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Biotransformation and pharmacokinetics of prodrug 9-(β-D-1,3-dioxolan-4-yl)-2-aminopurine and its antiviral metabolite 9-(β-D-1,3-dioxolan-4-yl)guanine in mice

Konstantine K. Manouilov [a], Lidia S. Manouilova [a], F. Douglas Boudinot [b], Raymond F. Schinazi [c], Chung K. Chu [a,*]

[a] *Department of Medicinal Chemistry, College of Pharmacy, University of Georgia, Athens, GA 30602-2352, USA*
[b] *Department of Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA 30602, USA*
[c] *Emory University/Georgia Research Center for AIDS and HIV Infection, VA Medical Center, Decatur, GA 30033, USA*

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Abstract

9-(β-D-1,3-Dioxolan-4-yl)guanine (DXG) exhibits potent antiviral activity against human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV) in vitro. However, since DXG possesses limited aqueous solubility, a more water soluble prodrug of DXG, 9-(β-D-1,3-dioxolan-4-yl)-2-aminopurine (APD), was synthesized. The purpose of this study was to characterize the pharmacokinetics of APD and its antiviral metabolite DXG in mice. Female NIH-Swiss mice were administered 100 mg/kg APD intravenously or orally. Serum, brain and liver were collected at selected times following prodrug administration and concentrations of APD and DXG were determined by HPLC. APD was efficiently converted to parent nucleoside DXG following both intravenous and oral administration. Biotransformation of APD to DXG likely occurs in the liver and is mediated by xanthine oxidase. Similar pharmacokinetic profiles for DXG were observed following either route of administration in serum, liver and brain. These results demonstrate that APD appears to be a promising prodrug for the delivery of DXG. © 1997 Elsevier Science B.V.

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1. Introduction

It is critical to continue the search for effective antiviral agents with low toxicity and no cross resistance with currently available HIV agents. As

* Corresponding author. Tel.: +1 706 5425379; fax: +1 706 5425381; e-mail: dchu@rx.uga.edu

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a part of these efforts, various oxathiolane and dioxolane nucleosides have been synthesized and evaluated as potential anti-HIV agents (Kim et al., 1993a,b; Schinazi et al., 1994). Among dioxolane nucleosides, 9-(β-D-1,3-dioxolan-4-yl)-guanine (DXG) exhibited the most potent anti-HIV activity with a median effective concentration (EC_{50}) of 0.03 μM against HIV-1 in peripheral blood mononuclear (PBM) cells infected with strain LAV (Kim et al., 1993b). In addition, the EC_{50} of DXG for hepatitis B virus (HBV) DNA replicative intermediates or HBV virion synthesis inhibition in transfected HepG-2 cells was ≤ 0.09 μM (Schinazi et al., 1994). No marked cytotoxicity (IC_{50}) in PBM, Vero, and CEM cells or human bone marrow progenitor cells was observed up to 100 μM (Kim et al., 1993b). DXG was chemically stable and enzymatically inert to purine phosphorylase.

Similar to other guanine derivatives, DXG is quite insoluble in water. Therefore, it was necessary to synthesize a prodrug that was more water soluble than DXG. Further, the prodrug should be well absorbed following oral administration and easily converted to parent compound in vivo. Previous studies have shown that water soluble prodrugs 6-deoxyacyclovir (Krenitsky et al., 1984) and famciclovir (Harnden et al., 1989) improved the oral bioavailability of acyclovir and penciclovir, respectively. 2-Fluoro-2′,3′-dideoxyribofuranosylpurine (2-F-ara-ddP) was also investigated as a prodrug of 2-fluoro-dideoxyribofuranosylinosine (Shanmuganathan et al., 1994). These studies demonstrated that 6-deoxypurine prodrugs with improved aqueous solubility increased oral absorption and were readily converted to parent compound by the xanthine oxidase enzyme system. In this report, the pharmacokinetics of 9-(β-D-dioxolan-4-yl)-2-aminopurine (APD) and its antiviral metabolite, DXG, were characterized in mice.

