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Evaluation of cyclosporin–phenytoin interaction with observations on cyclosporin metabolites

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1 We have observed that patients on concurrent cyclosporin and phenytoin therapy required increased doses of cyclosporin to maintain therapeutic concentrations of this novel immunosuppressive drug. We have, therefore, studied the influence of phenytoin on the pharmacokinetics of oral cyclosporin in six healthy male subjects.
2 Cyclosporin concentrations in serum and whole blood were measured by high pressure liquid chromatography (h.p.l.c.) and radioimmunoassay (RIA). Concentrations of cyclosporin in whole blood were consistently higher than corresponding values in serum. Concentrations of cyclosporin determined by RIA were also consistently higher than those determined by h.p.l.c.
3 Irrespective of the biological fluid (serum or whole blood) or the type of drug analysis (h.p.l.c. or RIA), changes in cyclosporin kinetics following phenytoin administration exhibited similar patterns. Phenytoin significantly reduced the maximum concentration and the area under the concentration-time curve and significantly increased total body clearance of cyclosporin. There was a statistically significant reduction of cyclosporin half-life ($t_{½}$) in whole blood using h.p.l.c. analysis. However, there was no significant change in cyclosporin $t_{½}$ in serum following phenytoin administration, using either form of drug analysis.
4 Cyclosporin metabolites 17 and 18 were measured by h.p.l.c. in whole blood samples only, since these metabolites were found almost entirely in red blood cells. Phenytoin significantly reduced the C_{max} and AUC of both metabolites, but no significant change was observed in the $t_{½}$ of either.
5 Phenytoin enhanced the metabolism of antipyrine which was co-administered with cyclosporin to assess oxidative enzyme activity.
6 We conclude that patients undergoing organ transplantation should be carefully monitored if they require phenytoin or other drugs known to accelerate oxidative metabolism.

Keywords cyclosporin phenytoin interaction

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Introduction

Cyclosporin, a cyclic undecapeptide previously known as cyclosporin A and also known as cyclosporine in North America, is an effective immunosuppressant in organ transplantation (Borel *et al.*, 1977; Calne *et al.*, 1978, 1979; Powles *et al.*, 1980; Gluckman *et al.*, 1981; European Multicentre Trial, 1982; Canadian Transplant Study Group, 1984). Cyclosporin is also under evaluation as a treatment for several autoimmune diseases (Laupacis *et al.*, 1982; Stiller *et al.*, 1983). Although there is some uncertainty about the best biological fluid and analytical method for therapeutic drug monitoring (Robinson & Ketchum, 1983), measurements of cyclosporin concentrations in serum or whole blood appear useful as a guide to therapy. A trough cyclosporin concentration in serum between 50 and 300 ng/ml, measured by radioimmunoassay (RIA), is likely to provide adequate immunosuppression with minimum risk of toxicity (Keown *et al.*, 1981).

Several cyclosporin metabolites have been identified by chromatography (Beveridge, 1981). Two major oxidative metabolites, 17 and 18, have been isolated from urine (Donatsch *et al.*, 1981; Neiderberger, personal communication). Metabolite 17 is formed by hydroxylation of amino acid 1 and metabolite 18 contains both a hydroxyl group and a cyclic ether in the side chain of amino acid 1 (Figure 1). Both appear to exert much less immunosuppressive activity than the parent compound (Borel, personal communication).

We have observed that each of five patients requiring phenytoin therapy for convulsions needed substantial increases in cyclosporin dosage to maintain plasma concentrations in the range we considered therapeutic. Since these patients had recently undergone major surgery, were moderately to severely ill and were receiving varying doses of prednisone, we investigated the possible interaction in healthy volunteers whose metabolic status was unlikely to be influenced by any variable other than

