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Pharmacokinetics and Absolute Bioavailability of Cyclosporin Following Intravenous and Abomasal Administration to Sheep

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Abstract—Cyclosporin A pharmacokinetics were studied following intravenous and abomasal dosing in an open, crossover study in healthy, merino ewes. Five different doses of cyclosporin A were dispersed in milk and administered into the abomasum through a surgically inserted fistula which simulates oral administration. Cyclosporin A was well tolerated. Whole blood concentrations of cyclosporin A were measured by HPLC and mean clearance ($0{\cdot}45 \pm 0{\cdot}05$ L h^{-1} kg^{-1}), distribution volume ($4{\cdot}4 \pm 2{\cdot}0$ L kg^{-1}), mean residence time ($9{\cdot}6 \pm 4{\cdot}1$ h) and half-life ($12{\cdot}1 \pm 3{\cdot}1$ h) were calculated. Negligible cyclosporin A was excreted in urine or bile. Area under the curve increased proportionally with doses up to 26·3 mg kg^{-1}, but was curvilinear above this dose. Abomasal bioavailability at 6·4 mg kg^{-1} was $0{\cdot}26 \pm 0{\cdot}09$, and mean absorption time was $4{\cdot}7 \pm 11{\cdot}1$ h. Considerable pharmacokinetic variability was observed, particularly after abomasal administration. Cyclosporin A pharmacokinetics in sheep lie within the values reported in man after renal, bone marrow and cardiac transplantation.

Detailed studies of the clinical pharmacokinetics of cyclosporin A are impeded by several factors including ethical problems in healthy volunteers due to significant adverse effects, unpredictable changes in cyclosporin A disposition with time and the risk of interactions with other medication. Accordingly, rats (Hedayati et al 1992), rabbits (Sawchuk & Awni 1986) and dogs (Gridelli et al 1986) have been used to study cyclosporin A disposition. Rats and rabbits are often unsuitable for major pharmacokinetic studies, especially if large numbers of specimens are required for an extended period. Dogs are difficult to handle and require constant supervision and many authorities are now insisting that clinical studies be restricted to specific and expensive breeds such as the beagle.

The sheep offers a possible alternative for studying cyclosporin A pharmacokinetics. While sheep have been used in other drug studies the pharmacokinetics of cyclosporin A in these animals have not been reported before. We have investigated the absolute bioavailability and single dose pharmacokinetics of cyclosporin A in the merino ewe after administration by two routes, intravenous infusion and following administration into the abomasum, the glandular stomach of the sheep. The latter simulated oral administration in man and other monogastric animals.

Materials and Methods

Animals

Five healthy, adult merino ewes aged between 1 and 3 years, 31 to 36 kg, were housed in individual metabolic cages (Till & Downes 1963) in an air-conditioned room (22°C–25°C, r.h. 55%). During the acclimatization and treatment periods the animals were fed a high quality Lucerne hay diet and water was freely available. Four weeks before the study an abomasal cannula was surgically implanted into the abomasum of each sheep.

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Cyclosporin administration

For the abomasal study, the animals were assigned to five treatments (A through E) in a Knight's latin-square, crossover design in which the order of administration of different doses was unique with respect to rows, columns and diagonals of the treatment matrix. Treatments A (2·7 mg kg^{-1}), B (6·4 mg kg^{-1}), C (13·0 mg kg^{-1}), D (26·3 mg kg^{-1}) and E (41·0 mg kg^{-1}) were separated by a minimum washout period of 10 days. Animals were fasted for 24 h commencing at 0800 h on the day before cyclosporin administration. On the morning of the study the animals were weighed and an external jugular vein was cannulated using a 15G Dwelcath catheter (Tuta Laboratories, Lane Cove, NSW) which was kept patent by intermittent infusion with heparin saline solution. Cyclosporin A oral solution (100 g L^{-1}; Sandimmun, Sandoz Australia Pty Ltd, North Ryde, NSW) was drawn up in the measuring pipette supplied with the commercial product and dispensed according to the manufacturer's instructions. The required volume of oral solution was added to 20 mL of cow's milk in a 50 mL glass beaker and thoroughly dispersed by repeatedly withdrawing and expelling the liquids through a 50 mL syringe. The contents of the beaker were drawn into the same syringe and injected through a rubber septum into the abomasum. The beaker and syringe were quickly rinsed with a further 20 mL and then 10 mL of milk and the washings injected without delay. Three days before the intravenous study, a biliary cannula was inserted into the common bile duct and exteriorized and maintained as described previously (Caple 1977). On the morning of the study an 8 F/G urinary Foley catheter was inserted and a second cannula was positioned in the external jugular vein opposite to that used for blood collection.