2. Materials and methods

2.1. Chemicals

APD, DXG and 2′-fluoro-2′,3′-dideoxyinosine were synthesized with chemical purity greater than 99% (Kim et al., 1993a,b). Acetonitrile (HPLC grade) and all other chemicals (analytical grade) were purchased from J.T. Baker (Phillipsburg, NJ). Xanthine oxidase, grade IV from milk, was obtained from Sigma (St. Louis, MO).

2.2. Animals

Female NIH-Swiss mice (Harlan Sprague–Dawley, Indianapolis, IN) weighing 24–28 g were maintained in a 12-h light/dark constant-temperature (22°C) environment with free access to standard laboratory chow and water at the University of Georgia College of Pharmacy Animal Care Facility, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. Mice were acclimatized to this environment for 1 week prior to the experiments. Animal studies were approved by the University of Georgia Animal Care and Use Committee, and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

2.3. In vitro metabolism studies

To determine whether the conversion of APD to yield DXG was mediated by xanthine oxidase, stock solutions of APD (1 mM) and allopurinol (2 mM) were prepared in 0.05 M isotonic phosphate buffer (pH 7.4). From them three other solutions were prepared with 20 μM of APD in each and allopurinol at concentrations of 0, 50 and 500 μM. The reaction was initiated by the addition of 200 μl of xanthine oxidase (final activity in the incubation media was 0.25 U/ml, where 1 U is the amount of enzyme which converts xanthine to uric acid at the rate of 1.0 μmol/min at 25°C and pH 7.5). At specified time intervals, aliquots of 200 μl were withdrawn for the determination of APD and DXG.

To assess the in vitro bioconversion of APD, untreated mice were anesthetized with diethyl ether and sacrificed by exsanguination via a left

ventricle heart puncture. Serum was harvested from blood collected. The brain and liver were excised, rinsed with normal saline, blotted dry, and weighed. Brain and liver homogenates were prepared in 1:1 (w/v) ratio with isotonic phosphate buffer, pH 7.4. Samples of mouse serum, brain homogenate and liver homogenate containing 50 μM 2-ADP were incubated at 37°C in a shaker water bath. APD in liver homogenate was also incubated in presence of allopurinol (50 and 500 μM). Aliquots of 200 μl were removed at time zero and for up to 4 h after adding 2-ADP to measure the prodrug and DXG concentrations. First-order degradation rate constants (k) and half-lives ($t_{1/2} = 0.693/k$) for the in vitro studies in buffer, serum, liver homogenate and brain homogenate were determined by linear least-squares regression of the natural logarithm of concentration versus time values.

2.4. Pharmacokinetic studies

For pharmacokinetic studies, APD (12.5 mg/ml), dissolved in physiological saline, was administered to mice at a dose of 100 mg/kg intravenously (i.v.) via a tail vein over 1 min. For oral (p.o.) administration, APD (8.3 mg/ml), dissolved in water, was administered by gastric gavage. Mice were placed in individual cages and allowed food and water ad libitum. Three mice each were sacrificed at 0.08, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h after prodrug administration. Serum, brain and liver were collected and immediately processed for the determination of APD and DXG.

2.5. Assay methodology

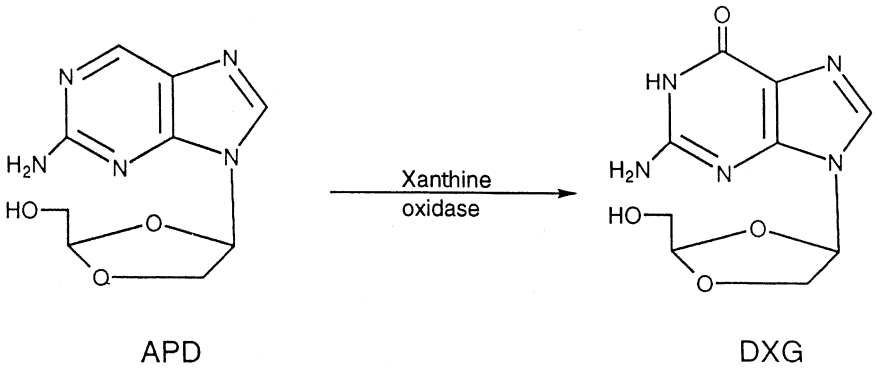
Brain and liver were homogenized in a 1:2 (w/v) ratio with ice cold water and centrifuged at $2000 \times g$ for 15 min at 4°C. Concentrations of APD and DXG in buffer, serum, and brain and liver homogenate were determined by high-performance liquid chromatography (HPLC) as previously described (Chen et al., 1997). The limit of quantification for APD and DXG was 1.0 μg/ml (or μg/g) and 0.1 μg/ml (or μg/g), respectively. The intra- and inter-assay relative standard deviations for each compound was less than 7% in all media.

