Br. J. clin. Pharmac. (1992), **33**, 39–43

The absorption site of cyclosporin in the human gastrointestinal tract

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1 An emulsion preparation of cyclosporin was administered locally to different parts of the small and large intestine by gavage: to the duodenum (opposite to the papilla of Vater), jejunum (150 cm distal to the teeth), ileum (300 cm distal to the teeth), and to the colon descendens (30 cm proximal to the anus).
2 The bioavailability of cyclosporin after these instillations was compared with that after oral administration of a hard gelatine capsule formulation.
3 Cyclosporin was found to be absorbed predominantly in the small intestine. This may have implications for dosage in patients with reduced absorptive surface area.

Keywords cyclosporin local administration intestinal absorption human

Introduction

Cyclosporin is a cyclic undecapeptide. Its therapeutic value has been established in transplantation, but additional indications include autoimmune diseases, rheumatoid arthritis, uveitis and psoriasis (Kahan, 1989). When given orally, only 30% of the dose is absorbed (Beveridge *et al.*, 1981; Frey *et al.*, 1988; Ptachinski *et al.*, 1986). Compared with other peptide drugs, however, the extent of absorption is high (Köhler *et al.*, 1987). Nevertheless, the absorption of cyclosporin is very variable and is affected by physiological and pharmaceutical factors such as bile (Mehta *et al.*, 1988; Venkatamaranan *et al.*, 1985), food (Gupta & Benet, 1990; Keown *et al.*, 1982; Ptachinski *et al.*, 1985), and vehicle (Johnston *et al.*, 1986) resulting in high inter- and intrasubject variability of pharmacokinetic parameters (Lindholm *et al.*, 1988).

Indirect evidence that the absorption of cyclosporin occurs preferentially from the small intestine comes from the observation that the dose required in children correlates with their estimated small bowel length (Whitington *et al.*, 1990). *In situ* investigations in rats and rabbits further support this hypothesis (Sawchuk & Awni, 1986; Tarr & Yalkowsky, 1989). In patients with impaired small bowel function the absorption of cyclosporin is reduced (Andrews *et al.*, 1987; Atkinson *et al.*, 1983, 1984; Williams *et al.*, 1987).

A beneficial effect of cyclosporin in Crohn's disease has been proposed (Brynskov *et al.*, 1989a). Therefore, two trials were performed with rectal administration of cyclosporin to the gut mucosa to circumvent potential adverse effects of treatment with oral cyclosporin. Topical application would avoid high concentrations in the systemic circulation (Brynskov *et al.*, 1989b; Sandborn *et al.*, 1990). However, the capacity of the colon to absorb cyclosporin has not been tested directly in humans.

Methods

Selection of subjects

The study was performed in 10 healthy male volunteers between the ages of 20 and 45 years, having body weights of 56–83 kg, and heights of 158–190 cm. The study was performed in accordance with the guidelines of the Declaration of Helsinki as revised in Tokyo (1975) and in Venice (1983). The study protocol and informed consent forms were approved by the Human Ethics Committee of the Kantonsspital Basel, University Clinic. All subjects gave written informed consent. Physical examination and measurements of blood pressure, pulse rate, ECG, and laboratory investigations (including creatinine clearance) revealed no clinically significant abnormalities.

Medication

An experimental microemulsion formulation providing good cyclosporin solubility after exposure to gastrointestinal fluids *in vitro* was used in the study. The formulation consisted of polyethylene glycol hydrogenated castor oil, middle chain triglycerides and low molecular weight glycols. The liquid formulation was

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either filled in 50 mg semi-solid, hard gelatine capsules or directly administered as a 150 mg solution. The dosage forms were prepared by Sandoz Pharma Ltd, Basel, Switzerland.

Cyclosporin treatment and study design

Each subject received 150 mg cyclosporin as a single oral dose according to a randomized open-label, five-period, Latin-square design. The wash-out period between the administrations was at least 7 days.

