathway. Finally, IsoddAMP and IsoddADP were also detected in the chromatogram (Fig. 3).

Inhibition of HIV-1 RT and human DNA polymerases by IsoddATP. Inhibition constants for IsoddATP and ddATP with human DNA polymerases α, β, and γ and HIV-1 RT were determined with activated calf thymus DNA as the nucleic acid substrate under identical conditions of pH, ionic strength, and divalent metal ion concentration. IsoddATP and ddATP were equally potent competitive inhibitors of HIV-1 RT, with K_i values of 16 ± 3 and 18 ± 2 nM, respectively. In contrast, significant differences were observed with the human enzymes. ddATP was a potent inhibitor of polymerases β and γ, with K_i values of 1.1 ± 0.2 and 0.018 ± 0.002 μM, respectively, whereas it was a relatively weak inhibitor of polymerase α, with a K_i value of 64 ± 8 μM (25). IsoddATP was a relatively weaker inhibitor of polymerases β and γ, with K_i values of 18 ± 2 and 0.36 ± 0.06 μM, respectively, whereas it was a more potent inhibitor of polymerase α, with a K_i value of 0.63 ± 0.08 μM.

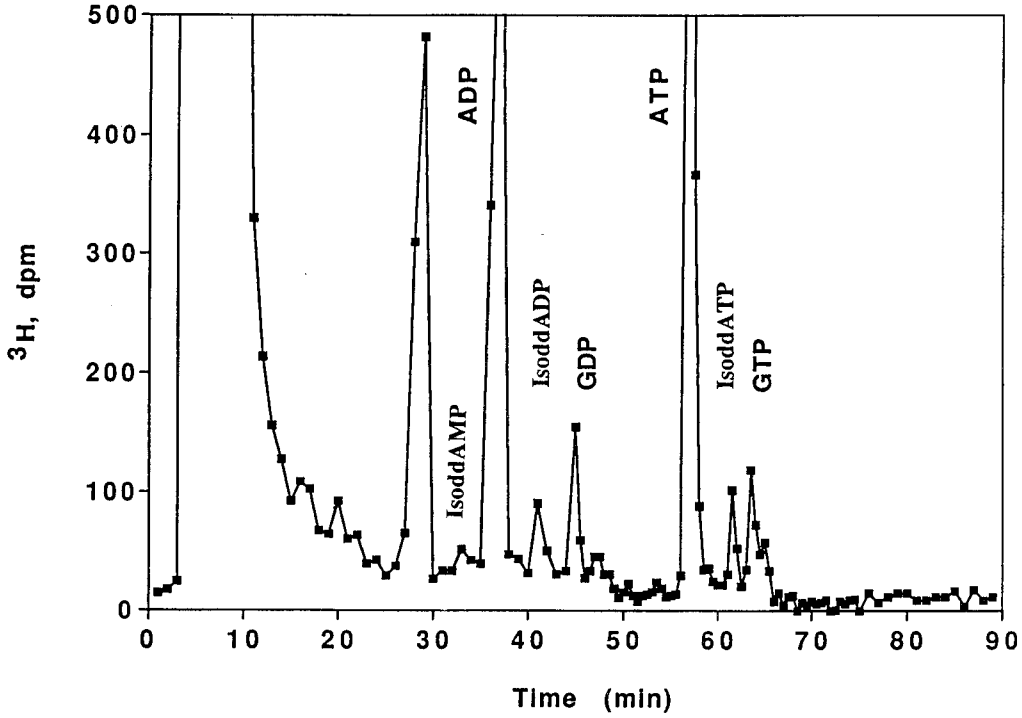

FIG. 3. Strong anion exchange HPLC chromatogram of an intracellular extract of CEM cells following incubation with 1 μM [^{3}H]IsoddA for 24 h. The experiment was performed as described in Materials and Methods.

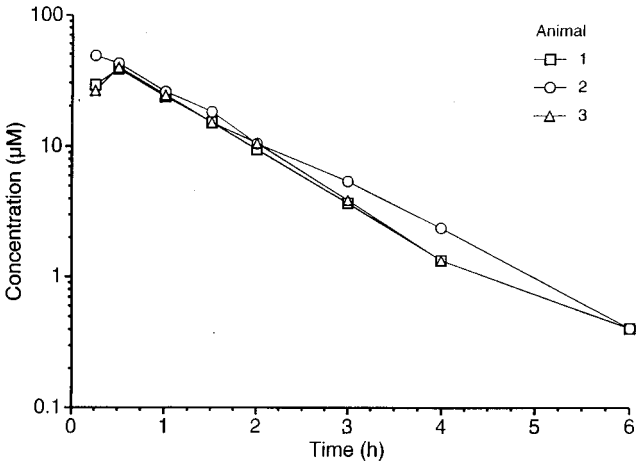

FIG. 4. IsoddA levels in rat plasma after a 25-mg/kg oral dose. The experiment was performed as described in Materials and Methods.

Deamination of IsoddA by adenosine deaminase. The substrate kinetic constants were determined for adenosine, dideoxyadenosine (ddA), and IsoddA with calf intestinal adenosine deaminase. The K_m for IsoddA was 250 ± 30 μM, and the V_{max} was (7.8 ± 0.5) × 10^{-5} μmol/min per unit of enzyme. The corresponding values for adenosine and ddA were 30 μM and 1.3 μmol/min per unit of enzyme and 160 μM and 0.8 μmol/min per unit of enzyme, respectively. Thus, the relative substrate efficiency (V_{max}/K_m) of IsoddA was 0.0008% of that of adenosine. This substrate efficiency is also much lower than that of ddA (11.5% of that of adenosine).