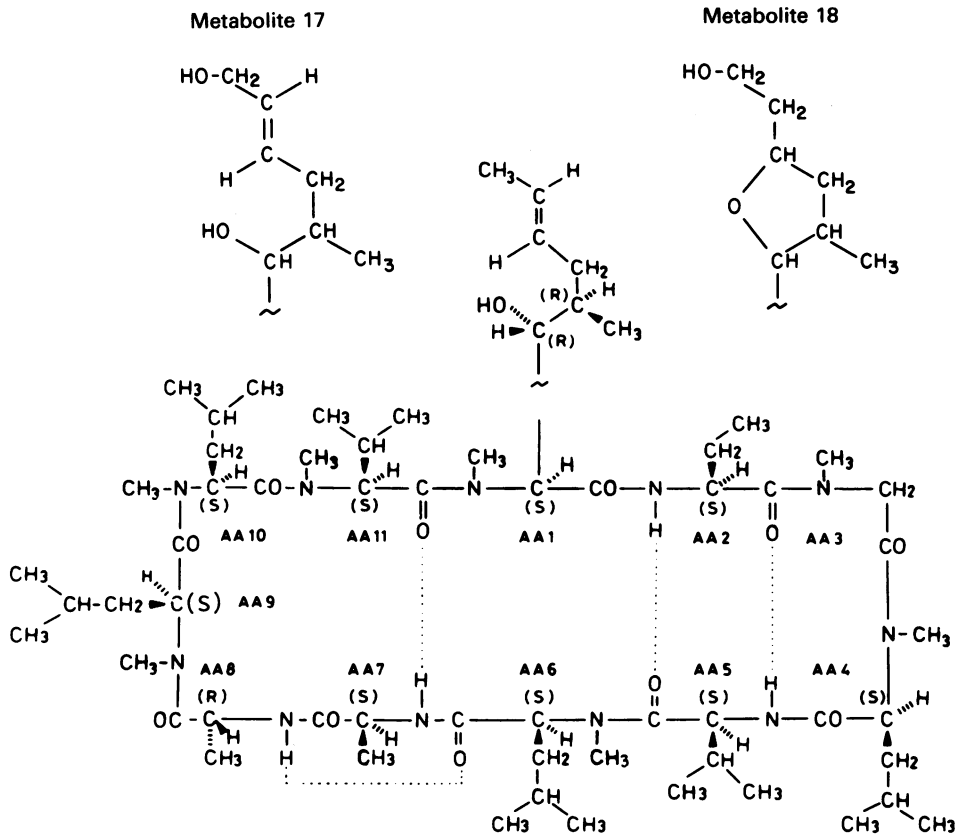

Figure 1 Molecular structure of cyclosporin. The structure of amino acid 1 (AA1) has been separated from the rest of the molecule to demonstrate the changes which occur in the oxidation of cyclosporin to metabolites 17 and 18.

phenytoin administration. We have also examined the influence of phenytoin on the disposition of metabolites 17 and 18 in these healthy subjects.

Methods

We studied six healthy men, 19–30 years old and weighing 63–89 kg, who consented in writing to a protocol approved by our ethics review committee. All volunteers drank small amounts of alcohol occasionally. Three were non-smokers; the others smoked from two to 10 cigarettes daily. They were asked to maintain their usual eating, drinking and smoking habits throughout the study.

After an overnight fast, the volunteers received a single oral dose of cyclosporin 15 mg/kg in chocolate milk. At the same time they were given oral antipyrine 10 mg/kg. Blood samples were collected in plain Vacutainer® tubes before and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after taking the medications. All sera were separated at room temperature (19–21°C). Whole blood and serum samples were stored at −20°C. Phenytoin was given orally in a dose of 300 mg daily, starting the following day. On day 5, serum phenytoin concentrations were measured and the dose increased if the level was below 10 mg/l (39.6 mmol/l). Three volunteers required an increase in phenytoin dose to 400 mg/day. Phenytoin was continued for a total of 9 days at which time phenytoin concentrations were within the desired range. On Day 10, one day after the last dose of phenytoin, the fasting volunteers received second doses of cyclosporin and antipyrine. Further blood samples were drawn at the times described above.

