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Kinetics of the decarboxylation of foscarnet in acidic aqueous solution and its implication in its oral absorption

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Summary

The kinetics of decarboxylation of the antiviral foscarnet (trisodium phosphonoformate) to form carbon dioxide and phosphorous acid was studied in aqueous solutions at 37°C over the pH range 0.2–3.6, using a direct spectrophotometric method as well as an HPLC procedure. The variation of the rate of degradation with pH could be accounted for in terms of a spontaneous decarboxylation of the undissociated acid form of foscarnet with a first-order rate constant of 0.10 min^{-1}. Half-lives for the degradation were 29 min, 231 min and 51 h at pH 1, 2 and 3, respectively. It was estimated that in the gastric pH range 1–2 and with a gastric emptying half-time of 50 min, 18–63% of an ingested dose would be decomposed in the stomach. It is concluded that intragastric degradation may be of significance for the absorption of foscarnet upon peroral administration.

Introduction

Foscarnet (trisodium phosphonoformate) is an antiviral agent with in vitro activity against all known human herpesvirus and some retrovirus including human immunodeficiency virus (HIV) (Sandström et al., 1985; Sarin et al., 1985; Öberg, 1983, 1989). This pyrophosphate analogue inhibits herpesvirus and retrovirus replication by inhibiting viral DNA synthesis and is currently undergoing clinical trials for the treatment of serious viral infections in patients with a deficient immune system. It has recently been marketed in several countries in the form of an aqueous solution for intravenous infusion.

Studies of the pharmacokinetics of foscarnet have shown that the drug is poorly and variably absorbed following oral administration. The extent of absorption of foscarnet in six HIV infected patients to which the drug was given orally as an aqueous solution varied between 12 and 22% (Sjövall et al., 1988). An absolute bioavailability of 5–16% in beagle dogs and 71–100% in rabbits has been reported after oral administration in saline solution (Ritschel et al., 1985) whereas a degree of absorption of 20–30% has been observed in rats after oral administration of aqueous drug solutions (Sjövall et al., 1988). The absorption mechanism of the highly hydrophilic foscarnet has recently been investigated in rats with in situ gastrointestinal loops and intestinal brush-border mem-

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Scheme 1.

brane vesicles (Tsuji and Tamai, 1989). It was shown that the absorption occurred primarily in the upper small intestine and was negligible from the stomach and large intestine. Evidence was furthermore presented to suggest that the absorption of foscarnet occurs mainly by a carrier-mediated process via the phosphate transport system existing in the intestinal brush-border membrane.

A limited capacity or the saturation of this carrier system may be a major cause for the poor absorption of foscarnet in man (Sjövall et al., 1988). An additional factor contributing to the incomplete and variable absorption pattern could, however, be acid-catalyzed degradation of the drug in the stomach. This factor was put forward by Ritschel et al. (1985) to explain the much higher absorption of foscarnet observed in rabbits (which have a high gastric pH) than in dogs.

Foscarnet is known to decompose in acidic solution to form carbon dioxide and phosphorous acid (Scheme 1) (Nylén, 1924). The kinetics and mechanism of the decarboxylation of foscarnet have been studied by Warren and Williams (1971) but only in strongly acidic solutions (0.4–3 M perchloric acid). To assess the potential impact of degradation of foscarnet in the stomach on its oral bioavailability we have investigated the kinetics of decarboxylation of the drug under conditions similar to those found during oral absorption.

Materials and Methods

Chemicals

Foscarnet was purchased from Sigma (St. Louis, U.S.A.). All other chemicals and solvents used were of analytical grade.

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostatically controlled cell compartment, using 1-cm quartz cells. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. High-performance liquid chromatography (HPLC) was performed with a system consisting of a Waters pump Model 510, a variable-wavelength UV detector Waters type Lambda Max 481 and a Rheodyne 20 μl loop injection valve. A reversed-phase Chrompack ChromSep steel column (100 × 4.6 mm) packed with Microspher C18 3 μm particles was used.

Kinetic measurements

All kinetic measurements were carried out in aqueous buffer solutions at 37 ± 0.2°C. Hydrochloric acid and phosphate were used as buffers. A constant ionic strength (μ) of 0.5 was generally maintained for each buffer solution by adding a calculated amount of potassium chloride.