2.6. Pharmacokinetic analysis

Mean serum, brain and liver prodrug and drug concentration versus time data from the pharmacokinetic studies were analyzed by a non-compartmental technique. The area under the serum, brain or liver nucleoside concentration versus time curve (AUC) and the first non-normalized moment (AUMC) were determined by Lagrange polynomial interpolation and integration from time zero to the last sample time (Rocci and Jusko, 1983) with extrapolation to time infinity using the least-squares terminal slope (λ_z). Half-life was calculated from $0.693/\lambda_z$. For intravenously administered APD, total clearance (CL_T) was calculated from Dose/AUC and steady-state volume of distribution (V_{ss}) from [Dose $\times$ AUMC]/AUC^2. Relative tissue exposure (r_e) for the compounds was calculated from AUC_{tissue}/AUC_{serum}. Absolute oral bioavailability of APD and relative oral bioavailability of DXG were calculated from $AUC_{p.o.}/AUC_{i.v.}$ for each compound, following the administration of prodrug.

3. Results and discussion

APD was stable in serum ($t_{1/2} = 123$ h) and brain homogenate ($t_{1/2} = 46.2$ h). However, in liver homogenate, APD was rapidly oxidized ($t_{1/2} = 0.34$ h) to yield stoichiometric levels of DXG. In the presence of 0.25 U/ml of xanthine oxidase in phosphate buffer, concentrations of APD declined with half-life 2.2 h. Allopurinol inhibited the biotransformation of APD to DXG in phosphate buffer in a dose dependent manner. There was no bioconversion of APD in samples containing 500 μM of allopurinol. Similarly, allopurinol inhibited the metabolism of APD in liver homogenate. These results suggest that xanthine oxidase present in the liver is the principal enzyme responsible for the bioconversion of APD to DXG (Scheme 1).



Scheme 1.

Concentrations of APD in serum following intravenous and oral administration of 100 mg/kg of the prodrug are illustrated in Fig. 1A. Pharmacokinetic parameters for APD and DXG are presented in Table 1. Following intravenous administration, serum concentrations of APD declined rapidly with half-life of 0.28 h. Total clearance of APD was 1.42 l/h/kg and the steady state volume of distribution was 0.64 l/kg. Following oral administration, APD was rapidly absorbed with peak concentrations achieved by 15 min (Fig. 1A). Subsequently, serum concentrations of APD declined with a half-life of 0.85 h. Bioavailability of APD after oral administration was 41%.

APD was detected in brain only within the first 15 min following intravenous administration, but not detected at all after oral administration of the prodrug. No APD was detected in liver after either route of administration. Thus, although the volume of distribution value suggests that APD distributes intracellularly, the prodrug does not appear to distribute into brain to a great extent. Consistent with the in vitro stability studies, drug distributed to the liver appears to be rapidly metabolized.

Pharmacokinetic profiles of DXG in serum after intravenous and oral administration of APD are shown in Fig. 1B. In each case, DXG rapidly achieved a maximum concentration of approximately 55 μg/ml and nucleoside concentrations remained relatively constant at that level for 3 h. DXG serum concentrations subsequently declined with similar half-lives following intravenous and oral administration (Table 1). The area under the DXG serum concentration versus time curve was similar following both routes of administration. Thus, although the absolute oral bioavailability of APD was less than 50%, the relative availability of DXG was the same regardless of the route of administration. These results suggest that the prodrug undergoes extensive first-pass metabolism yielding DXG following oral administration. As suggested by results of the in vitro studies, the biotransformation of APD to DXG likely occurs in the liver. The AUC of DXG in serum was considerably greater than that of APD indicating efficient bioconversion of the prodrug.

