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Evidence for pre-hepatic metabolism of oral cyclosporine in children

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1 The pharmacokinetics of cyclosporine were investigated before renal transplantation in 20 children aged 1.1 to 16.8 years. Cyclosporine was given as a single oral dose (10 mg kg^{-1}) or as a 4 h i.v. infusion (3 mg kg^{-1}).
2 The blood drug concentration was measured by both specific and nonspecific monoclonal radioimmunoassays.
3 The mean oral availability of cyclosporine was 20.6% (range 10.8–34.1%).
4 The mean ratio of AUCs measured by nonspecific and specific r.i.a. was 1.96 (range 1.4–2.7) after oral administration and 1.43 (range 1.1–2.0) after i.v. administration. The mean difference between the ratios was 38.5% ($P = 0.0001$). The ratio of $AUC_{nonspecific}$ to $AUC_{specific}$ was inversely related to blood drug clearance ($r = 0.57$; $P = 0.009$).
5 The findings are suggestive of presystemic, pre-hepatic metabolism of cyclosporine which could contribute to the low, and highly variable bioavailability of this drug.

Keywords cyclosporine A bioavailability metabolism children

Introduction

The oral absorption of cyclosporine is incomplete and highly variable for reasons which are not completely known (Grevel, 1986). Bile is essential for dissolution of this highly lipid-soluble drug and absorption appears to be confined to the upper part of the small intestine (Grevel *et al.*, 1986). Cyclosporine is 30% bioavailable after oral administration in adults (Ptachcinski *et al.*, 1986b) and a similar figure is reported for children after renal transplantation (Kahan *et al.*, 1986; Ptachcinski *et al.*, 1986b).

In dogs presystemic metabolism contributes to the low bioavailability of oral cyclosporine (Gridelli *et al.*, 1986), and they produce similar metabolites to man (Maurer & Lemaire, 1986). In humans cytochrome P-450 IIIA, which is responsible for the major products of cyclosporine metabolism, is found in enterocytes (Watkins *et al.*, 1987), but presystemic metabolism of cyclosporine has yet to be demonstrated conclusively.

In the present investigation we have sought indirect evidence for pre-hepatic, presystemic metabolism of cyclosporine in children.

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Methods

Subjects

Twenty children were investigated prior to renal transplantation. Twelve suffered from congenital nephrotic syndrome. The urethral valve was the cause of renal failure in four children, glomerulonephritis in two and polycystic renal diseases or nephritis in the remaining two patients. All children were on peritoneal dialysis (CCPD or CAPD).

The children were 1.1 to 16.8 years old (geometric mean 3.4 years). They were receiving standard medication for their renal insufficiency, including vitamin D analogues ($n = 17$), antihypertensive agents ($n = 12$), digitalis ($n = 8$), phosphate binders ($n = 8$), frusemide ($n = 6$), ion exchange resins ($n = 6$), recombinant human erythropoietin (r-HuEPO; $n = 7$), and warfarin ($n = 2$). Blood haemoglobin concentration and haematocrit were measured on the day of study. None of the subjects had any signs of hepatic dysfunction. Small-bowel length was estimated by interpolation from height according to Siebert (1980).

Informed consent from the parents, and from children old enough to give it, was obtained before entry to the study. The study protocol was approved by the Ethics Committee of the Children's Hospital.

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Study Protocol

Cyclosporine was given as a 4 h intravenous infusion (3 mg kg^{-1}) or as a single oral dose (10 mg kg^{-1}) in a randomized cross-over design with a drug free interval of 2 to 48 days (median 4.1 days). The drug (Sandimmun®) was administered in the morning to the fasting subjects. The oral dose was prepared immediately prior to administration by mixing the drug solution with a small amount of fruit juice (mixed berry syrup) in a syringe. The syringe was used to administer the dose and then flushed with additional fruit juice.

Cyclosporine concentration was measured in whole blood samples taken at 0, 1, 2, 3, 4, 6, 9, 12, 16 and 24 h after the oral dose and before, during (2 h) and at the end of the i.v. infusion (4 h) and at 0.5, 1, 2, 3, 6, 9, 12, 16 and 24 h thereafter. Blood was collected from an indwelling canula, kept patent with an obturator or a slow glucose infusion. EDTA was used as the anticoagulant and the samples were stored frozen until analyzed.

Analytical methods

Cyclosporine was measured in duplicate by specific and nonspecific monoclonal radioimmunoassays ('Sandimmun® Kit', Sandoz Ltd, Basel, Switzerland). The inter-assay coefficient of variation at the mean concentration of 381 $\mu g\ l^{-1}$ was 6.1% ($n = 10$) for the specific r.i.a. and 9.8% for the non-specific. The 4 h sample for four children after oral and i.v. administration was also assayed by h.p.l.c. (Wallemacq & Lesne, 1987). The mean specific r.i.a./h.p.l.c. concentration ratio was 1.16 (± 0.04 s.e. mean) and the correlation coefficient between the specific monoclonal r.i.a. and the h.p.l.c. assay was 0.994 ($P = 0.0001$).

