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Reactivity of Valganciclovir in Aqueous Solution

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ABSTRACT The rates of hydrolysis of valganciclovir to ganciclovir and L-valine and isomerization of the R and S diastereomers of valganciclovir in aqueous buffer solution from pH 3.8 to 11.5 were determined at 37°C. The kinetics of hydrolysis were first order for at least two half-lives in neutral and basic solutions. In acidic solutions where less than 10% degradation occurred, the rate of hydrolysis was determined assuming a first-order loss in drug. At 37°C and pH 7.08, the half life is 11 h. The maximum stability at the pH values studied occurred at pH 3.81 with a half life of 220 days. The kinetics of the approach to equilibrium for the isomerization were first order and the ratio of the R:S isomer at equilibrium was 52:48. Isomerization was approximately 10 fold faster than hydrolysis over the pH range studied with a half-life at pH 7.01 of 1 h. The maximum stability toward isomerization ($t_{1/2} > 533$ h) occurs at a pH below 3.8. The pH-rate profile for the hydrolysis and the isomerization reaction are best described by hydroxide ion catalyzed mechanisms. In acidic and neutral solutions, the hydroxide reacts with the protonated form of the drug, while in basic solutions, the hydroxide reacts with the neutral form of the drug.

KEYWORDS Valganciclovir, Hydrolysis, Isomerization, Ganciclovir, Prodrug

INTRODUCTION

Valganciclovir hydrochloride, 1, is the active ingredient in Valcyte™ and is the valinate ester prodrug of ganciclovir (Curran & Noble, 2001; Jung & Dorr, 1999; Nestor et al., 1996). The increased bioavailability of valganciclovir is related to its recognition as a substrate by the intestinal peptide transporter PEPT1 (Sugawara et al., 2000). Valcyte™ is sold as a 450 mg film-coated tablet.

Valganciclovir hydrochloride is an aminoacyl ester that has a pKa of 7.6 resulting from protonation on the amino group of the valine moiety (Physician's Desk Reference®, 2004). Protonation of the amino group of the drug may greatly enhance the rate of hydrolysis of valganciclovir hydrochloride to ganciclovir under physiological conditions. If this rate of hydrolysis is rapid compared to valganciclovir hydrochloride rate of absorption, the hydrolytic stability of the drug may potentially affect its bioavailability. Additionally, valganciclovir exists as a mixture of R and S diasteriomers. Prior to development, it was necessary to assess the rate of equilibration of the isomers to determine if a single isomer formulation could be prepared. In this

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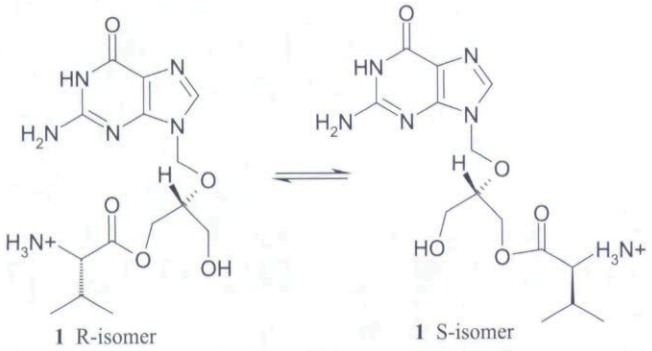

FIGURE 1 Diastereomers of Valganciclovir.

manuscript, we determined the rates of hydrolysis of valganciclovir to ganciclovir and L-valine in aqueous buffer solution and examined the rate of equilibration of the two diasteriomers (Fig. 1).

MATERIALS AND METHODS

Materials

Valganciclovir (mixture of R and S diastereomers) and enriched R isomer were obtained from Chemical Development, Roche Palo Alto (Palo Alto, CA, USA). Buffers were prepared from analytical grade reagents obtained from Mallinckrodt (Phillipsburg, NJ, USA) or Aldrich Chemical Company (St. Louis, MO, USA). Buffers were potassium phosphate (pH 6.5–7.5), potassium acetate (pH 5–6), citric acid (pH 3.8), boric acid (pH 8–9), and potassium hydroxide (pH 11.5).