Whole blood and serum cyclosporin concentrations were measured by high pressure liquid chromatography (h.p.l.c.) (Carruthers *et al.*, 1983) and RIA (Donatsch *et al.*, 1981). The limits of detection for cyclosporin by h.p.l.c. and RIA were 30 ng/ml and 25 ng/ml, respectively. Concentrations of metabolite 17 were measured by h.p.l.c. using standards prepared from a pure sample of the metabolite provided by Sandoz, Basel. In the absence of a pure sample of metabolite 18, the identity and measurement of this metabolite was based on information about its relative retention time and the assumption that its extinction coefficient and extraction were identical to that of metabolite 17. Antipyrine concentrations were measured by the h.p.l.c. method of Eichelbaum & Spannbrucker (1977).

The following pharmacokinetic measurements were calculated using standard techniques: maximum concentration (C_{max}), time to maximum concentration (t_{max}), area under the concentration-time curve from 0 to 24 h (AUC), elimination half-life ($t_{½}$) and oral clearance (CL_o). The data are expressed as mean ± s.d. Results before and after phenytoin administration were compared using Student's paired *t*-test.

Results

The effects of phenytoin on whole blood and serum concentrations of cyclosporin are shown in Figures 2 and 3, respectively. The concurrent changes in metabolite 17 and 18 concentrations are shown in Figure 4.

The measured concentrations of cyclosporin, and consequently derived pharmacokinetic data, varied with the type of sample (blood or serum) and the method of analysis (Tables 1 and 2). Nevertheless, the effect of phenytoin was essentially the same. If we consider the data obtained from h.p.l.c. measurements of whole blood, C_{max} was decreased from 1325 ± 178 to 831 ± 193 μg/l after phenytoin therapy ($P < 0.01$), there was no significant change in t_{max} (4.21 ± 1.0 to 3.3 ± 1.6 h) and the AUC was reduced from 10.4 ± 1.7 to 5.5 ± 2.1 mg l^{-1} h ($P < 0.01$). There was a decrease in the mean elimination $t_{½}$ of cyclosporin from 5.1 ± 1.1 to 3.7 ± 1.1 h ($P < 0.05$) and the oral clearance was increased from 110.4 ± 24.7 to 228.0 ± 87.0 l/h ($P < 0.02$). There were similar changes in the pharmacokinetics of cyclosporin in serum but the change in elimination $t_{½}$ after phenytoin administration was not significantly different from control.

Metabolites 17 and 18 were readily measurable in whole blood but were almost undetectable in serum. There was a significant decrease in C_{max} and AUC of both metabolites after phenytoin administration ($P < 0.05$), but no change in t_{max} or $t_{½}$ (Table 3).

Antipyrine AUC was decreased from 239.5 ± 77.7 to 163 ± 38.2 mg l^{-1} h ($P < 0.02$). The elimination $t_{½}$ of antipyrine was reduced from 9.5 ± 2.9 to 6.3 ± 1.4 h after phenytoin administration ($P < 0.02$).

Discussion

The apparent effect of phenytoin on cyclosporin levels in patients was confirmed by the study in volunteers. There was a significant decrease in