The rates of decarboxylation of foscarnet were followed spectrophotometrically by monitoring the absorbance decrease accompanying the decarboxylation at 236 nm. Foscarnet shows an absorption maximum at this wavelength, the molar absorptivity being 160 $M^{-1}\,cm^{-1}$. Upon decarboxylation this absorption disappears almost completely, the absorption of a totally degraded reaction solution being less than 5% of the initial value. Reactions were performed in 2.5 ml aliquot portions of buffer solution in a thermostated quartz cuvette and were initiated by adding 200 μl of a stock solution of foscarnet (0.067 M) in water. The pH of the stock solution was about 3.5, achieved by addition of an appropriate amount of 1 M hydrochloric acid. The final concentration of foscarnet in the reaction solutions was 5×10^{-3} M. It was checked that the pH of the reaction solutions remained constant during degradation. Pseudo-first-order rate constants were determined from linear plots of $\log(A_t - A_\infty)$ vs time, where A_t and A_∞ are the absorbance readings at time t and at infinity, respectively, or by using the method of Guggenheim (1926). In the case of slower reactions, the reaction solutions (40 ml) were kept at 37°C in a water bath and at various intervals, the absorbance of an aliquot of the solution was measured at 236 nm.

The rates of decomposition of foscarnet were also in some cases followed by using a reversed-phase HPLC method involving retainment of foscarnet as an ion pair with tetrahexylammonium ion. The mobile phase was methanol-0.005 M sulphuric acid (5 : 95 v/v) with the addition of 2 mM tetrahexylammonium hydrogen sulphate. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 236 nm. Under these conditions foscarnet showed a retention time of 2.5 min. Quantitation of the compound was carried out by measuring the peak heights in relation to those of standards chromatographed under the same conditions. The reactions were initiated by adding 100 μl of a stock solution of foscarnet in water to 10 ml of pre-heated buffer solution in screw-capped test tubes, the final concentration of foscarnet being 5×10^{-4} M. The solutions were kept in a water bath at 37°C and at appropriate intervals samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual foscarnet against time.

Results and Discussion

Kinetics of degradation

The kinetics of decarboxylation of foscarnet was studied in hydrochloric acid solutions at 37°C over the pH range 0.20–2.0 and in phosphate buffer solutions at pH 2.5–3.6. At constant pH and temperature the degradation displayed strict first-order kinetics over more than four half-lives. Typical first-order plots of the degradation followed by the direct spectrophotometric method are shown in Fig. 1. In a few cases the rates of degradation were also followed by HPLC in order to verify the utility of the more convenient UV method. The pseudo-first-order rate constants (k_{obs}) determined by using the two different methods were identical within $\pm 5\%$.

The rate constants obtained are listed in Table 1. It can be seen that changes in the phosphate buffer concentration at pH 2.55 had no effect on the rate of degradation. It is further seen that changes in the ionic strength of the reaction solutions effected through addition of potassium chloride have a small effect, the rates being decreased with increasing ionic strength. This salt effect most likely arises from a dependence of the ionization constants of foscarnet upon the ionic strength.

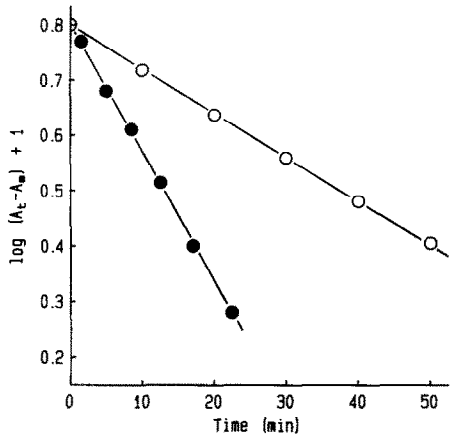


Fig. 1. Plots showing the apparent first-order kinetics of degradation of foscarnet in aqueous solution at pH 0.51 ($\mu = 0.5$) (●) and pH 1.23 ($\mu = 0.09$) (○) at 37°C.

The pH dependence of the rate of decarboxylation of foscarnet at 37°C is shown in Fig. 2 in which the logarithm of the observed pseudo-first-order rate constants has been plotted against pH. A plateauing is observed at low pH whereas the

TABLE 1

Rate data for the decarboxylation of foscarnet in aqueous solution at 37°C

Medium	μ	pH	k_{obs} (min^{-1})	$t_{1/2}$ (min)
HCl	0.86	0.20	7.14×10^{-2}	9.7
	0.5	0.51	5.28×10^{-2}	13.1
	1.0	0.92	2.73×10^{-2}	25.4
	0.5	0.92	2.90×10^{-2}	23.9
	0.32	0.92	2.98×10^{-2}	23.3
	0.19	0.92	3.40×10^{-2}	20.4
	0.09	1.23	1.84×10^{-2}	37.7
	0.5	1.23	1.66×10^{-2}	41.7
	0.5	1.57	1.01×10^{-2}	68.6
	0.5	1.92	4.15×10^{-2}	2.8 h
Phosphate (0.02 M)	0.5	2.55	6.14×10^{-4}	18.8 h
(0.05 M)	0.5	2.55	6.21×10^{-4}	18.6 h
	0.5	3.04	2.11×10^{-4}	54.7 h
	0.5	3.60	3.48×10^{-5}	332 h