Kinetics

Hydrolysis

The rates of hydrolysis of valganciclovir were determined at 37°C and ionic strength of 0.15 M (potassium chloride). Rate constants for hydrolysis were determined by following the decrease of the drug concentration with time by HPLC at 256 nm. Aqueous solutions were prepared in 0.01 M buffer with a final concentration of 100 μg/mL. The reaction mixtures were placed into HPLC vials and allowed to equilibrate to 37°C in the thermostated compartment of the HPLC (Hewlett-Packard 1090, Palo Alto, CA, USA). The temperature in the sample holder was monitored with a Fluke thermocouple (TE-139). The kinetics were followed by injecting 25 μL of the reaction mixture onto the HPLC at predetermined time intervals. The decrease of the drug concentration with time was followed by monitoring the sum of the diastereomers peak areas that elute at around 19 min and 22 min.

Kinetics of the hydrolysis at pH 3.8 were followed by withdrawing aliquots at time intervals from a reaction vial that was kept at 37°C in a water bath and storing the reaction samples at −18°C. Samples were allowed to warm to room temperature and assayed by HPLC.

Isomerization

The rates of isomerization of valganciclovir were determined at 37°C and an ionic strength of 0.15 M (potassium chloride). Rate constants for isomerization were determined by following the decrease of the R-diastereomer of the drug relative to the total drug concentration with time by HPLC at 256 nm. Aqueous solutions were prepared in 0.01 M buffer with a final concentration of 200 μg/mL. The reaction mixtures were placed into a water bath kept at 37°C. Aliquots (50 μL) were withdrawn at predetermined time

TABLE 1 HPLC Methodology

Parameter	Description
Equipment	Hewlett Packard 1090
Column	Zorbax SB-C18 5 μm, 4.6 × 250 mm
Mobile phase	Isocratic, 10:90 (1 g/L ammonium phosphate monobasic adjusted to pH 3 with phosphoric acid):acetonitrile
Mobile phase flow rate	1.0 mL/min
Injection volume	25 μL
Detection wavelength	256 nm
Temperature	Ambient
Approx. retention time	R: 19 min S: 22 min
Acquisition time	30 min

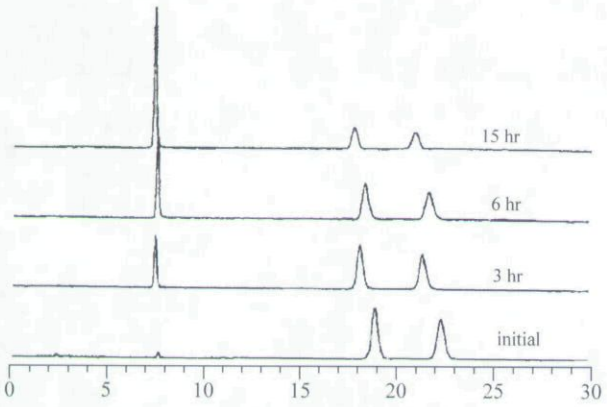

FIGURE 2 HPLC Chromatograms of Valganciclovir Hydrolyzed at pH 7.08 at 37°C. The Diastereomers Elute at Around 19 min and 22 min. Ganciclovir Elutes at 8 min. Valine Is Not Observed.

intervals and the reaction was quenched in citrate buffer (pH 2.9). Samples were assayed by HPLC.

First-order rate constants for the hydrolysis or isomerization were obtained by fitting the concentration vs. time data to an exponential equation using KaleidaGraph (Synergy Software, Reading, PA). The values for the second-order rate constants and the dissociation constant were obtained by best fitting the pH-rate profile using the non-linear regression function in KaleidaGraph.

High Performance Liquid Chromatography Methodology

Samples were analyzed by high performance liquid chromatography (HPLC) using the equipment and parameters described in Table 1.