Cyclosporin A (6·4 mg kg^{-1}) was diluted with 250 mL of sterile 5% w/v dextrose saline solution, a vehicle in which it is known to be stable (Ptachcinski et al 1986a), and infused through the second cannula at a constant rate over 60 min via a paediatric micro-drip administration set (Baxter Healthcare Pty Ltd, Toongabbie, NSW).

Blood, urine and bile sampling

During the abomasal study, blood (3 mL) was drawn into polypropylene EDTA collection tubes at 20-min intervals up to 1 h, 30-min intervals up to 4, 5, 6, then every 2 h to 12, 18 and 24 h, then every 12 h to 72 h after dosing. For intravenous studies, blood sampling was performed immediately after termination of the infusion, then at 20-min intervals up to 4 h, at 4·5, 5·0, 6·0, 7·0, 8·0, 10·0 and 12·0 h, and then at 12-h intervals up to 72 h. Urine and bile was collected at 4-h intervals up to 12 h, from 12 to 24 h, then at 24-h intervals up to 72 h. The volume of urine and bile collected during each interval was measured and recorded, a 10 mL specimen was retained and the remainder discarded. Blood, urine and bile were stored at −75°C.

Analytical

Concentrations of cyclosporin A in whole blood, urine and bile were measured by reversed-phase HPLC (Charles et al 1988). The assay is linear from 45 μg L^{-1} to at least 2000 μg L^{-1} ($r > 0{\cdot}999$; within-day and between-day CV < 9·0%). The accuracy, precision and specificity of the assay has been assessed through routine participation in international quality assurance programs (Pippinger 1985; Johnston et al 1986). Serum biochemistry, urinalysis and haematology was performed on specimens drawn immediately before and after each of the abomasal studies and the intravenous study.

Pharmacokinetics

In the abomasal study the observed peak cyclosporin A concentration (C_{max}) and the observed time to peak (t_{max}) were recorded. Elimination half-life ($t_{\frac{1}{2}}$) was calculated by dividing the slope of the terminal phase (obtained by linear regression) into the natural logarithm of 2. Area under the curve (AUC_∞), and area under the first moment curve ($AUMC_\infty$) from zero time to infinity were calculated by numerical integration using the log trapezoidal rule. Non-compartmental pharmacokinetic parameters were calculated as previously described (Yamaoka et al 1978; Perrier & Mayersohn 1982).

$$MRT = \frac{AUMC_\infty}{AUC_\infty} \quad (1)$$

$$MRT_{iv} = MRT - \frac{T}{2} \quad (2)$$

$$MAT = MRT_{ab} - MRT_{i.v.} \quad (3)$$

where MRT is the mean residence time after abomasal (ab) and intravenous (i.v.) administration, T is the infusion duration and MAT is the mean absorption time after abomasal administration.

Total clearance (CL) and steady-state distribution volume (Vd_{ss}) were determined at a dose (D) of 6·4 mg kg^{-1}. For abomasal dosing the parameters CL/F and Vd_{ss}/F were reported.

$$CL = \frac{D}{AUC_\infty} \quad (4)$$

$$Vd_{ss} = CL \cdot MRT \quad (5)$$

The absolute bioavailability (F) of cyclosporin A was determined at a dose of 6·4 mg kg^{-1} using the relationship:

$$F = \frac{AUC_{\infty,ab}}{AUC_{\infty,i.v.}} \quad (6)$$

Results

All animals remained healthy throughout the study. Mean cyclosporin A concentrations in blood following intravenous administration and abomasal administration are presented in Figs 1, 2, respectively. Pharmacokinetic parameters calculated following administration of five different abomasal doses are contained in Table 1, while Table 2 shows the