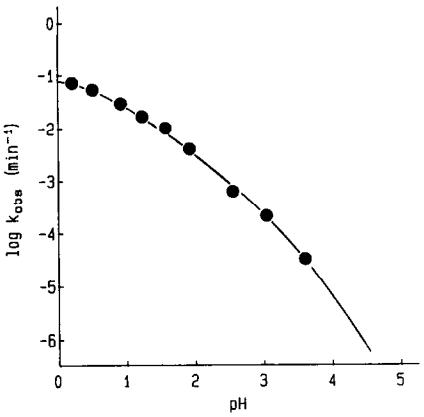


Fig. 2. The pH-rate profile for the decarboxylation of foscarnet in aqueous solution ($\mu = 0.5$) at 37°C. The points are experimental data whereas the full line is constructed from Eqn 1.

rate decreases sharply with decreasing pH for pH > 1.

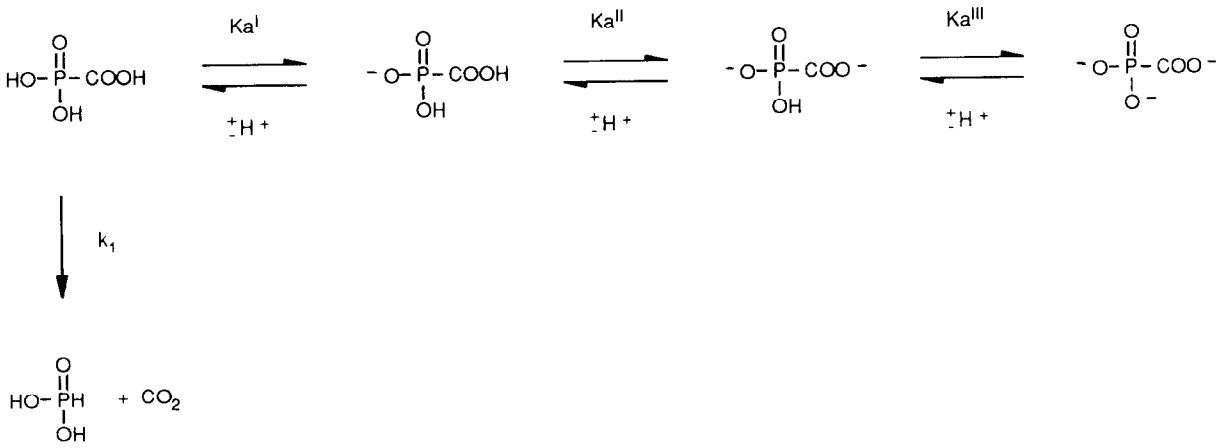
Foscarnet contains three acidic groups with pK_a values of 0.5 (P-OH), 3.4 (COOH) and 7.3 (P-OH) (Warren and Williams, 1971) and can therefore exist in the undissociated acid form and in three anionic forms. The shape of the pH-rate profile obtained (Fig. 2) suggests that the decarboxylation can solely be ascribed to a spontaneous or uncatalyzed reaction of the undissociated acid form (Scheme 2). According to this interpretation the following rate expression can be formulated:

$$k_{obs} = k_1 \frac{a_H^3}{a_H^3 + K_a^I a_H^2 + K_a^I K_a^{II} a_H + K_a^I K_a^{II} K_a^{III}} \quad (1)$$