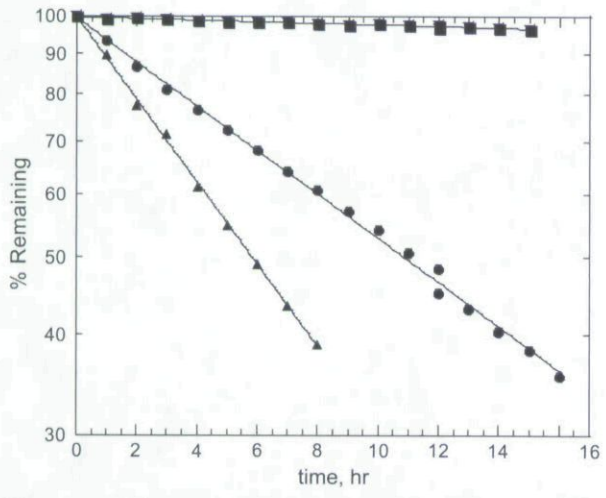

FIGURE 3 First-order Plots for the Hydrolysis of Valganciclovir at 37°C and pH 5.1 (■), 7.08 (●), and 9.66 (▲).

TABLE 2 First-Order Rate Constants for the Hydrolysis of Valganciclovir in Aqueous Buffers at 37°C

pH	k_{obs} (h^{-1})	$T_{1/2}$ (h)
3.81	0.00013	5300
5.11	0.0022	315
6.05	0.0154	45
6.49	0.028	25
7.08	0.060	11
7.44	0.076	9
7.87	0.085	8
8.95	0.11	6
9.66	0.21	3
11.48	3.82	0.2

pH Determination

pH solution measurements were made at 37°C using an Orion Triode™ pH meter (model 611, Orion Research Inc., Beverly, MA, USA) electrode calibrated with aqueous standard buffer solutions.

RESULTS AND DISCUSSION

Hydrolysis Reaction

The aqueous stability of valganciclovir was examined at 37°C from pH 3.8 to pH 11.5. The degradation of the drug was shown to correspond to the hydrolysis of the drug to ganciclovir and valine. Figure 2 shows typical HPLC chromatogram of a sample hydrolyzed at pH 7.08.

The reaction was found to be first order in drug concentration (Fig. 3) and the rate constants (k_{obs}) are summarized in Table 2. Examination of the data in Table 2 shows that the drug is relatively stable in acidic solutions with a half life of 220 days at pH 3.8. In

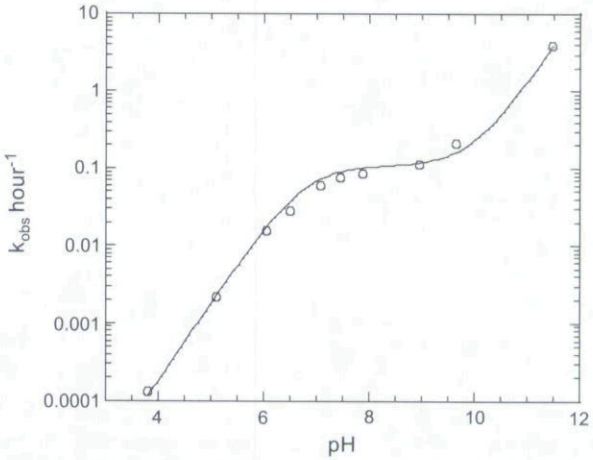

FIGURE 4 pH-rate Profile for the Hydrolysis of Valganciclovir.

