Br. J. clin. Pharmac. (1987), **24**, 329–334

Cyclosporin–phenytoin interaction: re-evaluation using metabolite data

M. ROWLAND & S. K. GUPTA
Department of Pharmacy, University of Manchester, Manchester M13 9PL

1 Freeman *et al.* (1984) evoked enzyme induction to explain the lower plasma concentration of cyclosporin following phenytoin co-administration in man.
2 We have examined the whole blood concentration data of two metabolites of cyclosporin, as well as unchanged drug, all measured by h.p.l.c., associated with the above mentioned study.
3 Phenytoin produced no significant effect on either the terminal half-life of both metabolites or the ratio of area under the concentration-time curve of metabolite to parent drug.
4 These data strongly suggest that phenytoin reduces the absorption of cyclosporin; they do not generally support the idea that phenytoin induces cyclosporin metabolism.

Keywords cyclosporin phenytoin interaction

Introduction

Freeman *et al.* (1984) reported that phenytoin induces the metabolism of cyclosporin in a group of young healthy adult volunteers. This conclusion was based primarily on an observed reduction in the area under the blood cyclosporin concentration-time curve, following a standard oral dose of cyclosporin, when given following a regimen of 300–400 mg phenytoin daily for nine days, compared to when given alone. Also measured in the study were two major metabolites of cyclosporin, designated by them as metabolites 17 and 18. In this paper we report analysis of these metabolite data and suggest that phenytoin primarily reduces the absorption of cyclosporin rather than induces its metabolism.

Methods

Details of the study, analytical methods used and data obtained are described by Freeman *et al.* (1984). Specifically considered here are the concentrations of unchanged cyclosporin and two of its metabolites, in whole blood, all measured by h.p.l.c.

Theory

The extent of absorption, or bioavailability (F), of an oral dose of drug is related to the corresponding total area under the plasma (or blood) drug concentration-time profile ($\mathrm{AUC}(d)$), by the relationship

$$F \cdot \mathrm{Dose} = \mathrm{CL} \cdot \mathrm{AUC}(d) \qquad (1)$$

where CL is the clearance of the drug.

If a fraction, f_m, of the absorbed dose is converted to a metabolite m, then from mass balance considerations

$$f_m \cdot F \cdot \mathrm{Dose} = \mathrm{CL}(m)\, \mathrm{AUC}(m) \qquad (2)$$

where $\mathrm{CL}(m)$ is the clearance of the metabolite and $\mathrm{AUC}(m)$ is the corresponding total area under the plasma (or blood) metabolite concentration-time profile.

Correspondence: Professor M. Rowland, Department of Pharmacy, University of Manchester, Manchester M13 9PL

It follows from the above that

$$\frac{AUC(m)}{AUC(d)} = \frac{f_m \cdot CL}{CL(m)} = \frac{CL_m}{CL(m)} \quad (3)$$

where CL_m is the formation clearance of the metabolite ($CL_m = f_m \cdot CL$).

If, between two treatments (A and B) the only difference is in bioavailability (with no change in the clearances of both drug and metabolite or in f_m) then by reference to equations 1 and 2, it is apparent that

$$\frac{F_A}{F_B} = \frac{AUC(d)_A}{AUC(d)_B} = \frac{AUC(m)_A}{AUC(m)_B} \quad (4)$$

and that

$$\frac{AUC(m)_A}{AUC(d)_A} = \frac{AUC(m)_B}{AUC(d)_B} \quad (5)$$

If, on the other hand, clearance of either drug and/or metabolite changes, then (unless the ratio $CL_m/CL(m)$ remains the same) the equalities in equations 4 and 5 no longer hold.

Data analysis

The area under the experimental concentration-time curve (AUC(0,*t*)) was calculated by the linear trapezoid approximation, and the area beyond the last observation (C^*) was calculated as the ratio C^*/k, where k is the terminal rate constant estimated by regression analysis on the decline phase data. The sum of these two areas was taken as an estimate of the total area under the curve.

The data were compared by paired *t*-test and ANOVA, using a level of $P < 0.05$ as significant.