Cyclosporin was administered locally to different parts of the small and large intestine by gavage. The following administration sites were compared: A) duodenum (opposite to the papilla of Vater), B) jejunum (150 cm distal to the teeth), C) ileum (300 cm distal to the teeth), and D) colon descendens (30 cm proximal to the anus). These administrations were compared with an oral administration of three 50 mg capsules, which were swallowed with 150 ml water.

Fourteen hours before the small intestinal administrations, all subjects were intubated in the supine position with a modified one-lumen gastro-intestinal tube (Cartmill feeding tube, Hollander Medizin-Technik, Cham, Switzerland) having an inner diameter of approximately 1.5 mm, an outer diameter of *circa* 2.5 mm, and length of 3.5 m. The tube was placed under fluoroscopic control with the tip in the duodenum. For duodenal administration the tube was fixed externally to the skin. For the other administrations the tube was allowed to be transported distally by peristaltic gut movement. On the day of administration, the positions of the tubes were checked by fluoroscopy and adjusted if necessary. Retrograde insertion of tubes into the colon was done 15 min before drug administration: all subjects underwent rigid sigmoidoscopy for the placement of the gastrointestinal tube. Intubation was done with minimal air insufflation, but without enemas or cathartics. The tube was placed under direct vision in the left colon about 30 cm proximal to the anus and fixed externally to the skin.

Gavage administrations were carried out in the supine position. Drug solutions were drawn from ampoules by a syringe and injected into the tubes. The ampoules were then rinsed with 1.5 ml of 0.9% w/v saline solution, which was also drawn from the ampoules and injected into the tubes. Next an additional 15 ml (10 ml for colon) of saline solution was injected into the tubes over 15 min. Subjects remained intubated in the supine position for 4 h, after which the tubes were carefully removed. Prior to the study, the adsorption of cyclosporin to the tubing was measured after injection of the drug formulation through the tube and was found to be less than 5% of the dose.

All administrations were done after an overnight fast of 10 h with only water allowed. Only caffeine-free and alcohol-free beverages were allowed for the first 24 h after cyclosporin administrations. Subjects were not allowed to smoke during the study until 12 h after drug intake. Food was not allowed for the first 4 h after each administration and drinking was restricted to 50 ml water h^{-1}. A standard liquid lunch, which contained a caloric content of 2090 kJ (500 ml Ensure®, Abbott Lab., Cham, Switzerland), was given 4 h after cyclosporin administration. Four hours later a light snack (one piece of cake and an apple) was given. Eleven hours after medication a standard dinner was given.

Cyclosporin assay

Blood samples for the measurement of cyclosporin were drawn before and up to 32 h after drug administration. Blood was collected in EDTA polystyrene tubes and immediately deep frozen at −20° C pending analysis. The whole blood samples were analyzed for cyclosporin concentrations using the specific monoclonal Sandimmun® radioimmunoassay (Ball *et al.*, 1988), which has an assay limit of 15 ng ml^{-1}. Samples were measured in duplicate for unknown and controls and in triplicate for standard curves (25 to 1600 ng ml^{-1}). The within- and between-assay variability (CV (%)) was found (n = 22) to be 3.5 to 2.5% and 3.8 to 5.0%, respectively.

Creatinine clearance was measured daily (over periods of 0–24 h and 24–48 h, respectively) in each treatment period. Blood pressure and pulse rate were measured in the sitting position at regular intervals.

Pharmacokinetic and statistical analysis

The following pharmacokinetic parameters were determined: Maximum whole blood concentrations (C_{max}) and the time of their occurrence (t_{max}) were compiled from the raw data. Areas under the blood drug concentration-time curve (AUC) up to 32 h were computed using the linear trapezoidal rule. Blood drug concentrations below the assay limit of 15 ng ml^{-1} were assumed to be zero.

Unweighted blood cyclosporin concentration-time curves in each individual were fitted by a linear two-compartment model with apparent zero-order absorption and first order elimination from the central compartment using NONLIN (Statistical Consultants, 1986). The following parameters were determined: the exponents of the biexponential disposition function (λ_1, λ_2), the duration of cyclosporin absorption (T), and the zero order rate constant describing the absorption of the bioavailable dose (k_0).

