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Absorption kinetics of cyclosporin in the rat

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Abstract—Cyclosporin was administered (6 mg kg^{-1}, i.v.) over 15 min, or (10 mg kg^{-1}) by gavage, to two groups of 5 rats. Following i.v. infusion, cyclosporin exhibited triphasic behaviour with mean ± s.e.m. disposition half-lives of 9·0 ± 1·3 min, 4·0 ± 0·5 h and 16·0 ± 1·7 h. Following oral administration, peak blood concentration (C_{max}) of 1290 ± 93 ng mL^{-1} was reached after 5 h, when cyclosporin absorption essentially ceased. The absolute bioavailability (F) of cyclosporin was 24·0%. Standard laboratory rat chow consisting of 2% corn oil did not appear to alter cyclosporin absorption kinetics.

Cyclosporin, a fungal undecapeptide, is a potent immunosuppressant which is currently the drug of choice in preventing graft rejection and graft vs host disease in organ transplantation (Borel 1988; Venkataramanan et al 1989). The widespread use of cyclosporin and its success in transplantation has led to a steady accumulation of basic pharmacokinetic data in man. Animal pharmacokinetic data, particularly in the rat, are relatively scarce, owing primarily to analytical difficulties in measuring unchanged cyclosporin. The high sensitivity of the earlier polyclonal antibody radioimmunoassay (RIA) is outweighted by its lack of specificity; the HPLC assay, on the other hand, offers specificity but lacks sufficient sensitivity to enable small samples to be analysed. The introduction of a specific monoclonal antibody RIA (Sandimmun-Kit, Sandoz Ltd, Switzerland) which exhibits little cross-reactivity with cyclosporin metabolites (Ball et al 1988) has overcome such analytical problems. We have studied the pharmacokinetics of cyclosporin in the rat. Specifically, we report data on the rate and extent of oral absorption, with reference to an i.v. dose.

Materials and methods

Animal experiments. Male Sprague-Dawley rats (250 ± 10 g, Charles River, UK), housed on a 12 h light/dark cycle, were fasted for about 6 h before cyclosporin administration and for a further 10 h thereafter. A positive control group consisting of 5 rats with access to standard laboratory rat chow (CRM Food, Biosure, UK) was used. Water was freely available. Six hours before commencement of the investigation, a single Silastic catheter (cat no. 602-135, Dow Corning, USA) was inserted into the right carotid artery of each rat for arterial blood sampling. An additional jugular vein catheter, for slow infusion of the drug, was inserted into those rats receiving cyclosporin i.v. Surgery was performed under light anaesthesia (Fluothane, ICI Ltd, UK) and animals were allowed to recover before drug administration.

For oral administration, 1·0 mL of the original oral solution (100 mg mL^{-1}, Sandoz Ltd, Switzerland) was diluted to 20·0 mL with olive oil BP to obtain a concentration of 5 mg mL^{-1}. A single dose (10 mg kg^{-1}) was administered by gavage to 5 rats.

For i.v. administration, 1·0 mL of the original concentrated infusion (50 mg mL^{-1}, Sandoz Ltd) was diluted to 20·0 mL with normal saline solution BP, to obtain a concentration of 2·5 mg mL^{-1}. A single i.v. dose (6 mg kg^{-1}) was infused slowly over 15 min to 5 rats.

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Cyclosporin determination. Blood samples (50–100 μL) were collected serially into small plastic vials containing an 8% anticoagulant solution of EDTA (20 μL mL^{-1} blood) over 56 to 82 h. Each sample was immediately capped, vortex mixed and stored in the dark at −23°C. The total volume of blood taken (~1·0 mL) comprises only a small fraction of the total blood volume. Between collections, the cannula was flushed with a small volume of heparin solution (100 units mL^{-1} in normal saline (0·9% NaCl)).

Cyclosporin was measured by using a monoclonal specific antibody (Sandimmun-Kit, Sandoz Ltd, Switzerland). Radioactivity was measured by liquid scintillation counting (LKB Model 1218 Rackbeta, LKB-Wallac, Finland) with Optiphase, Highsafe II scintillant. Quench correction was by the external standard ratio method.