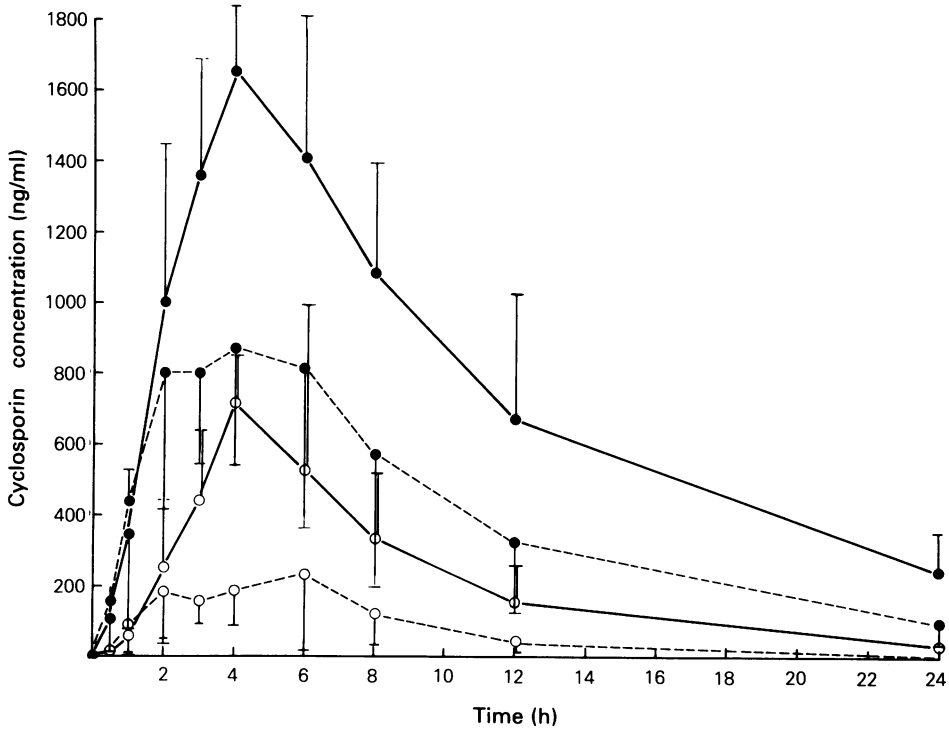

Figure 2 Cyclosporin concentrations measured by RIA before (continuous line) and after phenytoin administration (broken line) in whole blood (•) and serum (○).

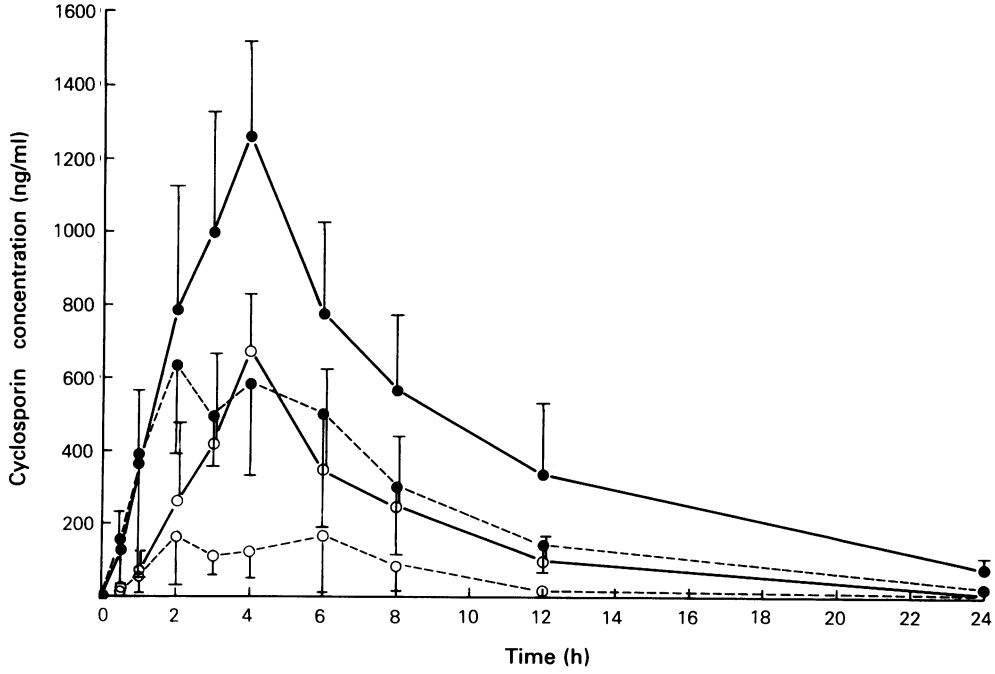

Figure 3 Cyclosporin concentrations measured by h.p.l.c. before (continuous line) and after phenytoin administration (broken line) in whole blood (•) and serum (○).