The specific monoclonal antibody is specific for unchanged cyclosporine A. The nonspecific monoclonal antibody also detects many of the metabolites of cyclosporine. Cross-reactivity with unchanged cyclosporine is 100% and with the major metabolites M1, M17, and M21 it is 34%, 76% and 6%, respectively. Cross-reactivity is also found with M8 (24%), M16 (50%) M18 (57%), M26 (55%) and <20% with metabolites M9, M10, M13 and M25 (Instruction Leaflet, 'Sandimmun® Kit', Sandoz Ltd, Basel, Switzerland).

Pharmacokinetic calculations

The pharmacokinetic variables were calculated using standard noncompartmental methods (Gibaldi, 1984). Values of the elimination rate constant (k_{el}) were determined from the slope of the terminal log-linear phase of the blood cyclosporine concentration (C)-time (t) curve by regression analysis. The terminal elimination half-life ($t_{½}$) was calculated from $t_{½} = \ln 2/k_{el}$. The total AUC was calculated using the linear trapezoidal rule with extrapolation to infinity using the ratio of the last observed data point and k_{el}. The average cyclosporine concentration was calculated as AUC / 24. The bioavailability (F) after oral administration was calculated from the ratio of oral to i.v. AUC normalized for dose. Blood clearance (CL_b) was calculated from i.v. dose divided by AUC_{iv}. All pharmacokinetic variables were calculated from data obtained with the specific monoclonal r.i.a. for cyclosporine.

The ratio of AUC values measured up to the last observed concentration determined using the nonspecific and specific assays ($AUC_{nonspecific}/AUC_{specific}$) was used as an index of cyclosporine metabolism. This ratio underestimates metabolite formation, because cross-reactivity of the metabolites with the nonspecific antibody is variable. Nevertheless, there is a correlation between the concentration of the main cyclosporine metabolite M17 and concentration measured using the nonspecific assay (Wenk *et al.*, 1989).

Statistical calculations

The results are given as mean and s.d. except where indicated. The distributions of the variables were checked for skewness and the values were log transformed where appropriate. Least-squares regression was used to assess any linear relationships between dependent and independent variables. The significance of the regressions was determined by the F test. The significance of differences between means of the variables was assessed with the paired t-test. A P value less than 0.05 was used to reject the null hypothesis.

Results

Blood cyclosporine concentration-time data after i.v. and oral administration are shown in Figure 1. Although the oral dose was three times larger than the i.v. dose, the oral AUC was considerably smaller than that after i.v. infusion. Consequently the average specific cyclosporine

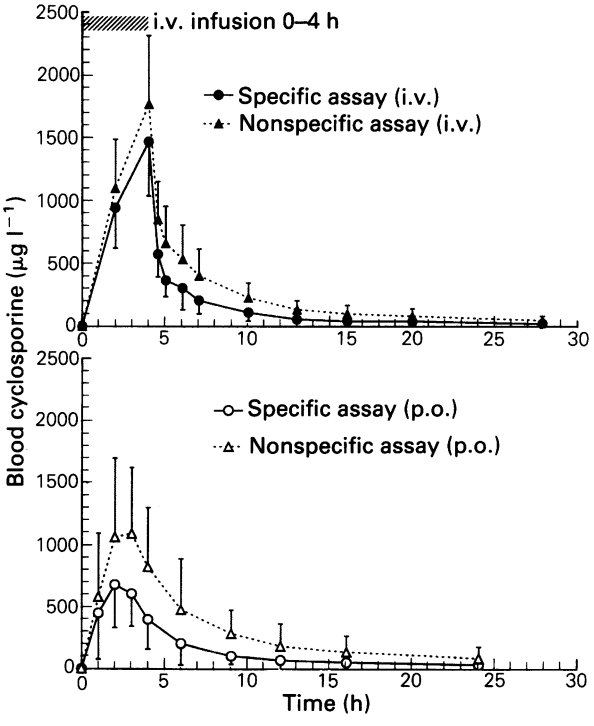

Figure 1 Mean (± s.e.mean) blood cyclosporine concentrations in 20 children on peritoneal dialysis after a single i.v. (3 mg kg^{-1}) or oral (solution 10 mg kg^{-1}) dose, measured by specific and nonspecific monoclonal r.i.a.