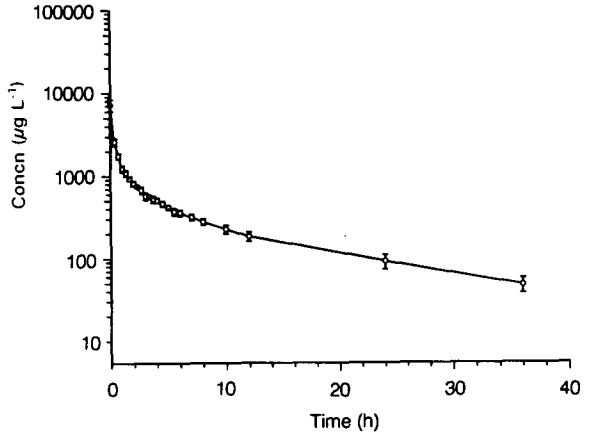

FIG. 1. Mean (±s.e.) concentrations of cyclosporin A in whole blood following termination of a 60 min intravenous infusion of cyclosporin A (6·4 mg kg^{-1}) to five sheep.

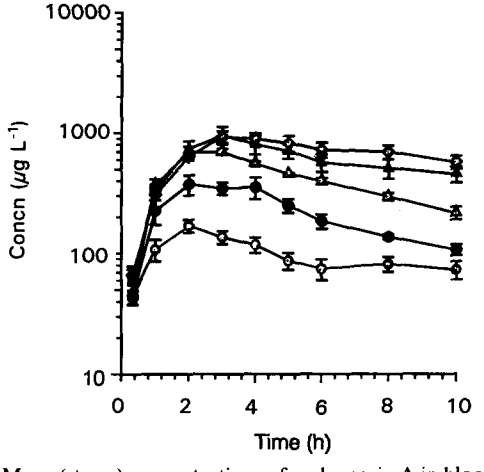

FIG. 2. Mean (±s.e.) concentrations of cyclosporin A in blood after abomasal administration to five sheep; 2·7 (○), 6·4 (●), 13·0 (△), 26·3 (▲), 41·0 (◇) mg kg^{-1}.

Table 1. Pharmacokinetic parameters following abomasal administration of cyclosporin A to five sheep.

Dose (mg kg^{-1})	AUC_∞ (μg L^{-1} h)	C_{max} (μg L^{-1})	t_{max} (h)	MRT (h)	$t_{\frac{1}{2}}$ (h)
2·7	1525±1068	182±43	3·0	10·8± 9·1	7·5±7·5
6·4	3789±1878	447±177	2·5	14·3±11·8	10·8±10·4
13·0	7303±1784	757±153	3·0	13·3± 4·1	10·4± 3·3
26·3	14015±4464	977±347	3·0	19·3± 5·7	13·4± 6·0
41·0	17014±4265	1012±272	4·0	19·8± 2·3	13·0± 4·8

Mean ± s.d.

Table 2. Pharmacokinetic parameters following administration of abomasal and intravenous cyclosporin A (6·4 mg kg^{-1}) to five sheep.

Route	AUC_∞ (μg L^{-1} h)	F	MRT (h)	MAT (h)	CL/F (L kg^{-1} h^{-1})	Vd_{ss}/F (L kg^{-1})	$t_{\frac{1}{2}}$ (h)
Abomasal	3789±1878	0·26±0·09	14·3±11·8	4·7±11·1	1·94±0·65	22·3±7·4	10·8±10·4
Intravenous	14306±1605	1·00±0·00	9·6±4·1	—	0·45±0·05	4·4±2·0	12·1±3·1

Mean ± s.d.

results of pharmacokinetic analyses from the abomasal and intravenous data at a dose of 6·4 mg kg^{-1}. There was considerable inter-animal variation in cyclosporin A kinetics, particularly at the lower abomasal doses.

Only non-quantifiable traces of cyclosporin A could be detected in urine and bile by either route of administration.