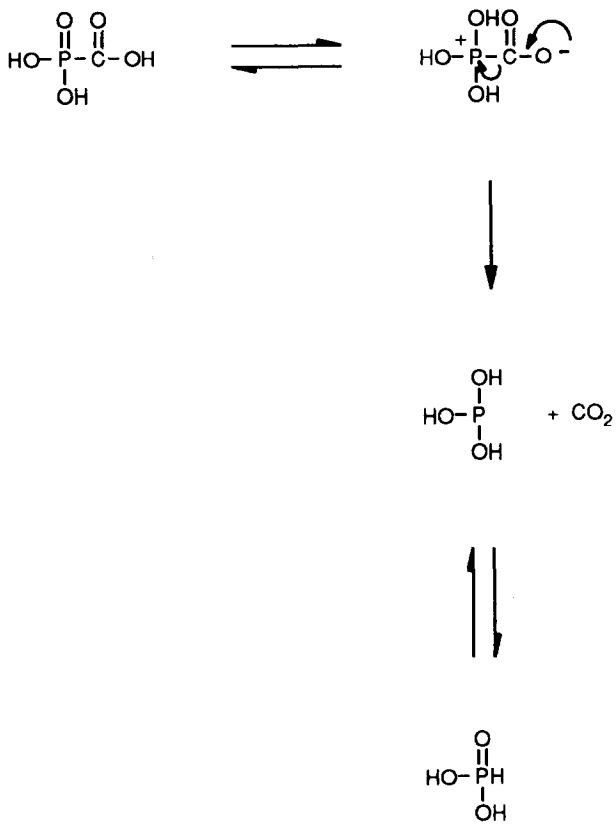
where k_1 is the first-order rate constant for the spontaneous decomposition of the undissociated acid form of foscarnet, a_H represents the hydrogen ion activity and K_a^I, K_a^{II} and K_a^{III} are the ionization constants of foscarnet. The fraction in Eqn 1 denotes the fraction of foscarnet occurring in the undissociated acid form as a function of a_H.

Using a value of 0.10 min^{-1} for k_1 and the K_a values (i.e., 10^{-pK_a}) mentioned above, the full curve drawn in Fig. 2 was constructed from Eqn 1. The good agreement seen between the experimental data and the full curve shows that Eqn 1 and hence Scheme 2 adequately account for the degradation kinetics in the pH range investigated.

As suggested by Warren and Williams (1971), a most likely mechanism for the spontaneous decarboxylation of foscarnet is a mechanism involving unimolecular decomposition of a zwitterion as depicted in Scheme 3. In addition to the spontaneous reaction, these authors reported the occur-



Scheme 2.



Scheme 3.

rence of an acid-catalyzed reaction in strongly acidic solution (up to 3 M perchloric acid). The present data show that such a reaction is without importance at pH values greater than 0.2.

The unreactivity of any ionized species of foscarnet makes the compound highly stable in weakly acidic, neutral and alkaline solutions where only a very minor fraction of the reactive undissociated acid form occurs. Thus, it can be predicted from Eqn 1 that at pH 5 and 37°C the pseudo-first-order rate constants for the degradation would be 7.7×10^{-8} min^{-1}, corresponding to a half-life of degradation of 17 years.

Bioavailability aspects

To assess the possible significance of the decarboxylation of foscarnet for the stability of the drug in the stomach after oral administration, important factors to be considered include the gastric acidity and the gastric emptying rate. These parameters vary among and within individuals and are influenced by numerous factors such as the emotional state and food ingestion (Griffith et al., 1968; Cooke, 1975; Mayersohn, 1979). The normal gastric pH is within the range 1–2 and the mean gastric emptying half-time is about 50 min (Heading et al., 1973; Theodorakis et al., 1980), in elderly people being about 160 min (Evans et al., 1980). Assuming the gastric emptying process to be a first-order process, the percentage of the amount of foscarnet surviving decarboxylation in the stomach can be calculated using the following equation for two parallel processes:

$$\% \text{ undegraded foscarnet} = \frac{k_{ge}}{k_{ge} + k_d} \times 100 \qquad (2)$$

where k_{ge} is the rate constant associated with the gastric emptying process (0.014 min^{-1} as derived from a $t_{1/2}$ value of 50 min) and k_d denotes the pseudo-first-order rate constant for the degradation of foscarnet. From Eqn 1, k_d is calculated to be 0.024 and 0.0030 min^{-1} at pH 1 and 2, respectively.

The values derived in this way are 37% for pH 1 and 82% for pH 2. With a gastric emptying half-time of 160 min the corresponding figures are 15% for pH 1 and 59% for pH 2. Thus, it appears that intragastric degradation of foscarnet may be of significance for the absorption of the drug following peroral administration, especially at low gastric pH and/or long gastric emptying times. The poor and variable bioavailability reported for foscarnet upon oral administration to man (Sjövall et al., 1988) can at least partly be attributed to decarboxylation of the drug in the stomach. Since foscarnet is not absorbed from the stomach (Tsuji and Tamai, 1989), it would be of interest to examine the oral bioavailability of the compound when given in an enteric-coated formulation or when given after medically induced low gastric acidity. An alternative way of increasing the bioavailability may be development of passively absorbed lipophilic and acid-stable prodrug forms of this highly hydrophilic agent. Various P- or C-esters of foscarnet including acyloxymethyl esters have been prepared (Norén et al., 1983; Iyer et al.,

1989; Strid et al., 1989) but the oral bioavailability of such compounds has not been reported.

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