Table 1 Pharmacokinetic parameters describing the fate of cyclosporine in 20 children studied before renal transplantation

	Mean	*s.d.*	*Range*
$t_{½,z}$ (h)	9.3	6.2	2.8 – 23.1
CL_b (ml min^{-1} kg^{-1})	8.6	2.5	5.3 – 13.1
F	0.21	0.08	0.11 – 0.34

concentration after oral administration (mean 158 μg l^{-1}; s.d. 72.1) was considerably lower than that after intravenous administration (mean 214 μg l^{-1}; s.d. 56.4; $t = 4.03$; $P = 0.0007$). Values of pharmacokinetic variables are shown in Table 1.

Mean values of haemoglobin concentration and haematocrit were 72.8 g l^{-1} (s.d. 13.7) and 0.22 (s.d. 0.042) (i.v. study) and 75.6 g l^{-1} (s.d. 19.0) and 0.23 (s.d. 0.053) (oral study). These were not significantly different for the two modes of administration. The mean estimated small-bowel length was 377 cm (95% CI 362–394 cm).

The proportion of metabolites, measured as the ratio of $AUC_{nonspecific}$ to $AUC_{specific}$ was larger after oral than intravenous administration (mean difference 38.5%; range 5.9 to 87.2) (Figure 2). The mean ratio was 1.43 (s.d. 0.25) after intravenous administration and 1.96 (s.d. 0.32) following oral administration ($t = -9.23$; $P = 0.0001$).

The possibility that this difference was related to dissimilar cyclosporine concentrations and not the route of administration was assessed by pooling the concentration data from all patients and plotting cyclosporine concentrations measured by the specific assay against those measured by the nonspecific assay (Figure 3). The pooled concentration ranges were comparable and the plots were linear after both oral ($r = 0.95$, $P = 0.0001$) and intravenous ($r = 0.94$, $P = 0.0001$) administration. However, the concentration measured non-specifically tended to be higher after oral than after intravenous administration.

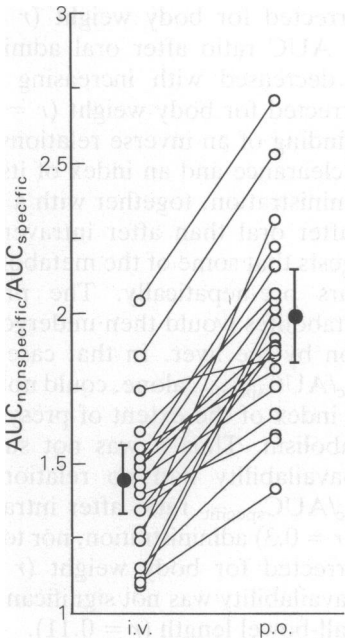

Figure 2 Individual (○) and mean (●) ± 95% CI values of the ratio of cyclosporine AUC measured by monoclonal nonspecific and specific r.i.a. after single i.v. and oral doses in 20 children on peritoneal dialysis.

The possibility of age-related changes, independent of body-size, was also considered. The dose of cyclosporine expressed as mg kg^{-1} did not correlate with the logarithm of age (i.v. $r = -0.02$; p.o. $r = -0.15$). Oral bioavailability was independent of age ($r = 0.11$). Blood cyclosporine clearance after intravenous administration, corrected for body weight, was inversely related to log(age) ($r = -0.60$, $P = 0.005$). The ratio $AUC_{nonspecific}/AUC_{specific}$ was significantly related to log(age)after oral ($r = 0.50$, $P = 0.03$) but not after intravenous administration (i.v. $r = 0.44$, $P = 0.055$).

The $AUC_{nonspecific}/AUC_{specific}$ ratio after i.v. administration was not significantly related to cyclosporine