Pharmacokinetics. The pharmacokinetics of IsoddA fit well a linear single-compartment model in the elimination phase, measurable up to 6 h postdose. Rapid absorption of the 25-mg/kg dose occurred (Fig. 4), giving an apparent maximum concentration of 42.0 ± 5.6 μM and a time to maximum concentration of 0.4 ± 0.1 h, although the complete absorption phase was not well described because of the limited number of samples in this phase. IsoddA was quickly eliminated from plasma, with a mean half-life of 0.8 ± 0.1 h. The mean area under the plasma concentration-time curve, extrapolated to infinity, was 58.5 ± 7.4 μM · h. The mean UR of IsoddA in rats was 87 ± 3% of the dose, and there was no measurable amount of drug after 24 h and no observed metabolites, including the hypoxanthine analog. Similar results were obtained with mice. The mean UR after intravenous dosing was 91 ± 21% of the dose, and that after oral dosing was 73 ± 5% of the dose. The oral bioavailability in mice, calculated as the ratio of the mean recoveries after oral and intravenous dosings, was 81%. No metabolites were observed in the mouse urine. Concentrations of IsoddA in rat brain homogenates were below the limit of detection (0.5 μM), and no metabolites were observed. The protein binding of 50 μM IsoddA in rat and mouse plasma was approximately 5%.

DISCUSSION

The isomeric dideoxynucleoside IsoddA exhibited activity in vitro against HIV-1 (strain IIIB), HIV-2 (strain ZY), and HIV-1 clinical isolates. This compound retained anti-HIV activity whether analyzed in MT4 cells or PBLs. Moderate resistance to IsoddA was observed with AZT-resistant clinical isolates of HIV-1. IsoddATP inhibited HIV-1 RT with a K_i of 16 ± 3 nM, a value comparable to that of ddATP (18 ± 2 nM). Interestingly, the combination of IsoddA plus AZT, ddI, or FTC was synergistic, on the basis of isobologram analysis. IsoddA was active against HBV in vitro but was minimally selective in an in vivo murine model of HBV infection.

IsoddA-resistant HIV-1 (HXB2) was selected in vitro in the presence of drug. The IC_{50} for passaged virus was four- to eightfold higher than that for wild-type HXB2. Nucleotide sequence analysis of the RT-coding region of the HIV-1 *pol* gene indicated that the change in sensitivity to IsoddA was due to a single amino acid change at codon 184 from Met to Val (M184V). M184V has been reported previously to be selected by passage of HIV in the presence of ddI (16), 3TC (5, 15, 35, 44), and FTC (44). This mutation confers a fivefold increase in the IC_{50} of ddI and cross-resistance to ddC (16).

IsoddA was inefficiently phosphorylated in human T-lymphocytic CEM cells. The intracellular level of IsoddATP was approximately 4.4 nM after the cells were incubated with 10 μM IsoddA for 24 h. By way of comparison, the intracellular level of ddATP is 56 nM following incubation of MOLT-4 cells with 5 μM ddI (2). The intracellular half-life of IsoddATP was estimated to be 9.4 h. This is shorter than the value of 24 h for ddATP, which was measured in human T cells that were incubated with ddI under similar conditions (1). On the other hand, the half-lives of the 5′-triphosphates of ddC, AZT, and 3′-deoxy-2′,3′-didehydrothymidine are approximately 3 h in MOLT-4 and CEM cells (17, 42).

IsoddA was well absorbed orally (81% bioavailability in mice) and rapidly eliminated from the plasma via renal excretion (>70% of the dose recovered in both mouse and rat urine as IsoddA). No metabolites of IsoddA were found in the urine in these in vivo studies; however, small amounts of what might have been the hypoxanthine analog were detected in in vitro studies with labeled drug. Consistent with these findings, IsoddA was a very inefficient substrate for adenosine deaminase. Further, transposition of the nucleobase from the natural 1′ position to the 2′ position stabilizes IsoddA to acid hydrolysis, distinguishing it from the 2′,3′-dideoxypurine anti-HIV agents. Unfortunately, no BP by IsoddA was detected.

IsoddA was not toxic to human leukemic cells or HBV-producing 2.2.15 cells derived from transfected HepG2 cells in vitro. IsoddATP was a surprisingly potent inhibitor of human DNA polymerase α, with a K_i (0.63 μM) that was 50% of the K_m for dATP (25). IsoddA was a relatively weak inhibitor of the mitochondrial DNA polymerase γ and the repair enzyme, polymerase β IsoddA was toxic to human granulocyte-macrophage (CFU-GM) and erythroid (BFU-E) progenitor cells, with IC_{50}s of 12 and 11 μM, respectively. By comparison, the IC_{50}s of AZT were 7 and 0.4 μM, respectively, and those of ddI (12) were >400 and 34 ± 4 μM, respectively. The clinical hematopoietic toxicity of some nucleosides, including AZT, has been associated with the ability to inhibit colony formation of human bone marrow progenitor cells in vitro (40). Therefore, if sustained plasma IsoddA levels in humans were to approach these concentrations, hematopoietic toxicity might occur.

In summary, IsoddA was active in vitro against HIV-1, HIV-2, and HIV-1 clinical isolates. IsoddA was also active against HBV in vitro; however, it lacked substantial selective activity in an in vivo HBV model. Combinations of the compound with AZT, ddI, or FTC showed synergistic inhibition of HIV-1. Upon oral administration to rats, the compound was very well absorbed and rapidly eliminated, and no catabolites were detected; however, no BP by IsoddA was detected. IsoddA-resistant HIV-1 produced by passage of the virus in the presence of the compound had a mutation known to cause resistance to ddI, ddC, 3TC, and FTC. Furthermore, IsoddA

was toxic to granulocyte-macrophage (CFU-GM) progenitor cells and erythroid (BFU-E) progenitor cells.

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