Discussion

Sheep have been widely used in many areas of medical research because they are more similar to man than many other laboratory animals (Hecker 1983). Abomasal emptying and intestinal motility in sheep is controlled by duodenal feedback processes similar to those in man (Ruckebusch et al 1991). Sheep have a large blood volume, are docile and easy to handle, and are inexpensive to procure and maintain. The simple, direct administration of cyclosporin A through an external fistula into the abomasum (true stomach) provides a closer representation of the gastric anatomy of man, compared with the complex system in the ruminant, and is equivalent to oral dosing. This technique also minimizes drug loss through spillage or regurgitation which can occur when liquid dosage forms of drugs are administered by mouth to animals. Abomasal cyclosporin A was administered to the sheep as a dispersion in milk which is one of the main vehicles recommended for clinical use. Doses used in the study encompassed those used most frequently for the prevention of graft rejection and for treatment of autoimmune diseases.

Two higher doses (26·3, 41 mg kg^{-1}) were also administered to determine if there is any dose-dependency in the disposition of cyclosporin A as well as to provide information on possible toxicity. Non-compartmental (statistical moment) methods were used to estimate pharmacokinetic parameters due to the differences in the number of identifiable, log-linear phases in the cyclosporin A blood level data following administration by either route. Further, double peaks and shoulders in some of the data precluded the use of computer curve-fitting models containing sums of exponentials.

The absence of cyclosporin A in sheep urine or bile indicates that, as in man and other mammals, renal and biliary clearance represent negligible routes of cyclosporin A elimination. Mean clearance (0·45 L kg^{-1} h^{-1}), distribution volume (4·4 L kg^{-1}) and absolute bioavailability (0·26) of cyclosporin A in sheep were similar to that reported for man following renal, cardiac and bone marrow transplantation (Ptachcinski et al 1986b; Shaw et al 1987; Gupta et al 1987; Luke et al 1992). The mean, terminal cyclosporin A half-life in sheep following intravenous administration (12·1 h) and oral administration (7·5–13·4 h) lies within the range (2·9–16·5 h) quoted for patients (Gupta et al 1987). A large Vd_{ss} probably reflects extensive partitioning of hydrophobic cyclosporin A into lipid-containing tissues. Compared with the present data, lower CL and Vd_{ss} have been reported for the rat (Hedayati et al 1992), while in dogs Vd_{ss} was larger but terminal half-life and F were similar to the sheep data (Gridelli et al 1986).

The abomasal administration of a wide range of abomasal doses was useful on several counts. First, any deviation from pharmacokinetic linearity can be readily detected from a plot of AUC_∞ vs dose. There was a rank order increase in both C_{max} and AUC_∞ with abomasal dose. The relationship between mean AUC_∞ and dose was linear up to and including 26·3 mg kg^{-1}, but the rate of increase tended to diminish at the highest dose (41 mg kg^{-1}). Non-linearity was also observed when mean C_{max} values were plotted against dose; however, curvature was noted above 6·4 mg kg^{-1}, reaching a plateau at 26·3 mg kg^{-1}. Dose-dependent absorption of cyclosporin A has been reported previously in man (Ptachcinski et al 1986b; Reymond et al 1988) and in rodents (Nooter et al 1984; Ueda et al 1984), but it is uncertain if the present data reflect incomplete dissolution or precipitation of the lipophilic cyclosporin A in intestinal fluids, the presence of saturable carrier mechanisms in the sheep intestinal mucosa, or both. t_{max} appeared unaffected by increasing dose. Second, the additive properties of statistical moments require linear pharmacokinetics (Cutler 1987), as does the determination of absolute bioavailability, F. Accordingly, the dose-ranging study was necessary to confirm an appro-

priate dose (6·4 mg kg^{-1}) for the determination of both the MAT and F from abomasal and intravenous data; this dose is commonly used in clinical work. Third, the experimental design enabled an assessment of possible dose-related cyclosporin A toxicity over a wide range of doses. Full chemical pathology profiles, urinalyses and differential blood counts were performed before and after the administration of each cyclosporin treatment. All animals remained well throughout the entire study and pathology and haematology data were unremarkable after each experiment. Physical and histological examination at post-study autopsy revealed no abnormalities.

In conclusion, the similarity between cyclosporin A pharmacokinetics in sheep and man and the lack of adverse effects of cyclosporin A suggests that this is a good animal model to use in further cyclosporin studies.

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