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6-Deoxyacyclovir: A xanthine oxidase-activated prodrug of acyclovir

(antiviral chemotherapy/aldehyde oxidase)

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ABSTRACT **Acyclovir {9-[(2-hydroxyethoxy)methyl]guanine} is an acyclic guanine nucleoside analogue that is widely used clinically as an antiherpetic agent. Its limited absorption in humans after oral administration prompted the search for prodrugs. A congener, referred to as 6-deoxyacyclovir {2-amino-9-[(2-hydroxyethoxy)methyl]-9*H*-purine}, was synthesized and found to be 18 times more water soluble than was acyclovir. Surprisingly, this congener was readily oxidized to acyclovir by xanthine oxidase (EC 1.2.3.2). It was also oxidized by aldehyde oxidase (EC 1.2.3.1) largely to 8-hydroxy-6-deoxyacyclovir {2-amino-8-hydroxy-9-[(2-hydroxyethoxy)methyl]-9*H*-purine} and then to 8-hydroxyacyclovir {2-amino-6,8-dihydroxy-9[(2-hydroxyethoxy)methyl]-9*H*-purine}. 6-Deoxyacyclovir and the major products of its oxidation by aldehyde oxidase lacked appreciable activity against herpes simplex type I *in vitro*. On the basis of these results, it was apparent that the success of 6-deoxyacyclovir as a prodrug *in vivo* would depend upon how well its desired activation by xanthine oxidase competed with the nonactivating oxidations by aldehyde oxidase. In rats dosed orally with 6-deoxyacyclovir, absorption was extensive and the major urinary metabolite was acyclovir. In two human volunteers, urinary excretions of acyclovir were 5–6 times greater than those typically observed after administration of equivalent doses of acyclovir itself. The areas under the plasma concentration–time curves for acyclovir were also 5–6 times greater. Plasma levels of acyclovir peaked soon after ingestion of the prodrug, indicating rapid absorption and metabolic conversion. These results suggested that 6-deoxyacyclovir might have clinical usefulness as a prodrug of acyclovir suitable for oral administration.**

Acyclovir {9-[(2-hydroxyethoxy)methyl]guanine; Zovirax} is a clinically useful antiherpetic agent (1, 2). Intravenous (3, 4), oral (5), or topical (6, 7) administration provides effective therapy. Only 15–20% of the dose is typically absorbed in humans after oral administration (8). This degree of absorption is adequate for efficacy against herpes simplex infections (5). However, greater absorption might be important in therapy against less sensitive viruses such as varicella-zoster virus (9). The clinical experience to date clearly indicates that although acyclovir represents a major therapeutic advance in the treatment of herpetic infections, a means of enhancing gastrointestinal absorption would significantly extend its usefulness.

Considerable effort has been expended in attempts to find a prodrug that is well absorbed after oral administration and then converted to acyclovir. Esterification of the hydroxyl group of the (2-hydroxyethoxy)methyl moiety of acyclovir has been an approach taken by two separate laboratories (10, 11). Unfortunately, those esters that have been tested showed no significant improvement in absorption after oral dosing (unpublished results).

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The 6-deoxy-6-amino congener of acyclovir {2,6-diamino-9-[(2-hydroxyethoxy)methyl]-9*H*-purine} (11) has been studied in some detail as a prodrug. It is deaminated to acyclovir by adenosine deaminase [EC 3.5.4.4.] (12). Oral dosing of dogs and rats with this congener resulted in modest increases in acyclovir plasma levels relative to those achieved with acyclovir itself (13).

The prodrug described herein is a congener of acyclovir lacking the 6-hydroxy group. It is referred to as 6-deoxyacyclovir {2-amino-9-[(2-hydroxyethoxy)methyl]-9*H*-purine}. Its synthesis (Scheme I), oxidation by xanthine oxidase (Scheme II) and aldehyde oxidase (Scheme III), and preliminary pharmacokinetics in rats and in humans are described.