Data analysis. The following triexponential equation was fitted to individual i.v. data using an iterative weighted least-squares method,

$$C_t = \frac{R}{V_1}\left[(e^{-\lambda_1\tau} - 1)\cdot\frac{C_1'}{-\lambda_1}\cdot e^{-\lambda_1 t} + (e^{-\lambda_2\tau} - 1)\cdot\frac{C_2'}{-\lambda_2}\cdot e^{-\lambda_2 t} + (e^{-\lambda_3\tau} - 1)\cdot\frac{C_3'}{-\lambda_3}\cdot e^{-\lambda_3 t}\right] \quad (1)$$

where C_t is the concentration at any time t after stopping the infusion, τ is the duration of infusion, R is the infusion rate, V_1 is the initial volume of distribution, λ_1, λ_2, λ_3 are exponential coefficients, and C_1', C_2', and C_3' are the corresponding zero-time fractional coefficients (sum = 1) associated with a unit bolus dose.

The area under the blood concentration-time curve (AUC_{0-t}) was calculated by the linear trapezoidal approximation, and the area beyond the last observation (C*) was calculated as the ratio C^*/λ_3. The sum of these two areas was taken as an estimate of the total area under the curve ($AUC_{0-\infty}$).

Pharmacokinetic parameters such as the clearance (CL), volume of distribution at steady state (V_{ss}), and the disposition half-lives ($t_{\frac{1}{2}}$) were calculated from the data in the standard manner (Gibaldi & Perrier 1982). The maximum concentration (C_{max}) and the time to reach maximum concentration (t_{max}) were direct observations. The absolute bioavailability (F) was estimated by the following equation,

$$F = \frac{(AUC_{0-\infty})_{p.o.}}{(AUC_{0-\infty})_{i.v.}}\cdot\frac{Dose_{i.v.}}{Dose_{p.o.}} \quad (2)$$

where $(AUC_{0-\infty})_{p.o.}$ is the individual AUC following an oral dose and $(AUC_{0-\infty})_{i.v.}$ refers to the mean AUC after an i.v. dose.

The amount of cyclosporin absorbed after oral administration was determined by a numerical deconvolution method (Vaughan & Dennis 1978).

The absorption parameters in the fasted and fed states were compared statistically using a two-tailed Student's *t*-test taking $P < 0.05$ as statistically significant.

Results

The mean (± s.e.) blood concentration-time profiles are displayed in Fig. 1 and the mean (± s.e.) pharmacokinetic parameters derived from the profiles are shown in Tables 1 and 2.

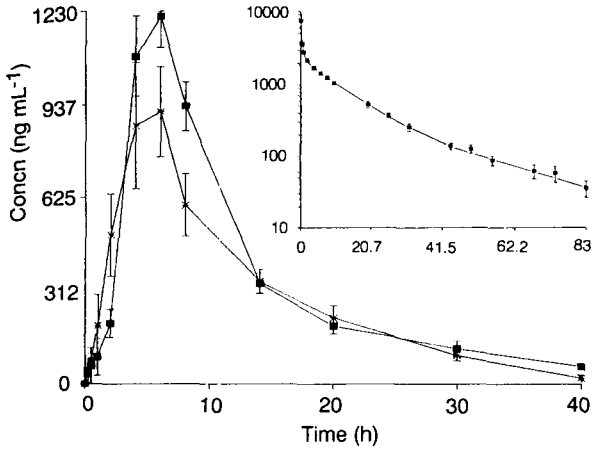

FIG. 1. Mean (±s.e.) whole blood cyclosporin concentration-time profiles following oral administration to fasted (■) (n = 5) and fed rats (+) (n = 5) and following i.v. infusion (n = 5) (inset).