For comparison of pharmacokinetic parameters, sample differences were first tested for normal distribution using the Wilk-Shapiro test and the homogeneity of variances by Levene's test (RS1 software package, 1988). If normal distribution of the data could not be rejected, samples were tested by two-way analysis of variance using a general linear model (GLM) (SAS software package, 1988). Otherwise, analysis of variance was applied to rank-transformed data using the Friedman test (Conover & Iman, 1981). In the event of significant differences analysis of variance was followed by application of the Newman-Keuls test for pairwise comparisons (SAS software package, 1988).

Results

All cyclosporin administrations were well tolerated by the subjects. No significant drug related adverse effects were noted. No clinically significant changes were

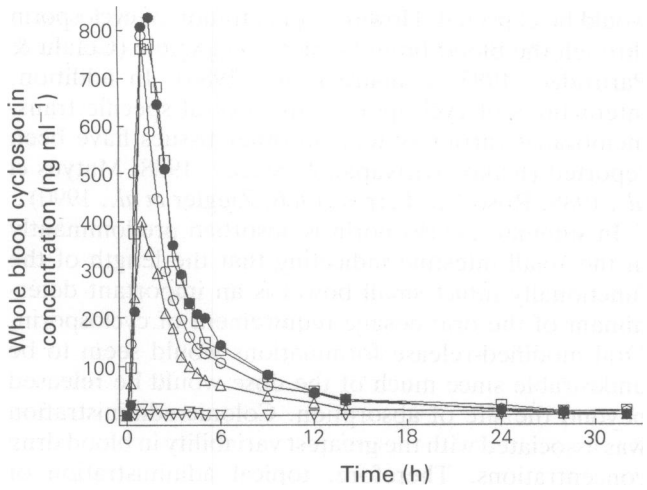

Figure 1 Median whole blood concentrations of cyclosporin after —●— oral administration of capsules; —○— local administration to the duodenum; —□— local administration to the jejunum; —△— local administration to the ileum; and —▽— local administration to the colon (n = 10; dose = 150 mg).

observed in physical and laboratory measurements including creatinine clearance.

The results are given as mean values (± s.d.). After all administrations (except that to the colon), cyclosporin was absorbed rapidly and blood drug concentration-time profiles were similar in shape (Figure 1). After oral administration of three 50 mg capsules the AUC was 3075 ± 535 ng ml^{-1} h. Using this as reference, absorption from the duodenum and the jejunum was comparable. In contrast, instillation of cyclosporin in the ileum and colon resulted in significantly decreased relative absorption (52 ± 34%; $P < 0.05$ and 53 ± 104%; $P < 0.01$, respectively). Absorption after the local administrations was quite variable. Coefficients of variation ranged from 44% (duodenum) to 196% in the colon. After colonic administration the relative AUC values for three subjects were 299%, 190%, and 37% while the remaining seven volunteers had negligible absorption (below 3%). However, these three subjects showed average absorption after the other administrations.

C_{max} values after colonic administration were significantly ($P < 0.05$) lower than those after local administration to the duodenum and jejunum and were comparable to those after oral administration. Although ileal administration also resulted in diminished peak blood drug concentrations, they were not significantly different from those following the other applications. No significant differences in t_{max} were observed after all treatments.

Pharmacokinetic parameters for duodenal (n = 9), jejunal (n = 8), ileal (n = 9), and oral administrations (n = 10) are shown in Table 1. The data obtained after colonic administration could not be fitted by the pharmacokinetic model.