EXPERIMENTAL PROCEDURES

Chemistry. Elemental analyses were performed by Atlantic Microlabs (Atlanta, GA) and were within ±0.4% of the theoretical values. NMR spectra were recorded at ambient temperature on a Varian FT 80A spectrometer. The 8-hydroxy derivative of acyclovir, 8-hydroxy-9-[(2-hydroxyethoxy)methyl]guanine, was synthesized by a procedure that will be published elsewhere.

*2-Amino-6-chloro-9-[(2-benzoyloxyethoxy)methyl]-9*H*-purine.* A suspension of 1.0 g (5.9 mmol) of 2-amino-6-chloropurine in 55 ml of dimethylformamide was heated on a steam bath until dissolved. The solution was cooled to room temperature, 1.9 ml (14.0 mmol) of triethylamine and 1.8 g (8.5 mmol) of 2-benzoyloxyethoxymethyl chloride were added, and the yellow solution was stirred at ambient temperature for 18 hr. The triethylammonium chloride precipitate was filtered off and washed with acetone and the combined washings and mother liquors were evaporated *in vacuo*. The residual oil was dissolved in a minimal amount of CH_2Cl_2, preabsorbed on silica gel, and applied to a column of 50 g of silica gel in CH_2Cl_2. The column was eluted initially with ether to remove unreacted by-products and then with 5% methanol in CH_2Cl_2. The fractions containing the desired 9-isomer (determined by TLC on silica gel in 2% methanol in $CHCl_3$; R_f = 0.2) were combined and evaporated and the residue was recyrstallized from benzene to give 0.9 g (44%) of product: mp 130–133.5°C; NMR ($CDCl_3$) δ 7.65 (m, 6H ArH and H_8), 5.53 (s, 2H NCH_2O), 5.37 (br s, 2H, NH_2), 4.17 (m, 4H, OCH_2CH_2O); UV at pH 7.0, λmax 223 nm (ε 27,400), 310 (5740). Anal. ($C_{15}H_{14}ClN_5O_3$) C, H, N.

6-Deoxyacyclovir. A mixture of 2.48 g (7 mmol) of 2-amino-6-chloro-9-[(2-benzoyloxyethoxy)methyl]-9*H*-purine, 250 ml of absolute ethanol, 1.9 ml (14 mmol) of triethylamine, and 0.6 g of 5% palladium on charcoal was shaken

Abbreviations: acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine; 6-deoxyacyclovir, 2-amino-9-[(2-hydroxyethoxy)methyl]-9*H*-purine; 8-hydroxy-6-deoxyacyclovir, 2-amino-8-hydroxy-9-[(2-hydroxyethoxy)methyl]-9*H*-purine; 8-hydroxyacyclovir, 2-amino-6,8-dihydroxy-9[(2-hydroxyethoxy)methyl]-9*H*-purine; $t_{1/2}$, elimination-phase half-life; AUC, area under the plasma concentration–time curve; C_{max}, peak drug concentration; t_{max}, time at C_{max}.

(Parr apparatus) under an initial pressure of 50 psi (1 psi = 6.89 kPa) of H_2 at room temperature for 20 hr. The mixture was filtered, 0.26 g of 5% palladium on charcoal and 1.9 ml of triethylamine were added, and the mixture was shaken under 50 psi of H_2 for 18 hr longer. The ethanolic solution was filtered through a pad of Celite and evaporated *in vacuo* and the resulting white solid was extracted with boiling benzene (4 × 100 ml). The benzene extracts were concentrated to 50 ml and stirred with 20 ml of 40% aqueous methylamine and 20 ml of methanol. The solution was evaporated to dryness on a steam bath and the residue was triturated with ether to remove the *N*-methylbenzamide. Recrystallization of the ether-insoluble material from absolute ethanol yielded 0.91 g (62%) of product: mp 187–189°C; NMR (Me_2SO-d_6) δ 8.64 (s, 1H, H_6), 8.22 (s, 1H, H_8), 6.60 (s, 2H, NH_2), 5.50 (s, 2H, NCH_2O), 4.69 (br s, 1H, OH), 3.50 (s, 4H, OCH_2CH_2O); UV at pH 1.0, λmax 247 nm (ε 5900), 312 (5460), at pH 7.0, 243 (7640), 305 (9170), at pH 13.0, 243 (8080), 305 (9390). Anal. ($C_8H_{11}N_5O_2$) C, H, N.

