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Prodrugs of peptides. 16. Isocyclosporin A as a potential prodrug of cyclosporin A

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Summary

The kinetics of degradation of isocyclosporin A (isoCyA) was studied in aqueous solution at pH 1–10 and in human plasma. At pH > 5 isoCyA was found to undergo a quantitative conversion to cyclosporin A (CyA) via a mechanism involving intramolecular aminolysis of the ester bond by the *N*-methyl amino group in the peptide. The pH-rate profile obtained for this *O*,*N*-acyl migration was accounted for in terms of spontaneous as well as hydroxide ion-catalyzed aminolysis. The half-life of the conversion of isoCyA to CyA was 21.7 h at pH 7.4 and 37°C and 12.1 h in 80% human plasma at 37°C. IsoCyA is suggested as a potential prodrug derivative of CyA which may be useful to improve the delivery characteristics of the parent drug.

Introduction

Cyclosporin A (CyA) (Fig. 1), a lipophilic, cyclic undecapeptide, is an immunosuppressive agent widely used to prevent allograft rejection in recipients of various organ transplants. However, the compound shows suboptimal delivery characteristics. Thus, its bioavailability following peroral administration is poor and highly variable (Ptachcinski et al., 1986; Kahan, 1989; McMillan, 1989) and parenteral administration is hampered due to the very low water solubility (0.03 mg ml^{-1} (Ismailos et al., 1991)) of the compound (Venkataram et al., 1990). Recently, CyA has attracted interest as an agent for treating psoriasis and other dermatologic disorders (Gupta et al., 1989; Griffiths and Voorhees, 1990) but, unfortunately, the degree of skin penetration of the drug is poor (Powles et al., 1988; Thomson and Payne, 1988; Griffiths and Voorhees, 1990).

A promising means to improve the delivery characteristics of CyA may be development of a prodrug or transport form with a better biphasic (water/lipid) solubility and which is capable of releasing the parent CyA in the blood or at the desired place in the organism. A potential prodrug of CyA may be isocyclosporin A (isoCyA) (Scheme 1). This cyclic isomer is formed from CyA under acidic conditions by an *N*,*O*-acyl migration (Rüegger et al., 1976; Fois and Ashley, 1991; Friis and Bundgaard, 1992). This isomeriza-

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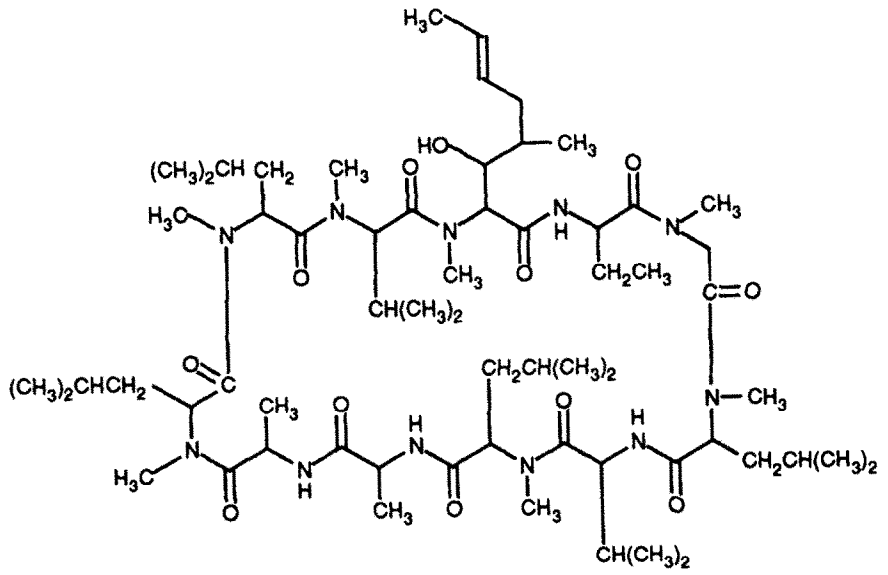


Fig. 1. Structure of cyclosporin A.