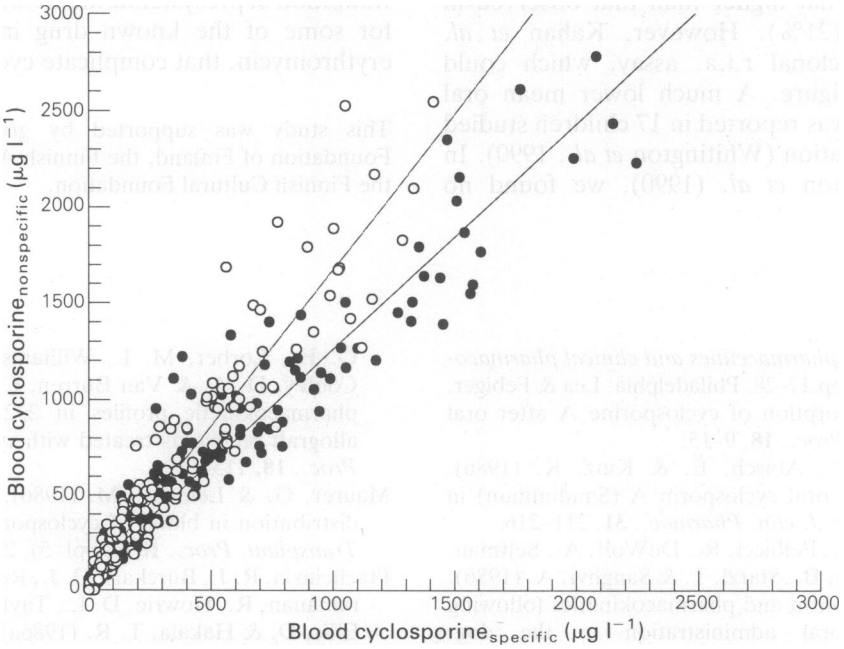

Figure 3 Relationship between blood cyclosporine concentrations measured by monoclonal specific and nonspecific r.i.a. after single i.v. (●) and oral (○) doses in 20 children on peritoneal dialysis.

clearance corrected for body weight ($r = -0.3$). In contrast, the AUC ratio after oral administration of cyclosporine decreased with increasing cyclosporine clearance corrected for body weight ($r = -0.57$, $P = 0.009$). The finding of an inverse relationship between cyclosporine clearance and an index of its metabolism after oral administration, together with a higher metabolic index after oral than after intravenous administration, suggests that some of the metabolism of cyclosporine occurs pre-hepatically. The pre-hepatically produced metabolites would then undergo further biotransformation by the liver. In that case the ratio of $AUC_{nonspecific}/AUC_{specific}$, alone, could not be expected to be a good index of the extent of presystemic cyclosporine metabolism. Thus it was not surprising that the oral bioavailability had no relationship to the $AUC_{nonspecific}/AUC_{specific}$ ratio after intravenous ($r = 0.07$) or oral ($r = 0.3$) administration, nor to cyclosporine clearance corrected for body weight ($r = 0.03$). In addition, bioavailability was not significantly related to the log of small-bowel length ($r = 0.11$).

Discussion

Our data suggest that presystemic, presumably prehepatic, metabolism is a factor contributing to the low and variable oral bioavailability of cyclosporine in children.

The time-course of the blood concentrations of cyclosporine after oral administration in the children we have studied was similar to that observed in adults, showing a rapidly attained peak and a sharp decline thereafter. The mean oral bioavailability of cyclosporine in adults is about 30% (Ptachcinski *et al.*, 1986b). In two studies each of seven children after renal transplantation, the mean oral bioavailability of cyclosporine was 31% (Ptachcinski *et al.*, 1986a) and 52% (Kahan *et al.*, 1986), values somewhat higher than that observed in the present study (21%). However, Kahan *et al.* (1986) used a polyclonal r.i.a. assay, which could explain the higher figure. A much lower mean oral availability of 7.8% was reported in 17 children studied after liver transplantation (Whitington *et al.*, 1990). In contrast to Whitington *et al.* (1990), we found no correlation between small-bowel length and the oral availability of cyclosporine. In the children of the present study both the arithmetic (378 cm) and geometric (377 cm) means of small-bowel length were larger than the mean small-bowel length (341 cm) in the children studied by Whitington *et al.* (1990). It is possible that bile flow in children after liver transplantation is different and influences the dissolution of cyclosporine thereby making the length of the small-bowel more critical.

The low oral bioavailability of cyclosporine is generally thought to result from poor absorption. However, studies by Gridelli *et al.* (1986) in dogs have suggested that presystemic metabolism contributes. Thus, they measured cyclosporine by nonspecific r.i.a. and by h.p.l.c. and found that the mean r.i.a./h.p.l.c. ratios were higher after oral than i.v. administration. The highest cyclosporine + 'metabolite' AUC was measured in the portal vein after oral administration. However, the hepatic extraction ratio of parent drug and cyclosporine 'metabolites' was similar after i.v. and p.o. administration. These results suggested that the gastrointestinal tract may play a role in the metabolism of cyclosporine when the drug is administered orally (Gridelli *et al.*, 1986).

Our finding of a significantly higher nonspecific to specific r.i.a. - AUC ratio after oral administration is in agreement with the results of Gridelli *et al.* (1986), and suggests that cyclosporine undergoes presystemic metabolism in humans too. Further evidence in support of this hypothesis comes from the demonstration that enterocytes have cytochrome P-450 IIIA activity, the isoenzyme which is responsible for the major pathway of cyclosporine metabolism (Watkins *et al.*, 1987). The human gastrointestinal mucosa is also able to metabolize cyclosporine *in vitro* (Tjia *et al.*, 1990).

We suggest that the low bioavailability of cyclosporine in children is due to both poor absorption and prehepatic metabolism. Combined intra- and interindividual variability in these processes would explain the very large variability of oral cyclosporine bioavailability. Inhibition of presystemic metabolism might also account for some of the known drug interactions, e.g. with erythromycin, that complicate cyclosporine therapy.

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