Isolation of 8-hydroxy-6-deoxyacyclovir {2-amino-8-hydroxy-9-[(2-hydroxyethoxy)methyl]-9H-purine}. This reference compound was desired because it was suspected to be an intermediate in the oxidation of 6-deoxyacyclovir by aldehyde oxidase. Attempts to synthesize it by chemical methods were unsuccessful. Consequently, the following isolation from urine was undertaken. Rabbits were used since they have high levels of aldehyde oxidase (14).

Three 2-year-old male rabbits (New Zealand White, 3.5 kg) were given 175 mg of 6-deoxyacyclovir (intraperitoneally) daily for 6 days. One hour before each dose, the animals were treated with allopurinol (9 mg intraperitoneally) to decrease the extent of oxidation of 6-deoxyacyclovir by xanthine oxidase. The urine was collected daily, clarified by centrifugation, and stored at 3°C after addition of sodium azide (0.04%). Isolation of the desired product was monitored by HPLC as described below. Eleven liters of urine was adjusted to pH 10.3 with 14 M ammonium hydroxide and then applied to a Dowex 1/formate (X8) column (5 × 36 cm). The column was washed with 1 liter of water. The pH value of the total eluate was adjusted to 10.4 and this solution was applied to a second Dowex 1/formate column (10 × 15 cm). Although some product was retained by the first column, the second retained the greater portion. The second column was washed with 6 liters of 5 mM, 4 liters of 10 mM, and 4 liters of 30 mM formic acid. The product was then eluted with 35 mM formic acid. Fractions containing the bulk of it were combined and evaporated to dryness *in vacuo*. The residue was dissolved in 25 ml of a mixture of *n*-propanol/water, 30:70 (vol/vol), and applied to a polyacrylamide gel (P-2, Bio-Rad) column (5 × 90 cm) that had been preequilibrated with the solvent mixture. The product was eluted with the same mixture. After drying *in vacuo*, the residue was triturated with cold water to remove a slight amount of yellow color and then dried again to yield 260 mg of pure compound: mp 224°C, proton NMR (MeSO-d_6) δ 10.6 (br s, 1H, 7-NH), 7.76 (s, 1H, 6-H), 6.20 (s, 2H, 2-NH_2), 5.11 (s, 2H, NCH_2O), 4.6 (t, 1H, OH), 3.52 (m, 4H, OCH_2CH_2O); ^{13}C NMR (MeSO-d_6) δ 159.09 (^{13}C–^1H coupling, d $^3J_{C_2H_6}$ = 11.8 Hz, C_2), 151.09 (m, C_4), 113.39 (d $^2J_{C_5H_6}$ = 5.6, C_5), 134.79 (d $^1J_{C_6H_6}$ = 182.1, C_6) 153.07 (t $^3J_{C_8H_{A2}}$ = 4.2, C_8), 70.87 (m ^1J = 142.2, OCH_2CH_2, 68.60 (t ^1J = 160, NCH_2O), 59.93 (m ^1J = 140.3, CH_2CH_2OH). The structure of this compound can be completely assigned from these proton and carbon-13 NMR data. The proton spectrum showed all of the expected functions: amide NH, aromatic CH, -NH_2, -NCH_2O-, and -OCH_2CH_2OH. The carbon-13 chemical shifts are characteristic of a purine. The proton-coupled carbon-13 spectrum proves the positions of the functional groups and the chemical shift assignments. The aromatic proton is in the 6-position since the one-bond coupling constant ($^1J_{C_6H}$) is 182.1 Hz ($^1J_{C_{8-H}}$ would be >210 Hz) and $^3J_{C_2H_6}$ is 11.8 Hz, which is characteristic of such a coupling across the ring nitrogen. The side chain is in the 9-position since both C_8 and C_4 show small couplings to the methylene protons, whereas C_5 does not. UV at pH 1.0, λmax 311 nm (ε 5700), λmin 265 (680), at pH 13.0, λmax 254 (9230), 318 (7860), λmin 255 (5740), 278 (1010). Anal. ($C_8H_{11}N_5O_3$) C, H, N.