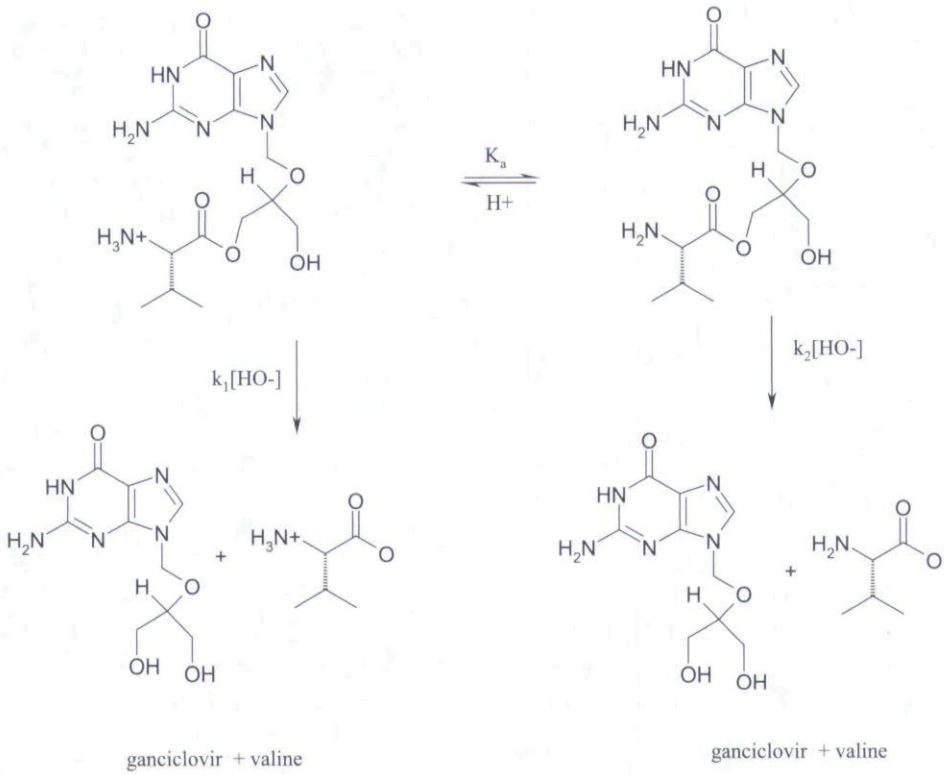

FIGURE 5 Reaction Scheme for the Hydrolysis of Valganciclovir to Ganciclovir and L-Valine.

neutral and basic solution, the drug is hydrolyzed to ganciclovir and valine with half-lives of 11 h and 3 h at pH 7.08 and 9.66, respectively.

The pH dependence of the reaction is shown in Fig. 4. The pH-rate profile is characterized by a curvilinear dependence of the rate constant on pH, changing from a proportional dependence on hydroxide concentration at low pH to little dependence at neutral pH. In strongly alkaline regions, the rate of hydrolysis is again proportional to the hydroxide concentration. The simplest interpretation of such dependence is shown in Fig. 5.

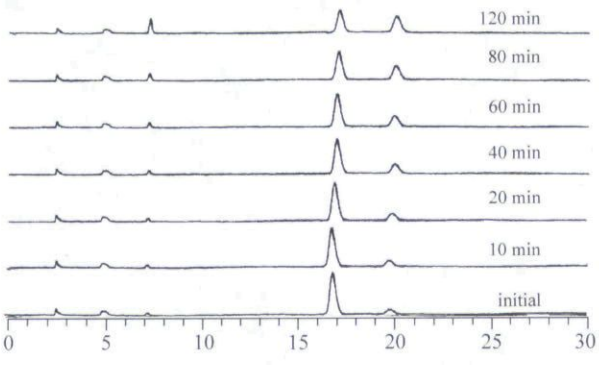

FIGURE 6 HPLC Chromatograms of the Isomerization of Valganciclovir at pH 7.98 at 37°C. The R-Diastereomer Elutes at 17 min and the S-Diastereomer Elutes at 20 min. Ganciclovir Elutes at 7 min.