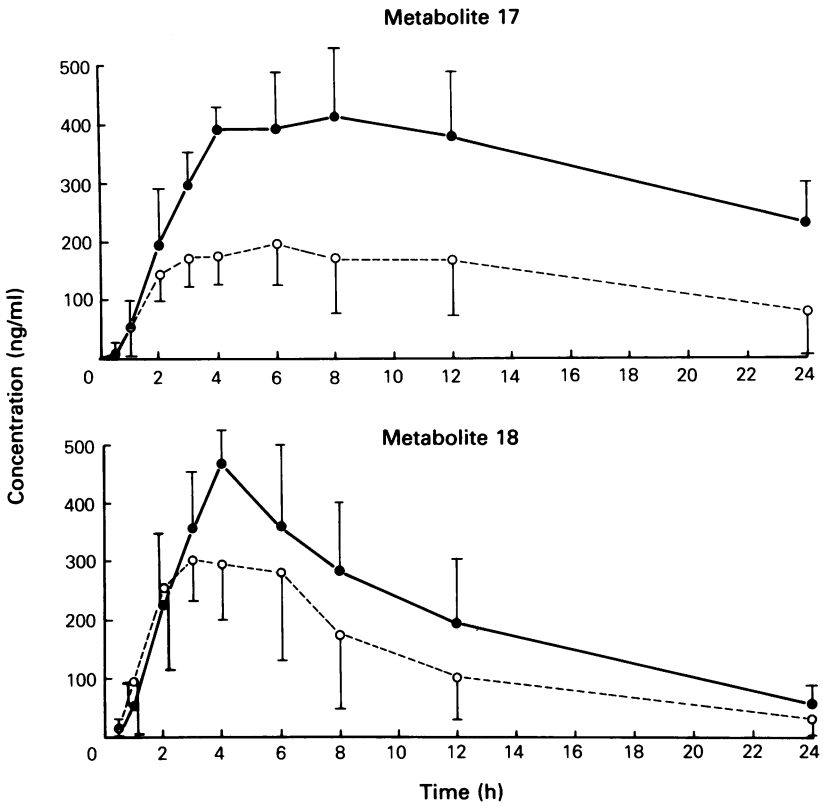

Figure 4 Concentrations of metabolite 17 and 18 in whole blood before (continuous line) and after phenytoin administration (broken line).

C_{max} and AUC and an increase in the clearance of oral cyclosporin after phenytoin administration. The AUC and C_{max} of metabolites 17 and 18 in whole blood were also lower after phenytoin administration.

We cannot offer a ready explanation for the relatively small, albeit significant, decrease in cyclosporin elimination $t_{½}$ in whole blood and the absence of any change of $t_{½}$ in serum, despite the much larger increase in apparent cyclosporin clearance in both whole blood and serum. Phenytoin may interfere with cyclosporin absorption but this is unlikely because volunteers received their final doses of phenytoin at least 24 h before cyclosporin administration. Other possible explanations are enhanced metabolism of cyclosporin during its absorption from the gastrointestinal tract or during its first pass through the liver, or an alteration of cyclosporin distribution in the presence of phenytoin. Likewise, we cannot readily explain the reduction in metabolite concentrations without any corresponding change in apparent elimination $t_{½}$. The lower metabolite concentrations may reflect enzyme induction of their further metabolism to unidentified metabolites. Further studies using intravenous cyclosporin are necessary to evaluate these hypotheses.

We have found that cyclosporin concentrations in whole blood samples from volunteers were higher than those in serum, irrespective of the type of analysis. This is consistent with the fact that approximately 50% of cyclosporin in whole blood is taken up by the red blood cell (Beveridge, 1982; Follath *et al.*, 1983), a process which appears to be temperature-dependent (Follath *et al.*, 1983).

The discrepancy between RIA and h.p.l.c. measurements has been reported previously (Donatsch *et al.*, 1981; Carruthers *et al.*, 1983; Kennedy *et al.*, 1984). Based on RIA studies with serum, Donatsch *et al.* (1981) attributed this to the cross reactivity of metabolite 17 with the cyclosporin antibody. However, since we found essentially no metabolite 17 or 18 in serum, the differences between RIA and h.p.l.c.