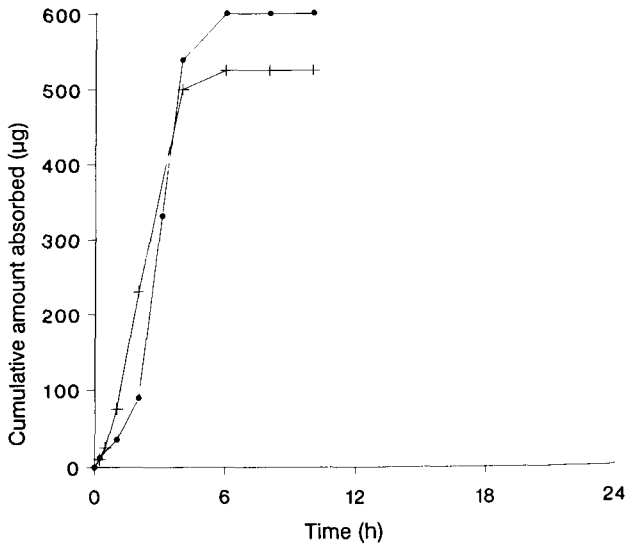

FIG. 2. Cumulative amount of cyclosporin absorbed with time after oral administration to fasted (●) and fed (+) rats.

Following infusion (Fig. 1. inset), cyclosporin exhibited multiphasic behaviour. The data were best fitted by a triexponential equation with half-lives of 9·0 ± 1·3 min, 4·0 ± 0·5 h and 16·0 ± 1·7 h. The blood clearance of cyclosporin ranged between 35·0 and 51·0 mL h^{-1} and the steady-state volume of distribution lay between 2·3 and 3·3 L kg^{-1}. The blood concentration-time profiles of cyclosporin exhibited a much higher degree of variability after oral than after i.v. administration (Fig. 1).

Following oral administration, a peak cyclosporin blood concentration of 1290 ± 93 ng mL^{-1} was reached after about 5 h. The cumulative amounts of cyclosporin absorbed with time after oral dosing under fasted and fed states are displayed in Fig. 2. Absorption was slow initially, then became more rapid, and ceased by about 6 h. The extent of absorption of cyclosporin was variable ($AUC_{0-\infty}$) = 15·1 ± 3·7 mg L^{-1} h and incomplete (24%). Standard laboratory rat chow did not appear to alter cyclosporin absorption kinetics (Table 1).

Table 1. Mean (±s.e.) pharmacokinetic parameters of cyclosporin following a 15 min i.v. infusion of cyclosporin (6 mg kg^{-1}) to five rats.

Parameter	Mean (±s.e.)
AUC (mg L^{-1} h)	38 (2·8)
CL (mL h^{-1} kg^{-1})	160 (13)
V_{ss} (L kg^{-1})	2·7 (0·2)
C_1	0·71 (0·09)
C_2	0·19 (0·01)
C_3	0·10 (0·03)
$t_{\frac{1}{21}}$ (min)	9·0 (1·3)
$t_{\frac{1}{22}}$ (h)	4·0 (0·5)
$t_{\frac{1}{23}}$ (h)	16·0 (1·7)

Table 2. Mean (±s.e.) absorption characteristics of cyclosporin after administration of a dose (10 mg kg^{-1}) by gavage to fasted and fed rats.

Parameter	Fasted (n = 5)	Fed (n = 5)	*t*-test
C_{max} (ng mL^{-1})	1290 (93)	1040 (183)	ns
T_{max} (h)	5·0 (0·5)	5·0 (1·0)	ns
AUC (mg L^{-1} h)	15·1 (3·7)	12·9 (2·5)	ns
F	24	21	—

ns, not significant.

Discussion

The i.v. study showed that in the rat, cyclosporin has a low clearance and a high volume of distribution, the latter being due to extensive distribution into many tissues, particularly fat (Atkinson et al 1983; Bernareggi & Rowland 1991).

As in other species, including man (Grevel et al 1986; Gridelli et al 1986), cyclosporin absorption in the rat is slow, incomplete and variable. This is best attributed to its poor aqueous solubility (0·04 mg mL^{-1} at 25°C, (Cavanak & Sucker 1986)) and high mol. wt of 1202 daltons (Wenger et al 1986), which limit its dissolution and possibly its intestinal permeability, respectively. The possibility of first pass hepatic loss can be discounted, given the low clearance of cyclosporin in the rat. The small intestine transit time of polystyrene microspheres in the rat is about 195 min (Saffran et al 1986) and if one includes consideration of mean gastric emptying time (about 2 h (Harris et al 1990)) the average time before orally administered material leaves the small intestine is approximately 5 h. This time coincides with the cessation of cyclosporin absorption (Fig. 2), suggesting that absorption is limited to the small intestine. This conclusion is consistent with that reported in dog (Gridelli et al 1986), and in man (Grevel et al 1986). The mean absolute bioavailability (F) of cyclosporin of 24% was estimated on the assumption that the mean $(AUC_{0-\infty})_{i.v.}$ and, hence mean clearance, was representative of all rats. This is reasonable given the relatively low coefficient of variability in clearance of about 18% among the animals receiving the drug intravenously. This value of F in rats is comparable with an absolute bioavailability value of 30% reported in healthy volunteers (Ptachcinski et al 1986).