Discussion

Compared with local administrations of cyclosporin to the duodenum and jejunum, the extent of local absorp-

Table 1 Mean ± s.d. (median) pharmacokinetic parameters of cyclosporin after local administration of cyclosporin by gavage and following oral administration of a capsule (150 mg dose)

	Duodenum	*Jejunum*	*Ileum*	*Colon*	*Oral*
AUC (0–32 h) (ng ml^{-1} h)	2837 ± 1391 (2384)	4344 ± 2754 (3513)	1474 ± 783 (1570)[1]	1377 ± 2731 (61)[2]	3075 ± 535 (2980)
F_{rel} (%)	93 ± 40 (83)	147 ± 95 (116)	52 ± 34 (47)	53 ± 104 (2)	100
C_{max} (ng ml^{-1})	1084 ± 941 (808)[3]	1157 ± 865 (936)[3]	489 ± 298 (427)	334 ± 529 (56)	881 ± 120 (849)
t_{max}[4] (h)	1.0 (0.3–2.0)	1.0 (0.3–2.0)	1.0 (0.5–3.0)	2.2 (0.3–5.0)	1.5 (1.0–2.5)
λ_1 (h^{-1})	1.56 ± 0.45 (1.00)	1.06 ± 0.22 (0.80)	1.73 ± 0.47 (0.98)	n.d.	1.14 ± 0.28 (0.88)
λ_2 (h^{-1})	0.16 ± 0.05 (0.13)	0.10 ± 0.02 (0.11)	0.18 ± 0.04 (0.17)	n.d.	0.16 ± 0.02 (0.15)
T (h)	1.1 ± 0.2 (1.0)	1.1 ± 0.1 (1.2)	1.2 ± 0.1 (1.2)	n.d.	1.3 ± 0.1 (1.3)
k_0 (mg min^{-1})	1.9 ± 0.4 (1.6)	1.5 ± 0.3 (1.2)	1.0 ± 0.2 (0.8)	n.d.	1.7 ± 0.2 (1.4)

AUC = area under the blood drug concentration-time curve; F_{rel} = AUC relative to oral AUC; C_{max} = maximum blood drug concentration; t_{max} = time of maximum blood drug concentration; λ_1, λ_2 = exponents of the biexponential disposition function; T = duration of cyclosporin absorption; k_0 = zero order rate constant describing the absorption of the bioavailable dose.

The results are the means ± s.d. (medians) for 10 subjects; n.d.: not determined.

[1]Significantly different from oral administration (non-parametric tests; $P < 0.05$).

[2]Significantly different from oral administration (non-parametric tests; $P < 0.01$)).

[3]Significantly different from colonic administration (parametric tests; $P < 0.05$).

[4]Medians (range).

tion from the terminal small intestine and colon was significantly decreased. Similar elimination kinetics after oral and small intestinal administrations indicated that this decreased bioavailability was caused by differences in the absorption process. The significantly decreased extent of absorption after ileal administration could be the result of a decreased absorption capacity.

These results suggest that in humans there is an absorption window for cyclosporin in the small intestine. They also provide experimental evidence for the hypothesis of Whitington *et al.* (1990) that cyclosporin dose requirements in children correlate with the estimated length of the small bowel. This hypothesis is further supported by *in situ* investigations in rats and rabbits (Sawchuk & Awni, 1986; Tarr & Yalkowsky, 1989).

Since cyclosporin is relatively lipophilic passive diffusion through biological membranes including the colonic enterocyte wall and the blood-brain barrier would be expected. However, penetration of cyclosporin through the blood-brain barrier is negligible (Cefalu & Pardridge, 1985; Lemaire *et al.*, 1988). In addition, interactions of cyclosporin with several specific transmembranal carrier systems in other tissues have been reported (Kukongviriyapan & Stacey, 1988; Matyus *et al.*, 1986; Rosoff & Terres, 1986; Ziegler *et al.*, 1990).

In summary, cyclosporin is absorbed predominantly in the small intestine indicating that the length of the functionally intact small bowel is an important determinant of the oral dosage requirement of cyclosporin. Oral modified-release formulations would seem to be undesirable since much of the dose would be released beyond the site of absorption. Colonic administration was associated with the greatest variability in blood drug concentrations. Therefore, topical administration of cyclosporin to the large intestine does not appear to be practical.

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(Received 11 June 1991, accepted 19 August 1991)