HPLC. The Microsorb C-18 column (15 cm) was purchased from Rainin Instrument (Woburn, MA). The eluant was 2% ethanol in 50 mM ammonium formate buffer (pH 3.5). Retention times at a flow rate of 0.5 ml/min were 700 sec for 8-hydroxy-6-deoxyacyclovir, 750 sec for acyclovir, 940 sec for 6-deoxyacyclovir, and 1120 sec for 8-hydroxyacyclovir {2-amino-6,8-dihydroxy-9[(2-hydroxyethoxy)methyl]9*H*-purine}.

Enzymology. Bovine milk xanthine oxidase (EC 1.2.3.2) was purchased from Boehringer Mannheim. Aldehyde oxidase (EC 1.2.3.1) was purified from rabbit liver according to a published method (14). Protein concentrations were determined spectrophotometrically by absorption at 260 and 280 nm (15).

Kinetic constants were determined at 25°C by using ferricyanide as the electron acceptor (16). Reaction mixtures contained 1 mM potassium ferricyanide, 0.04 mM EDTA, and 140 mM potassium phosphate buffer at pH 6.8. The reduction of ferricyanide was monitored at 420 nm ($\Delta\varepsilon$ = $-2080\ M^{-1}\cdot cm^{-1}$). In cases in which HPLC or spectral analysis of the products was required, ferricyanide was omitted and atmospheric oxygen was allowed to serve as the electron acceptor.

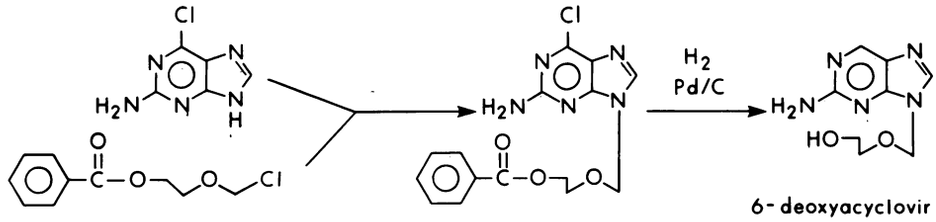
Disposition Studies. Compounds were administered to male Long–Evans rats (200 g) by gavage. Each rat was kept in a metabolic cage that separated urine and feces. Urinary concentrations of acyclovir were determined by a sensitive radioimmunoassay (17) that showed no appreciable cross-reactivity toward 6-deoxyacyclovir, its metabolites, or those of acyclovir.

Two healthy volunteers (D.S., male, 71 kg; E.C., female, 62 kg) were each given a single dose of 200 mg of 6-deoxyacyclovir dissolved in 100 ml of water. After drinking the solution, the subjects drank 100 ml of water. Blood samples were drawn in heparinized tubes at various times up to 14 hr after the dose via an indwelling venous cannula. Total urine was collected for 48 hr after dosing. Plasma and urine acyclovir concentrations were determined by a double antibody radioimmunoassay that utilizes a monoclonal antibody to acyclovir, is highly specific, and has little cross-reactivity with acyclovir metabolites or with 6-deoxyacyclovir. The details of this assay will be published elsewhere.

Pharmacokinetic analysis. The plasma concentration–time data for acyclovir were best fitted to a two-compartment model with first-order input by using the nonlinear least-squares regression program NONLIN (18). Values of the elimination-phase half-life ($t_{1/2}$), area under the plasma concentration–time curve (AUC), peak drug concentration (C_{max}), and time at C_{max} (t_{max}) were calculated from the model-derived parameters.