Concentrations of DXG in liver following intravenous and oral administration of the prodrug are depicted in Fig. 1C. DXG was formed from APD rapidly with peak levels seen at the first sampling time (5 min). Despite the different routes of administration, the relative exposure of DXG in liver was virtually identical. The AUC values of DXG in liver exceeded those in serum by 16–23%. Half-lives of DXG in liver were comparable to those in serum. These results further suggest that the metabolism of APD to DXG occurs in the liver.

Pharmacokinetic profiles of DXG in brain following prodrug administration are shown in Fig. 1D. The pattern of distribution of DXG was similar for both routes of administration, however brain concentrations up to 1 h after prodrug were generally higher after intravenous administration. The maximum DXG brain concentration of 5.1 $\pm$

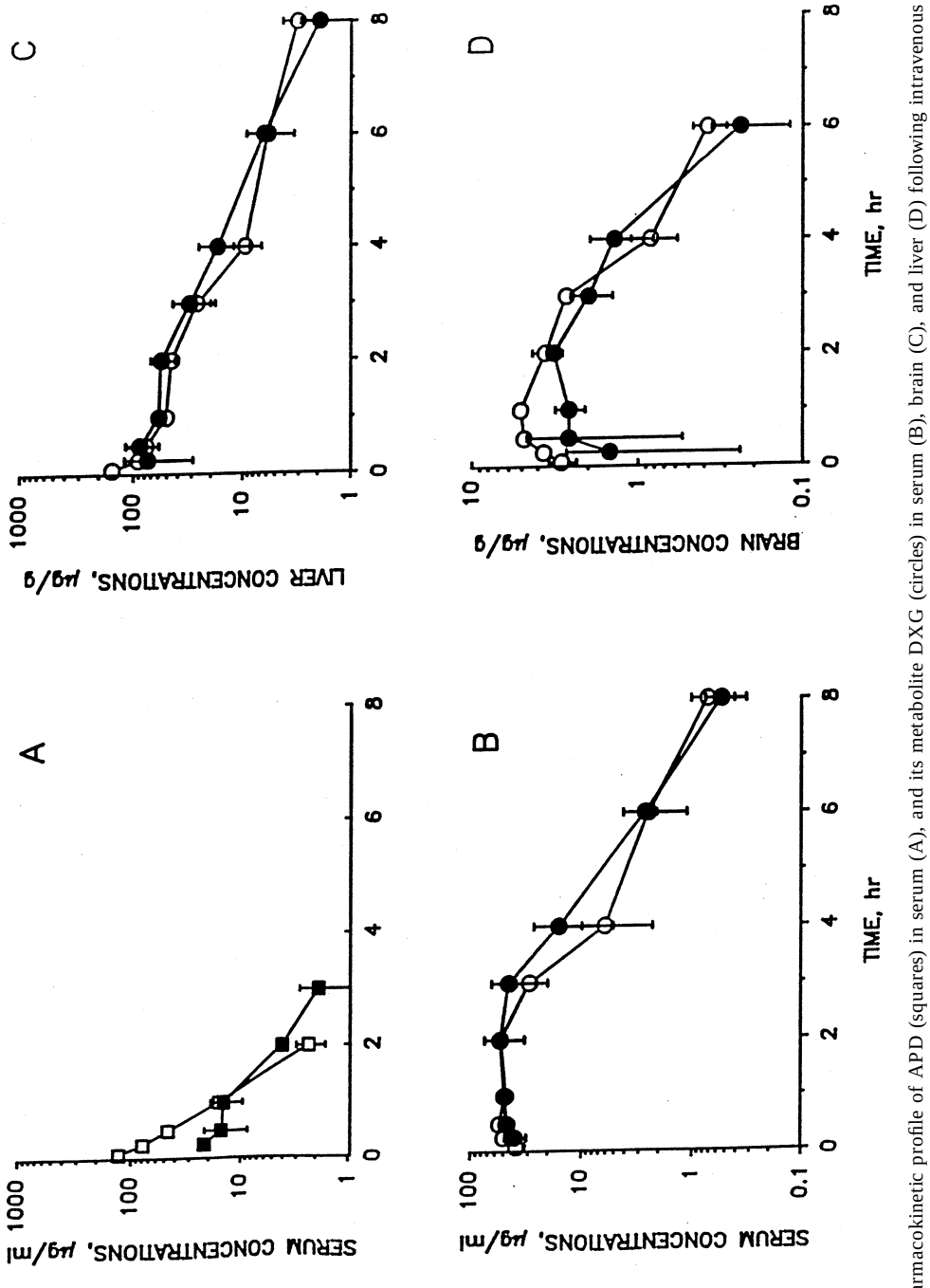


Fig. 1. Pharmacokinetic profile of APD (squares) in serum (A), and its metabolite DXG (circles) in serum (B), brain (C), and liver (D) following intravenous (●) and oral (▽) administration of 100 mg/kg APD to mice.

Table 1
Pharmacokinetic parameters of APD and DXG after intravenous and oral administration of 100 mg/kg APD to mice

Media	Compound	AUC (μmol•h/ml or g)		r_e		$t_{1/2}$ (h)	
		i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
Serum	APD	366.5 ± 9.53[a]	150.3 ± 14.6	1.0	1.0	0.28	0.85
	DXG	818.9 ± 35.5	930.1 ± 96.6			1.04	1.15
Liver	APD	ND[b]	ND	ND	ND	ND	ND
	DXG	1014.6 ± 31.9	1086.9 ± 85.9	1.23	1.16	1.56	1.21
Brain	APD	1.3[c]	ND	0.018	ND	0.22	ND
	DXG	74.3 ± 2.82	53.9 ± 4.66	0.091	0.058	1.45	1.13

[a] Mean ± S.E.
[b] None detected.
[c] Standard error could not be calculated due to limited number of data points.

0.33 μg/g after intravenous administration was significantly greater than that after oral administration (3.2 ± 0.36 μg/g). The AUC values in brain, however, were similar after both routes of administration (Table 1). Thus, APD yielded similar brain levels of DXG following either oral or intravenous administration.