Table 1 Influence of phenytoin on the pharmacokinetics of cyclosporin in whole blood. Data are presented as mean ± s.d.

	Control		*After phenytoin*	
	RIA	*h.p.l.c.*	*RIA*	*h.p.l.c.*
AUC (mg l^{-1} h)	18.1 ± 4.06	10.4 ± 1.71	9.8 ± 4.3**	5.5 ± 2.1**
C_{max} (μg/l)	1764 ± 274	1325 ± 178	1144 ± 272***	831 ± 193**
t_{max} (h)	4.7 ± 2.0	4.2 ± 1.0	3.2 ± 1.9	3.3 ± 1.6
$t_{½}$ (h)	7.2 ± 2.4	5.1 ± 1.1	5.5 ± 1.6	3.7 ± 1.1*
CL_o (l/h)	64.4 ± 16.8	110.4 ± 24.7	133.6 ± 57.0*	228.0 ± 87.0*

Compared with controls: *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

must result from the presence in serum of relatively high concentrations of other metabolites.

The rate of onset of the cyclosporin–phenytoin interaction in patients and its apparent duration after phenytoin was discontinued are consistent with changes in oxidative metabolism brought about by enzyme induction (Gelehrter, 1976; Marshall, 1978). The enhanced clearance of antipyrine in the healthy subjects is also consistent with enzyme induction, but does not prove that phenytoin increased cyclosporin metabolism in these individuals. The mechanism of the interaction clearly requires further evaluation. Nevertheless, we conclude that patients who undergo organ transplantation should be monitored with particular care if they require phenytoin therapy. Phenobarbitone, rifampicin or other drugs known to accelerate oxidative metabolism should also be administered with caution until the nature and extent of possible interactions with these drugs have been resolved. A recent report of enhanced metabolism of both cyclosporin and prednisone in a patient receiving antituberculous therapy with rifampicin and isoniazid (Langhoff & Madsen, 1983) likely resulted from the rifampicin. Without an appropriate increase in cyclosporin dosage, there is a risk that cyclosporin concentrations will fall below a therapeutic level and that the transplanted organ will be rejected.

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Table 2 Influence of phenytoin on the pharmacokinetics of cyclosporin in serum. Data are presented as mean ± s.d.

	Control		*After phenytoin*	
	RIA	*h.p.l.c.*	*RIA*	*h.p.l.c.*
AUC (mg l^{-1} h)	5.3 ± 1.6	3.9 ± 8.7	1.9 ± 1.1**	1.2 ± 0.7**
C_{max} (μg/l)	868 ± 317	783 ± 145	340 ± 168**	281 ± 140***
t_{max} (h)	5.0 ± 1.6	4.2 ± 1.0	3.5 ± 1.5	3.7 ± 2.0
$t_{½}$ (h)	4.3 ± 0.7	2.8 ± 1.5	4.4 ± 0.9	1.9 ± 0.6
CL_o (l/h)	227.3 ± 76.2	299.3 ± 94.5	766.1 ± 432.0*	1313 ± 858.8*

Compared with controls: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3 Influence of phenytoin on the pharmacokinetics of metabolites 17 and 18 in whole blood. Data are presented as mean ± s.d.

	Control		*After phenytoin*	
	Metabolite 17	*Metabolite 18*	*Metabolite 17*	*Metabolite 18*
AUC (mg l^{-1} h)	7.6 ± 1.7	4.8 ± 1.4	3.4 ± 1.6**	3.2 ± 1.4*
C_{max} (μg/l)	456 ± 83	493 ± 80	219 ± 54***	332 ± 114*
t_{max} (h)	7.3 ± 3.0	4.3 ± 0.8	6.3 ± 3.4	3.5 ± 1.5
$t_{½}$ (h)	17.2 ± 2.0	6.2 ± 0.4	14.2 ± 4.2	4.9 ± 2.4

Compared with controls: * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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