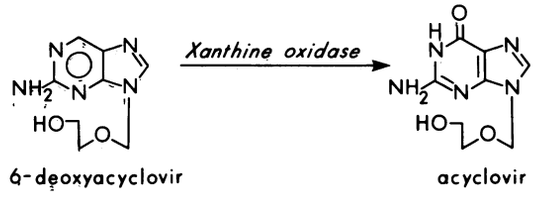
RESULTS AND DISCUSSION

Scheme I outlines the synthetic route for 6-deoxyacyclovir. The first step involved the reaction of 2-benzoyloxyethoxymethyl chloride with 2-amino-6-chloropurine. Both the 7- and 9-isomers were formed. This mixture was resolved by chromatography on silica gel. The 6-chloro group of the 9-isomer was then removed by catalytic hydrogenation. Treatment of the resulting product with methylamine hydrolyzed the benzoyl group to give 6-deoxyacyclovir. This congener was found to be 18 times more water soluble than acyclovir (49 vs. 2.7 mg/ml at 37°C).



Scheme I

Xanthine oxidase has a broad substrate specificity. However, previous studies demonstrated that 9-substitution of a variety of purines greatly diminished or obliterated their rate of oxidation by this enzyme (16, 19). It was therefore a surprise to find that 6-deoxyacyclovir was oxidized by xanthine oxidase from bovine milk at twice the rate of its 9-unsubstituted congener, 2-aminopurine (Table 1). The presence of this 9-substituent did, however, result in a large increase in the K'_m value over that of 2-aminopurine. The product of the oxidation of 6-deoxyacyclovir by xanthine oxidase had the UV spectrum of acyclovir (Fig. 1). The identity of the product was confirmed by its coelution with acyclovir during HPLC. Acyclovir was not further oxidized by xanthine oxidase (Table 1). Scheme II depicts the effects of xanthine oxidase on 6-deoxyacyclovir.



Scheme II

Acyclovir was a weak noncompetitive inhibitor of xanthine oxidase with xanthine as the variable substrate. The intercept K_i was 8.5 mM and the slope K_i was 0.98 mM. These relatively high inhibition constants make it appear unlikely that acyclovir could effectively inhibit its own formation from 6-deoxyacyclovir *in vivo*.

Aldehyde oxidase is closely related to xanthine oxidase both structurally and catalytically. Many purines are oxidized by both enzymes (16). 6-Deoxyacyclovir was found to be oxidized by aldehyde oxidase purified from rabbit liver

Table 1. Some kinetic constants with bovine milk xanthine oxidase

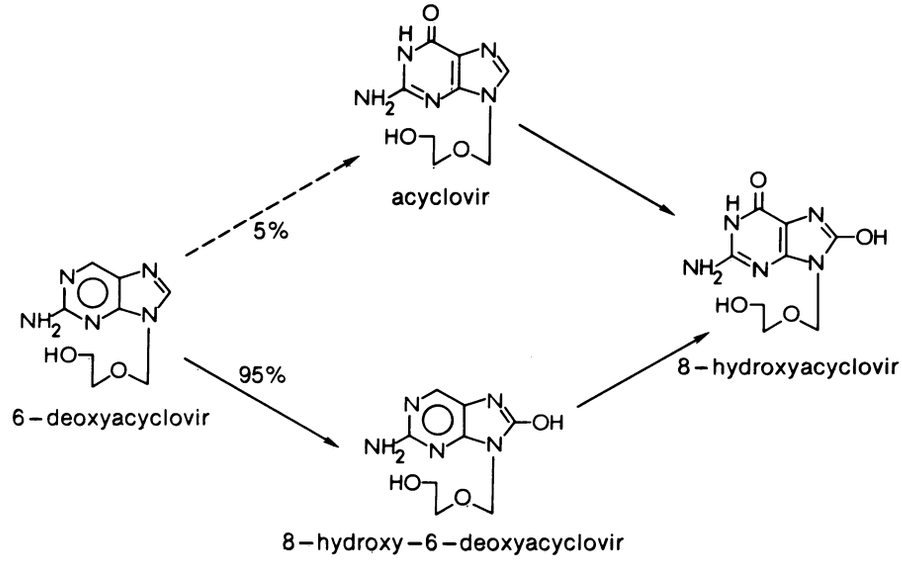
Substrate	K'_m, mM	V'_{max}, nmol/min per mg of protein
Xanthine	0.008	1060
2-Aminopurine	0.008	220
6-Deoxyacyclovir	0.9	500
8-Hydroxy-6-deoxyacyclovir	2.2	1.1
Acyclovir	—	<0.1 at 3.8 mM

K'_m, apparent K_m; V'_{max}, apparent V_{max}.