Although drug absorption, in general, is affected by gastrointestinal contents, few studies have looked at the influence of food on cyclosporin absorption (Ptachcinski et al 1985; Wood & Lemaire 1985; Keogh et al 1988). Attempts have been made to use standardized diets as far as possible, and the findings of reports in the literature imply that food generally has an

inconsistent effect on cyclosporin absorption. In this investigation, standard rat chow with 2% corn oil did not appear to alter the oral absorption of cyclosporin from the gastrointestinal tract. For ethical reasons, in laboratory investigations, one would not wish to subject animals to any greater degree of stress than is necessary. Our findings suggest that if one does not wish to fast animals before cyclosporin oral administration, standard rat chow is a suitable feed.

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Toloxatone pharmacokinetics in the plasma and cerebrospinal fluid of the rabbit

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Abstract—The pharmacokinetics of toloxatone (5 and 10 mg kg^{-1}, i.v.) was studied in anaesthetized rabbits. There was a biexponential decline in plasma concentration with time. No differences were observed in the pharmacokinetic parameters with the increase of the dose. The terminal half-life was short ($47{\cdot}4 \pm 2{\cdot}8$ and $41{\cdot}5 \pm 4{\cdot}2$ min for 5 and 10 mg kg^{-1}, respectively). The total clearance was 79 ± 18 mL min^{-1} after a dose of 5 mg kg^{-1} and 106 ± 40 mL min^{-1} after a dose of 10 mg kg^{-1}. The volume of distribution was $5{\cdot}8 \pm 2{\cdot}8$ (5 mg kg^{-1}) and $5{\cdot}4 \pm 1{\cdot}8$ L (10 mg kg^{-1}). The average percentage of toloxatone bound to plasma protein was 30% and was not affected by concentrations within the investigated range. In cerebrospinal fluid (CSF), the highest concentrations of toloxatone were obtained within 15 min after the end of the injection. The CSF level of toloxatone was equal to that of plasma unbound toloxatone and declined at a rate similar to toloxatone in plasma. These results suggest that the toloxatone passage through the blood–brain barrier may be completed by passive diffusion. In addition, the unbound plasma concentration would accurately reflect the toloxatone concentration in CSF and could be a useful tool for drug monitoring.

A new generation of reversible, selective MAO-A inhibitors has recently been developed with fewer potentially dangerous side effects. One of these, toloxatone (5-(hydroxymethyl)-3-(3-methylphenyl)-2-oxazolidinone), has been found to be an effective antidepressant and is now used for this indication. However, although metabolic studies with this drug (Malnoë & Strolin Benedetti 1975, 1979; Strolin Benedetti et al 1982) are amply documented, no data on the penetration of toloxatone into the cerebrospinal fluid (CSF) has been published.

The present study was carried out to determine the level of toloxatone in the plasma and CSF after an i.v. injection in anaesthetized rabbits, a species in which we are studying the effects of toloxatone and other monoamine oxidase type A inhibitors on the central monoamine systems.

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Materials and methods

Animals. Adult male Fauve de Bourgogne rabbits, 2·5–3·0 kg (Elevage Scientifique des Dombes, Châtillon sur Chalaronne, France), were housed individually in stainless steel cages and were maintained on a 14 h light/dark cycle with lights on from 0600 to 1900 h in a controlled environment (temperature 20–22 °C and relative humidity 40–65%). The animals were acclimatized for one week before the start of the experiments and had free access to a commercial diet (Alimentation UAR, Epinay sur Orge, France) and water.