Figure 5 describes a mechanism where the reaction of water with valganciclovir is not significant compared to the hydroxide ion catalyzed rate in the pH range of 4 to 11.5. In the acidic region, hydrolysis is due to the reaction of hydroxide with the protonated form of valganciclovir. At neutral pH where deprotonation occurs, both the neutral and protonated forms react with hydroxide ion. As the pH increases above pH 9, hydrolysis occurs by reaction of hydroxide with the less reactive neutral form of the drug. The pH

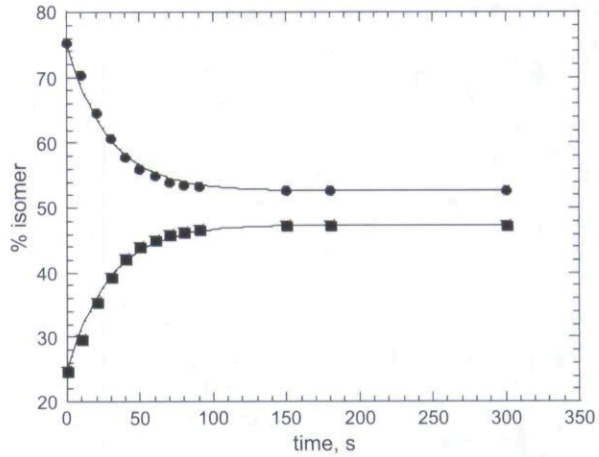

FIGURE 7 Time Dependence of the Approach to Equilibrium of the R-Diastereomers (●) and the S-Diastereomers (■) of Valganciclovir at pH 11.5, 37°C.

TABLE 3 First-Order Rate Constants for the Isomerization of Valganciclovir in Aqueous Buffers at 37°C

pH	k_{obs} (h^{-1})	$T_{1/2}$ (h)
3.81	0.0013	533
5.11	0.022	31.5
6.2	0.321	2.1
7.01	0.702	1
7.32	1.39	0.5
7.98	2.14	0.3
8.52	2.52	0.3
9.66	4.86	0.1
10.40	15.3	0.05
11.45	144	0.005

profile is described by Eq. 1, which is derived from Fig. 5.

$$k_{obs} = \frac{K_w}{[H+]}\left(\frac{k_1[H+] + k_2K_a}{[H+] + K_a}\right) \quad (1)$$

In Eq. 1, K_a is the ionization constant of the amine moiety of the valine group and K_w is $10^{-13.6}$, the autoprotolysis constant of water at 37°C. The solid line in Fig. 4 is calculated from Eq. 1 with values of $k_1 = 7 \times 10^5$ M^{-1} hr^{-1}, $k_2 = 1.3 \times 10^3$ M^{-1} hr^{-1}, and $K_a = 1.7 \times 10^{-7}$.

Isomerization Reaction

The rate of equilibration of diastereomers of valganciclovir was investigated. The equilibration corresponds to the isomerization shown in Fig. 1. Figure 6 shows a typical HPLC chromatogram of the reaction and percent remaining versus time is plotted in Fig. 7. The equilibration constant [R-diastereomers]/[S-diastereomers], was measured using equilibrium data and was shown to be pH independent in the pH region studied. First-order rate constants were calculated by fitting the R-diastereomers concentration relative to the total drug concentration vs. time to an exponential equation using 52% as the R-diastereomers concentration at equilibrium.

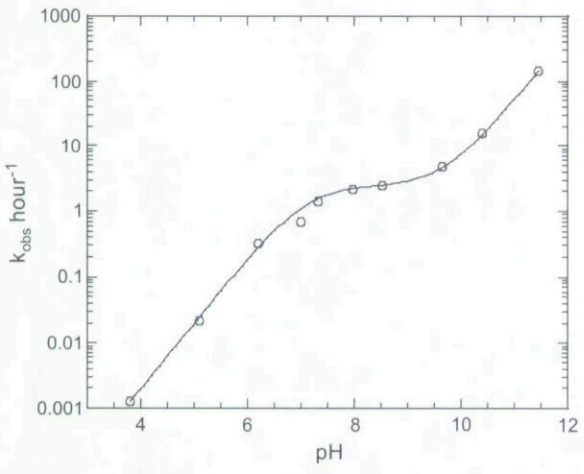

FIGURE 8 pH-Rate Profile for the Isomerization of Valganciclovir.