(Table 2, Scheme III). HPLC analyses just after the addition of aldehyde oxidase to solutions of 6-deoxyacyclovir showed that a mixture of 8-hydroxy-6-deoxyacyclovir (see *Experimental Procedures*) and acyclovir was formed initially. The ratio of the 8-hydroxy to the 6-hydroxy (acyclovir) products was 95 to 5. The final product after prolonged incubation had a UV spectrum (dashed line in Fig. 1) that was identical to that of a known metabolite of acyclovir, 8-hydroxyacyclovir (20). Furthermore, this final oxidation product was coeluted during HPLC with an authentic sample of 8-hydroxyacyclovir. The kinetic parameters for acyclovir as a substrate for aldehyde oxidase are also provided in Table 2. Its

Table 2. Some kinetic constants with rabbit liver aldehyde oxidase

Substrate	K'_m, mM	V'_{max}, nmol/min per mg of protein
2-Aminopurine	0.72	555
6-Deoxyacyclovir	0.06	1900
8-Hydroxy-6-deoxyacyclovir	0.63	17
Acyclovir	2.1	78



Scheme III

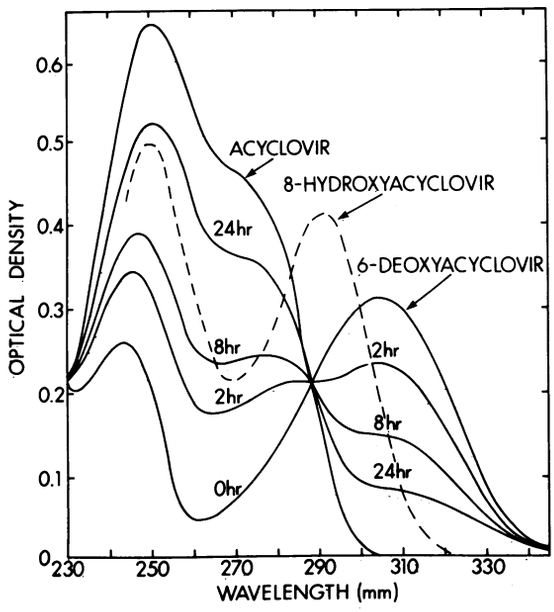


FIG. 1. UV spectra at various times during the oxidation of 6-deoxyacyclovir by xanthine oxidase (solid lines) and the spectra of the final product after exposure of 6-deoxyacyclovir to aldehyde oxidase (dashed line).

relatively inefficient substrate activity is consistent with the finding that 8-hydroxyacyclovir is a significant metabolite of acyclovir only in those species that have high levels of aldehyde oxidase (20, 21).

In contrast to acyclovir, 6-deoxyacyclovir did not show detectable activity against herpes simplex type I at 50 μg/ml in an *in vitro* system that lacked xanthine oxidase. The same result was obtained with the major oxidation products of 6-deoxyacyclovir by aldehyde oxidase, 8-hydroxy-6-deoxyacyclovir and 8-hydroxyacyclovir (P. Collins, personal communication). This lack of appreciable activity against herpes simplex type I suggested that the contribution of aldehyde oxidase to the oxidation of 6-deoxyacyclovir *in vivo* would be largely unproductive from a therapeutic standpoint. One would not predict from the relatively low K'_m value of the prodrug for aldehyde oxidase relative to that for xanthine oxidase (Tables 1 and 2) that acyclovir would be the major