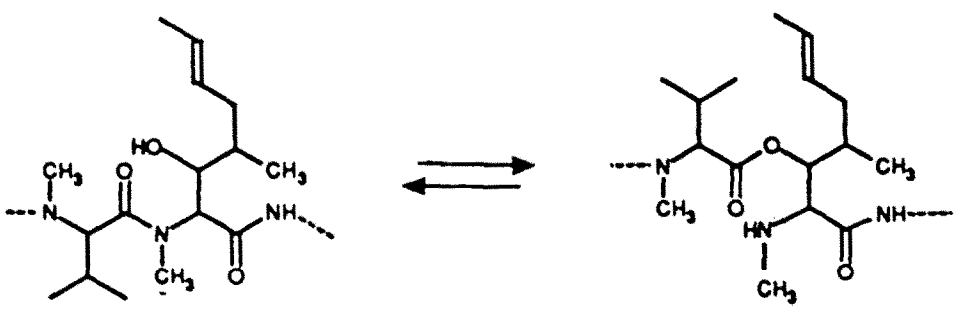
tion is reversed in boiling dioxane (Rüegger et al., 1976).

For evaluating the potential usefulness of isoCyA as a prodrug form it is important to determine the rate and extent of its potential conversion to the parent peptide under physiological conditions. In this work, we have examined the kinetics of the rearrangement of isoCyA to CyA in aqueous solution as a function of pH and temperature, and in human plasma.

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 and solvents used were of analytical or HPLC grade.



Scheme 1.

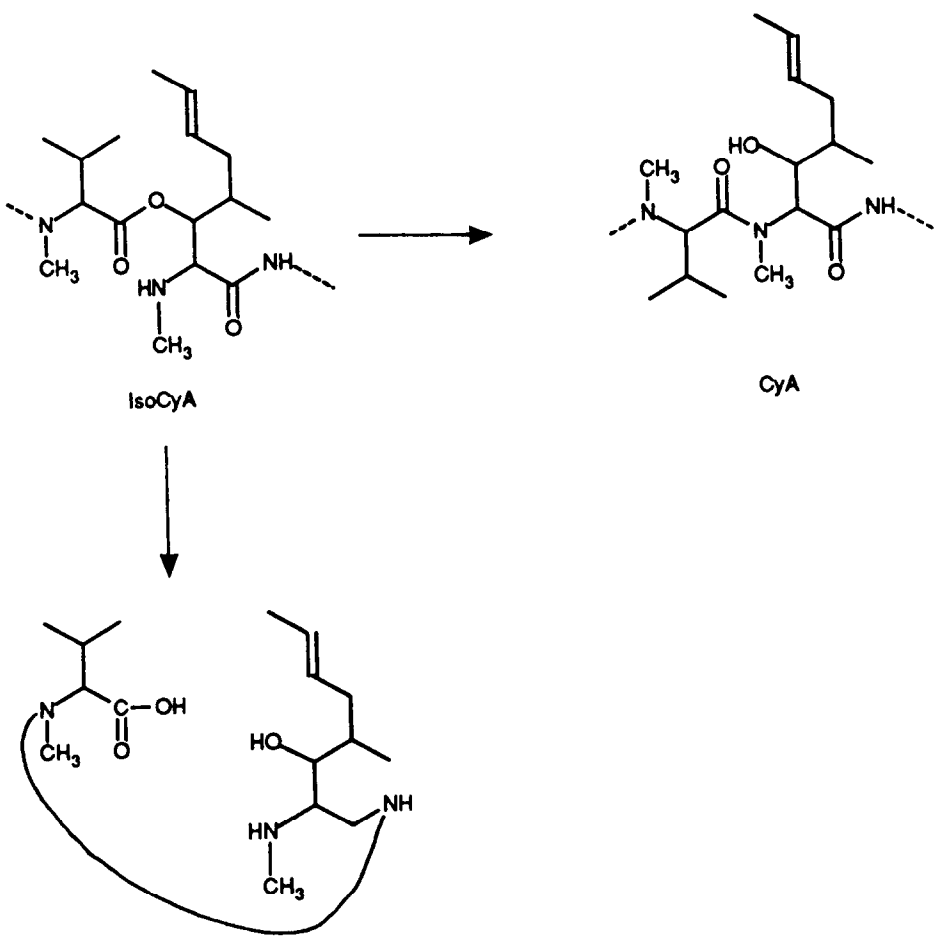
Apparatus

The HPLC equipment used was as described in the preceding paper (Friis and Bundgaard, 1992). Readings of pH were carried out on a Radiometer Type PHM 83 Autocal pH meter.

