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Kinetics of degradation of cyclosporin A in acidic aqueous solution and its implication in its oral absorption

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Summary

The kinetics of degradation of cyclosporin A was studied in aqueous solution at 37 and 60°C over the pH range 0.1–4.0. The predominant degradation reaction at pH < 2 was an isomerization or *N,O*-acyl rearrangement with formation of isocyclosporin A. A specific acid-catalyzed reaction dominated at pH < 0.5 whereas a spontaneous or water-catalyzed reaction contributed at higher pH values. Half-lives for the degradation were 63 and 79 h at pH 1.1 and 3.0, respectively, and at 37°C. It is concluded that intragastric degradation is of very minor importance for the absorption of cyclosporin A upon peroral administration.

Introduction

Cyclosporin A (CyA) (Fig. 1) is a neutral, lipophilic, cyclic undecapeptide that is widely used as an immunosuppressive agent to prevent the rejection of transplanted organs. A major problem with CyA therapy is the poor oral and variable bioavailability of the drug following peroral administration. The mean bioavailability is about 30% with interindividual variations ranging from 1 to 95% (Ptachcinski et al., 1986; Kahan, 1989; McMillan, 1989). The incomplete and variable systemic bioavailability has been attributed to dissolution-limited absorption, low intestinal wall permeability and first-pass metabolism by the liver and the gastrointestinal mucosa (Ptachcinski et al., 1986; Reymond et al., 1988; Ismailos et al., 1991; Tjia et al., 1991). One factor which has not been considered is acid-catalyzed degradation of the peptide in the stomach. Rüegger et al. (1976) showed several years ago that treatment of CyA with methanesulphonic acid in methanol or dioxane led to *N,O*-acyl migration of the *N*-methylvalyl group to the neighbouring secondary OH group with formation of isocyclosporin A (isoCyA) (Scheme 1). The isomerization is reversible, since isoCyA reverts into CyA in boiling dioxane (Rüegger et al., 1976).

We found that this rearrangement of CyA also occurs in aqueous acidic solutions and in this communication, we report the kinetics of CyA degradation in solutions of pH 0.1–4.0 and evaluate the potential impact of the acid-catalyzed

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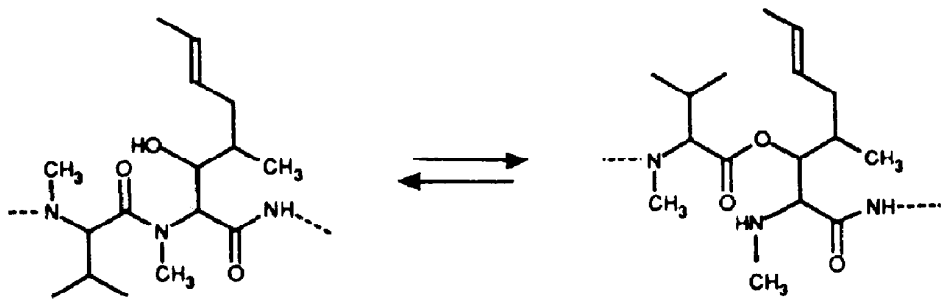


Scheme 1.

degradation on the bioavailability of the drug following peroral administration.

Materials and Methods

Chemicals

Cyclosporin A was obtained from Sandoz (Basle, Switzerland). Isocyclosporin A was prepared as described by Rüegger et al. (1976). All other chemicals were of analytical or HPLC grade.

Apparatus

High-performance liquid chromatography (HPLC) was performed with a system consisting of a Shimadzu Model LC-6A pump, a Shimadzu SPD-6A variable-wavelength UV detector, and a Rheodyne 7125 injection valve with a 20 μl loop. A reversed-phase Supelcosil LC-8-DB column (33 $\times$ 4.6 mm) (3 μm particles) equipped with a Supelguard 20 mm precolumn (both from Supelco Inc., U.S.A.) was used.