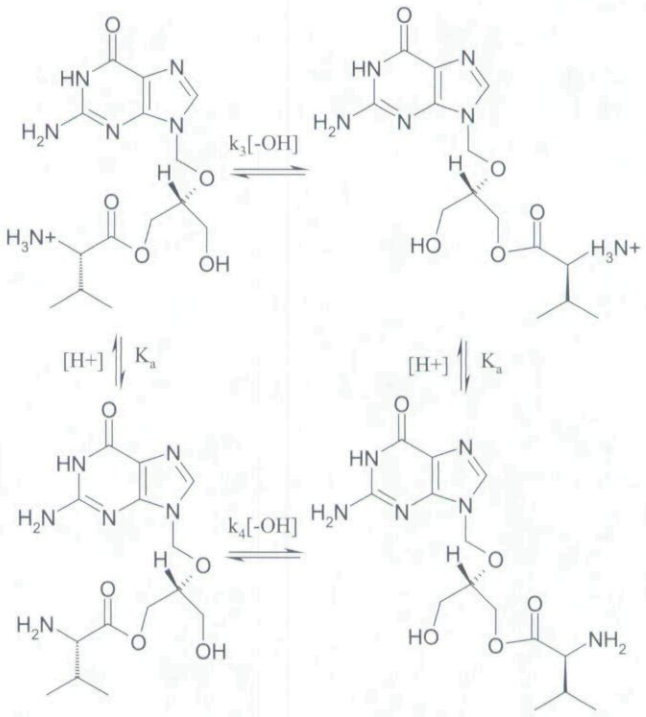

FIGURE 9 Reaction Scheme for the Isomerization of Valganciclovir.

First-order rate constants for the isomerization are summarized in Table 3. Comparison of the data in Tables 2 and 3 suggest that the isomerization reaction is about 10 fold faster than hydrolysis over the pH range studied. Figure 8 shows the pH-rate profile. The rate of isomerization displays a similar profile to the hydrolysis reaction. In acidic solution, isomerization is slow and the rates are proportional to hydroxide ion concentration. The rate becomes pH independent in neutral solution but increases with increasing pH in more basic solutions. The pH dependence can be interpreted in terms of the reaction scheme shown in Fig. 9 in which isomerization of both the neutral and protonated form of valganciclovir is catalyzed by hydroxide ion. In acidic solutions (pH<6), both isomers exist in the protonated form and hydroxide catalyzes the isomerization of the protonated species. Above pH 7.5, the diastereomers are neutral and hydroxide ion catalyzes the isomerization of this form.

The pH dependence derived from Fig. 9 can be fitted to Eq. 2. The solid line in Fig. 8 is calculated from Eq. 2. The values for of $k_3 = 9.7 \times 10^6\ M^{-1}\ hr^{-1}$, $k_2 = 2.4 \times 10^4\ M^{-1}\ hr^{-1}$, and $K_a = 8.1 \times 10^{-8}$.

$$k_{obs} = \frac{K_w}{[H+]}\left(\frac{k_3[H+] + k_4 K_a}{[H+] + K_a}\right) \qquad (2)$$

CONCLUSIONS

The kinetic results on the hydrolysis and isomerization of valgancicovir showed that both reactions had similar pH-rate profile with the isomerization reaction proceeding approximately 10 fold faster. The results suggest that stable aqueous solutions of valganciclovir can be prepared in aqueous solutions at acidic pHs but these solutions will exist as a mixture of isomers.

The long half-life of hydrolysis in acidic pH indicates that oral administration of valganciclovir will result in stable solution of the drug in upper GI. At neutral pH, the drug has a hydrolysis half-life of 11 h, which is considerably slower than its oral absorption rate with T_{max} of 1 h (Jung & Dorr, 1999), which suggests that hydrolytic stability of the drug should not affect its bioavailability.

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