Kinetic measurements

The conversion of isoCyA was studied in aqueous buffer solutions at 60 ± 0.2°C and, in some cases, at 37 ± 0.2°C. Hydrochloric acid, acetate, phosphate and borate solutions were used as buffers; the total buffer concentration was generally 0.02 M. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The reactions were initiated by adding 100 μl of a stock solution of isoCyA in acetonitrile to 10 ml of pre-heated buffer solution in screw-capped test tubes, the final concentration being about 5×10^{-6} M. The solutions were kept in a water bath at constant temperature and at appropriate intervals, samples were withdrawn and analyzed immediately by HPLC using the conditions described in the preceding article (Friis and Bundgaard, 1992). This HPLC assay allowed adequate separation of isoCyA and CyA. Pseudo-first-order rate constants for the degradation of isoCyA were determined from the slopes of linear plots of the logarithm of residual isoCyA against time.



Scheme 2.

Degradation studies in plasma were performed with 80% human plasma (diluted with 0.05 M phosphate buffer of pH 7.4) at 37°C. An aliquot (250 μl) of the reaction solutions was mixed with 250 μl of a 2% solution of zinc sulphate in methanol-water (1:1 v/v) in order to deproteinize the plasma. The mixture was centrifuged for 3 min at 13000 rpm and 20 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

The kinetics of degradation of isoCyA was studied in aqueous solution at 60°C over the pH range 1.1–9.8. At pH > 5 the predominant degradation reaction was found to be intramolecular aminolysis (*O*,*N*-acyl transfer) of the ester bond by the adjacent *N*-methyl amino group to yield CyA (Scheme 1). At pH 7.4 and 60 or 37°C, CyA was found to be formed in a yield greater than 90%. A competing reaction may be hydrolysis of the ester bond in isoCyA to yield a ring-opened structure (Scheme 2). The main degradation reaction of CyA in acidic solutions (pH < 2) is isomerization to isoCyA (Friis and Bundgaard, 1992) and therefore, the isomerization is in fact a reversible reaction. However, the rate of degradation of isoCyA at pH 1–4 was found to be much slower than that of CyA whereas the opposite is the case at pH > 5. In agreement with this, no CyA was observed to be formed at pH 1–4. The main productive degradation pathway possible for isoCyA at these pH values may be hydrolysis of its ester bond.

The influence of pH on the overall rates of degradation of isoCyA is shown in Fig. 2 where the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. Some buffers showed a slight catalytic effect and in these cases, the k_{obs} values used in the plot in Fig. 2 were obtained by extrapolation of the k_{obs} values determined at different buffer concentrations to zero buffer concentration. The shape of the pH-rate profile indicates that the degradation

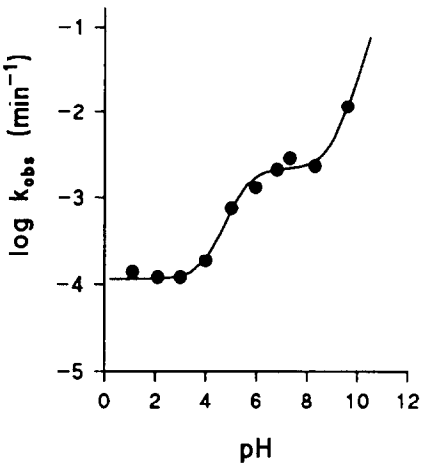


Fig. 2. pH-rate profile for the degradation of isocyclosporin A in aqueous solution (μ = 0.5) at 60°C.