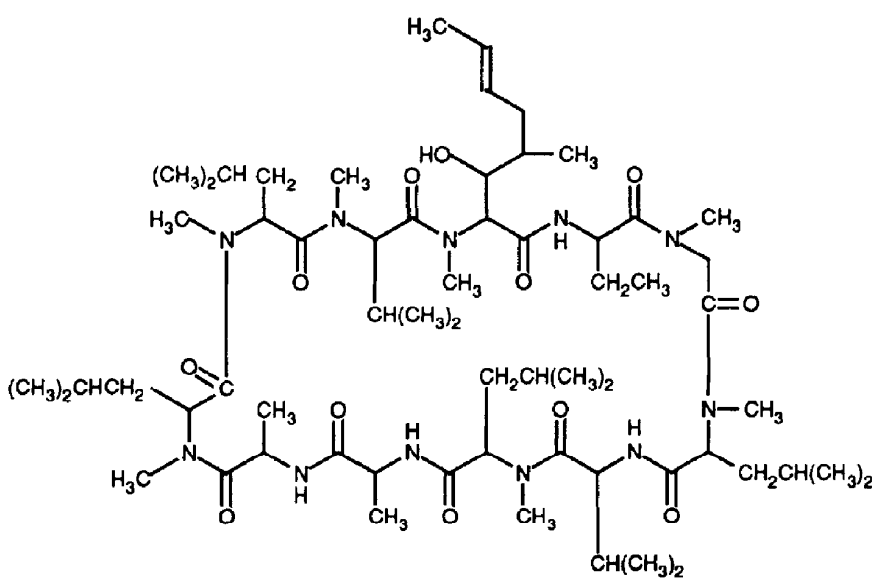


Fig. 1. Structure of cyclosporin A.

Kinetic measurements

The degradation of CyA was studied in standardized hydrochloric acid solutions (pH 0–2) and in a 0.02 M phosphate or acetate buffer solution of pH 3.0 and 4.0, respectively, at 37 or 60 ± 0.2°C. The ionic strength (μ) of the solutions was maintained at 0.5, when possible, by adding a calculated amount of potassium chloride.

The reactions were initiated by adding 100 μl of a stock solution of CyA in acetonitrile to 10 ml of the pre-heated buffer solution to give an initial concentration of about 5×10^{-6} M. The solutions were kept in screw-capped test tubes in a water bath. At appropriate times samples were taken and immediately analyzed by HPLC. The reversed-phase column was eluted at 70°C with a mobile phase consisting of acetonitrile-0.01 M phosphate buffer of pH 7.0 (1 : 1 v/v) with triethylamine added at a concentration of 10^{-3} M. The column effluent was monitored at 210 nm and the flow rate was 1.8 ml min^{-1}. Under these conditions, CyA showed a retention time of 8.5 min whereas isoCyA appeared after 12.2 min. Quantitation of the compounds was performed by measuring the peak heights in relation to those of standards, chromatographed under the same conditions. Pseudo-first-order rate constants for the degradation of CyA were determined from the slopes of linear plots of the logarithm of residual CyA against time.

Results and Discussion

The kinetics of degradation of CyA was studied in aqueous solutions at 60°C over the pH range 0.14–4.0. At constant pH and temperature, the disappearance of CyA displayed strict first-order kinetics over several half-lives. At pH < 2 the loss of CyA was accompanied by the formation of isoCyA in more than 80% yield as demonstrated by HPLC. No peaks other than that corresponding to isoCyA appeared in the chromatograms of completed reaction solutions. Further support for the formation of isoCyA in acidic solution was provided by an experiment in which the pH of the completed reaction solutions of pH 0.4 was adjusted to 8. The peak corresponding to isoCyA disappeared rapidly and a peak due to CyA reappeared. This behaviour is characteristic of the *N,O*-acyl rearrangement (Iwai and Ando, 1967). The kinetics of this reversed *N,O*-acyl transfer reaction has been reported in the following paper (Bundgaard and Friis, 1992). At pH 2–4 less isoCyA was formed than in the more acidic solutions. Since no other peaks were seen in the chromatograms the nature of the main degradation reaction remains unknown. A possible reaction may be addition of the hydroxyl group to the double bond in the C_9-amino acid, leading to a tetrahydrofuran derivative. Such a reaction has been shown to occur on treatment of CyA with 6 N HCl (Rüegger et al., 1976).