Chen et al. (1996) previously characterized the disposition of APD following oral administration to rhesus monkeys. However, since the prodrug was not administered intravenously, bioavailability could not be determined and assessment of clearance (CL/F) and volume of distribution (V/F) in this study was ambiguous. Nevertheless, similarities in the disposition of APD and DXG in mice and monkeys were noted. In both species, the apparent half-life of derived DXG was greater than that of prodrug, APD. Following oral administration of ADP to monkeys, the half-life of prodrug was 0.47 h and that of DXG was 1.45 h. A similar two- to four-fold greater half-life of DXG than APD was also seen in mice. The AUC values for DXG were greater than those of prodrug ADP in both species. In monkeys, the ratio of the AUC values of DXG to APD was 3.0; in mice this ratio was 2.4 and 6.7 following intravenous and oral administration, respectively. Clearance of APD in both species was high. The oral bioavailability of APD in mice was 41%. High clearance (CL/F) values (11 l/h/kg) after oral administration of APD in monkeys suggests low bioavailability of the prodrug in this species as well. However, significant first-pass metabolism of APD yielded relatively high levels of DXG.

Similar to results in mice, relatively low concentrations of APD were detected in cerebrospinal fluid of monkeys following oral administration of the prodrug. In monkeys, cerebrospinal fluid concentrations of DXG were approximately 10% those of DXG in serum. Similarly, the relative brain exposure of DXG in mice was 9.1 and 5.8% after intravenous and oral administration of APD, respectively. Thus, in general, the disposition patterns of APD and DXG in mice were similar to those in monkeys.

In conclusion, APD was efficiently converted to parent nucleoside DXG following intravenous and oral administration to mice. Biotransformation of APD to DXG likely occurs in the liver and is mediated by xanthine oxidase. Similar disposition profiles for DXG were observed following either route of administration in serum, liver and brain. These results, along with those reported for monkeys, suggest that APD appears to be a promising prodrug for DXG.

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