can be described by the following equation:

$$k_{obs} = k_0 \frac{a_H}{a_H + K_a} + (k'_0 + k_{OH} a_{OH}) \frac{K_a}{a_H + K_a} \quad (1)$$

where k_0 is the first-order rate constant for the spontaneous or water-catalyzed degradation of the protonated species, k'_0 denotes a first-order rate constant for the spontaneous degradation of the unprotonated species, k_{OH} is a second-order rate constant for the apparent specific base-catalyzed degradation of the latter species, K_a represents the ionization constant for the protonated amino group, and a_H and a_{OH} are the hydrogen ion and hydroxide ion activity, respectively. The latter was calculated from the measured pH at 60°C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = \text{pH} - 13.02 \quad (2)$$

The unbroken line in Fig. 2 was constructed from Eqn 1 and the following values of the rate and ionization constants were found: $k_0 = 1.2 \times 10^{-4}$ min^{-1}; $k'_0 = 2.2 \times 10^{-3}$ min^{-1}; $k_{OH} = 2.4$ min^{-1} M^{-1}; pK_a = 5.4. The value of pK_a for the amino group as determined kinetically is in the range

expected for a compound containing electron-attracting α-amino and β-ester substituents.

There are three possible kinetically indistinguishable mechanisms which may account for the shape of the pH-rate profile in the neutral pH-region: (a) intramolecular nucleophilic attack by the unprotonated *N*-methyl amino group on the ester carbonyl moiety; (b) intramolecular general base catalysis by the amino group of the attack by a water molecule on the ester bond; and (c) intramolecular general acid catalysis by the unprotonated amino group of the attack by hydroxide ion on the ester bond (general acid, specific base catalysis of hydrolysis). Since CyA was the sole or predominant product of degradation in the neutral pH range, mechanism (a) is indicated. In contrast, the other mechanisms involve the formation of an ester hydrolysis product.

Since CyA was also the main degradation product at pH 9–10 where the OH^--catalyzed reaction predominates, the latter reaction may be described as hydroxide ion-catalyzed aminolysis, i.e., hydroxide ions behave as a general base capable of removing a proton from the ester group-attacking amino group. Hydroxide ion catalysis has previously been described for similar reactions such as the ring closures of various amino acid esters and dipeptide esters (Pilbrant, 1969; Purdie and Benoiton, 1973; Caswell et al., 1981) as well as of various cephalosporins (Bundgaard, 1976) to form piperazine-2,5-diones. Alternatively, the apparent OH^--catalytic effect may represent a change in the rate-determining step as the pH varies (cf. Caswell et al., 1981).

The influence of temperature on the rate of isomerization of isoCyA to CyA was examined at pH 7.4 (0.02 M phosphate buffer) over the temperature range 37–60°C. Treatment of the rate data according to the Arrhenius equation gave an energy of activation of 54 kJ mol^{-1} and a frequency factor of 8.1×10^5 min^{-1}. The observed half-lives for the conversion were 21.7 h at 37°C, 7.5 h at 50°C and 4.0 h at 60°C.

The half-life of degradation of isoCyA in 80% human plasma at 37°C was found to be 12.1 h. This is somewhat shorter than the half-life observed in a pH 7.4 buffer (21.7 h), indicating a small catalytic effect by plasma. HPLC analysis of the plasma reaction solutions showed that isoCyA was converted to CyA to an extent greater than 90%. Thus, plasma enzymes do not appear to hydrolyze the ester bond of isoCyA at the expense of intramolecular aminolysis to form CyA.

Conclusions

The results of this study show that isoCyA is a potential prodrug of CyA. It is quantitatively converted to CyA under physiological conditions. The rate of this conversion is probably slower than desired but this may depend on the intended use of the prodrug. In contrast to CyA, isoCyA contains a free amino group and this feature may give rise to improved water solubility at weakly acidic pH values. Studies are in progress to compare the in vitro skin penetration properties of isoCyA and CyA.

Acknowledgements

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