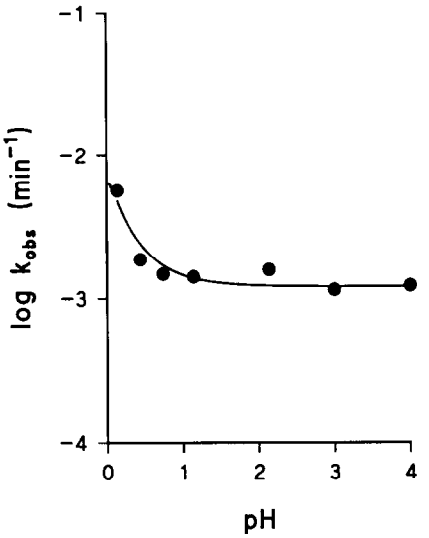
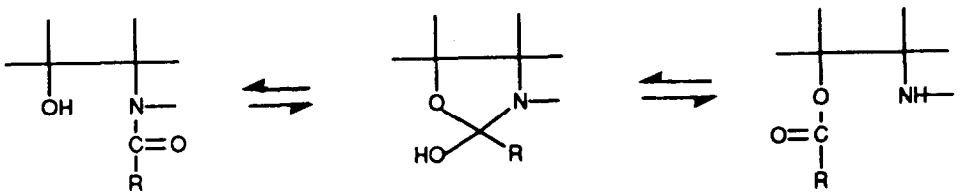


Fig. 2. pH-rate profile for the degradation of cyclosporin A in aqueous solutions at 60°C.

The pH dependence of the degradation of CyA at 60°C is shown in Fig. 2 in which the logarithm of the observed pseudo-first-order rate constants (Table 1) has been plotted against pH. The rate is independent of pH in the pH range 1–4 and increases at greater acidity. The following rate expression can be formulated:

$$k_{obs} = k_0 + k_{II} a_{II}$$



Scheme 2.

where k_H is a second-order rate constant for the specific acid catalyzed degradation, k_0 denotes the first-order rate constant for the spontaneous or water-catalyzed degradation and a_H is the hydrogen ion activity. The following values of these rate constants were found at 60°C: $k_H = 5.8 \times 10^{-3}$ M^{-1} min^{-1} and $k_0 = 1.3 \times 10^{-3}$ min^{-1}.

The *N,O*-peptidyl transfer reactions are generally thought to proceed through a hydroxyoxazolidine intermediate (Iwai and Ando, 1967) (Scheme 2). The observed shape of the pH-rate profile may indicate that a change in the rate-determining step occurs at pH around 1. Alternatively, a shift in the main route of degradation may take place at this pH.

To assess the possible significance of the isomerization of CyA for the stability of the drug in the stomach after oral administration, the degradation was also studied at 37°C at pH 1.1 and 3.0. At both pH values the rate decreased by a factor of 8 relative to that at 60°C, the half-lives being 63 h at pH 1.1 and 79 h at pH 3.0. The normal gastric pH is within the range 1–3 and the mean gastric emptying half-time is about 50 min (Theodorakis et al., 1980). It can readily be calculated that only 1–2% of an ingested dose of CyA undergoes degradation during passage through the stomach. Thus, degradation of CyA under acidic aqueous conditions appears to be of very minor importance for peroral bioavailability.

TABLE 1

Rate data for the degradation of cyclosporin A in aqueous solution at 60°C

Medium	μ	pH	k_{obs} (min^{-1})	$t_{1/2}$ (h)
HCl	1.0	0.14	5.6×10^{-3}	2.1
	0.5	0.44	1.9×10^{-3}	6.1
	0.5	0.74	1.5×10^{-3}	7.7
	0.5	1.14	1.4×10^{-3}	8.3
	0.5	2.14	1.6×10^{-3}	7.2
Phosphate (0.02 M)	0.5	3.00	1.2×10^{-3}	9.6
Acetate (0.02 M)	0.5	4.00	1.2×10^{-3}	9.6

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