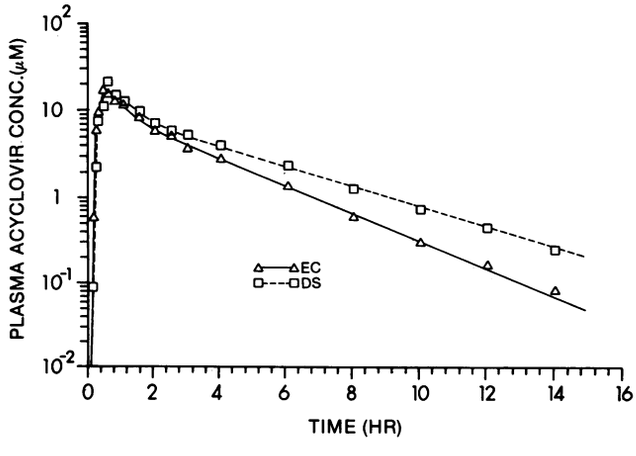


FIG. 2. Plasma concentration–time profiles of acyclovir after an oral dose of 200 mg of 6-deoxyacyclovir in two human volunteers. The solid and dashed lines are the NONLIN derived curves (18). The rectangles and triangles represent the experimental data points. E.C. (a female, 62 kg) and D.S. (a male, 71 kg) were volunteers.

Table 3. Acyclovir pharmacokinetic parameters in human volunteers after a single oral dose of 200 mg of acyclovir or 6-deoxyacyclovir

Acyclovir parameter	Treatment		
		6-Deoxyacyclovir	
	Acyclovir*	E.C.	D.S.
C_{max}, μM	1.4 ± 0.5	15.3	15.8
t_{max}, hr	1.2 ± 0.3	0.5	0.6
$t_{1/2}$, hr	2.9 ± 0.7	1.9	2.6
AUC, μM·hr	7.1 ± 2.3	34.4	45.1

*Ref. 8.

metabolite after the administration of 6-deoxyacyclovir *in vivo*.

The question of how well 6-deoxyacyclovir was absorbed after oral administration and how extensively it was oxidized to acyclovir was first addressed by studies in rats. This species was used because its limited ability to absorb acyclovir from the gastrointestinal tract (22) was similar to that of humans (8). In addition, the Long–Evans strain of rats had aldehyde oxidase levels that were 20% of those previously found with Sprague–Dawley rats (21). This made Long–Evans rats an even better model since human tissues have very low levels of aldehyde oxidase (21). Only 15% ± 3% (n = 3) of a dose of acyclovir of 25 mg/kg that was orally administered to Long–Evans rats was excreted in the urine over a 48-hr period. In contrast, when the same rats were given equivalent doses of 6-deoxyacyclovir, they excreted 66% ± 12% (n = 3) of the dose as acyclovir in 48 hr.

These encouraging results and a preliminary toxicological profile (W. Tucker, personal communication) similar to that of acyclovir (23) prompted a study of 6-deoxyacyclovir in human volunteers, each given a 200-mg oral dose. The 48-hr urinary excretion of acyclovir in two subjects was found to be 65% (D.S.) and 68% (E.C.) of the dose. For comparison, in six volunteers given a 200-mg oral dose of acyclovir, the mean urinary recovery was 12% ± 5% (24). Further, in 20 patients with normal renal function given intravenous doses of 0.5–15 mg of acyclovir per kg, the urinary recovery was 67% ± 15% (25).

Plasma levels of acyclovir were also studied in the volunteers dosed with 6-deoxyacyclovir. After the short absorption phase, the plasma acyclovir concentration–time profile exhibited a biphasic decline and the data were fitted by a two-compartment pharmacokinetic model. The model-derived curves superimposed over the experimental data points are illustrated in Fig. 2. The model-dependent pharmacokinetic parameters for the two volunteers are compared in Table 3 with the mean parameters for a group of volunteers given a 200-mg dose of acyclovir in an earlier study (8). Peak acyclovir plasma concentrations (C_{max}) after 6-deoxyacyclovir administration were observed earlier and were 11-fold higher than the mean peak level after similar doses of acyclovir. The $t_{1/2}$ values of acyclovir after either treatment were similar. The AUC for plasma acyclovir after 6-deoxyacyclovir dosing was 5- to 6-fold greater than the mean AUC after acyclovir treatment.

It is apparent from these preliminary studies in rats and in human volunteers that 6-deoxyacyclovir is readily absorbed after oral administration and is extensively oxidized to acyclovir. This pharmacokinetic profile suggests that 6-deoxyacyclovir might be superior to acyclovir itself for oral administration.

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