Results

Figure 1 shows a semilogarithmic plot of the whole blood concentrations of cyclosporin and the two metabolites in a representative subject, before and after phenytoin pretreatment. A marked reduction in all three species was clearly evident after phenytoin pre-treatment. In contrast, the terminal slope varied little between the control and phenytoin pre-treatment

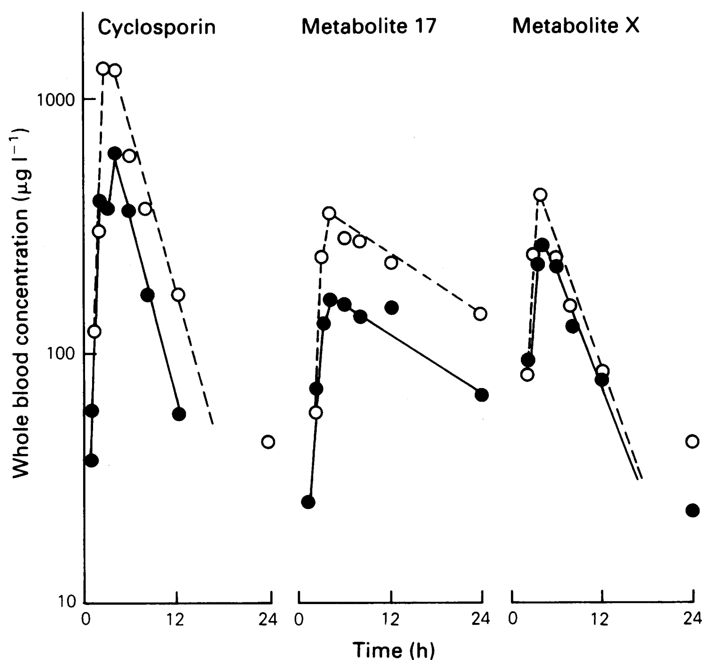

Figure 1 Semilogarithmic plots of the whole blood concentration of cyclosporin and two of its metabolites, metabolites 17 and X, in a subject who ingested cyclosporin (15 mg kg^{-1}) before (○) and after (●) phenytoin pre-treatment.

phases for all three compounds. The same effect was seen in all six subjects.

The estimated values for the terminal rate constant, and associated half-life, for all three compounds in the six subjects studied are listed in Table 1, with associated statistics. The control-phase mean terminal half-lives (derived from the mean elimination rate constant) associated with cyclosporin, metabolite 17, and metabolite 18 were 3.6, 17.5 and 6.4 h respectively. The corresponding phenytoin-phase values were 3.2, 13.8 and 6.5 h, respectively. For each compound the terminal half-life did not vary markedly among the subjects and no significant difference was found in the terminal slopes between the control and phenytoin pre-treatment phases.

Table 2 lists the calculated AUC until the last observation, the total AUC (from 0 to ∞) and the percent extrapolated AUC for all subjects for the three compounds, with associated statistics. For all three chemical species the total AUC was reduced by approximately 50% following phenytoin pre-treatment, although there were large inter-individual differences in the degree of reduction (ranging from very little in subject 1 to about 75% in subject 6). Also listed in Table 2 for each compound is the ratio of total AUC during the phenytoin pre-treatment phase to that observed during the control phase, as well as the ratio of AUC of metabolite to that of cyclosporin for both phases. There was no significant difference in any of these AUC ratios between phenytoin pre-treatment and control phase, with the exception of the AUC ratio for metabolite 18 (0.634 *vs* 0.500).

Discussion

The present data analysis confirms the marked reduction in the total AUC of cyclosporin, when given orally, caused by phenytoin pre-treatment to a group of young healthy adult volunteers (Freeman *et al.*, 1984). A similar observation has been made in organ-transplant patients receiving phenytoin whilst on oral cyclosporin therapy (Keown *et al.*, 1984). A reduction in cyclosporin AUC can be caused by a reduction in the amount of drug absorbed, by an increase in cyclosporin clearance, or by both. A definitive answer would be provided by intravenous cyclosporin data, but this would have increased substantially the demands on the study as all subjects would have had to receive four treatment periods. Consideration of both cyclosporin and metabolite data does, however, provide a useful insight into the likely explanation.

Before commencing any data analysis, the identity of one metabolite must be questioned,

Table 1 Terminal half-lives of cyclosporin, metabolite 17 and metabolite X in whole blood following oral administration of cyclosporin, before and after pre-treatment with phenytoin

Subject	*Cyclosporin*		*Metabolite 17*		*Metabolite X*	
	Control	*Phenytoin pre-treatment*	*Control*	*Phenytoin pre-treatment*	*Control*	*Phenytoin pre-treatment*
1	2.9	3.0	16.6	16.6	6.2	5.9
2	2.9	3.0	18.3	8.8	5.2	6.8
3	3.0	2.4	17.5	15.4	6.2	5.9
4	3.4	3.8	14.3	16.4	5.7	7.7
5	6.2	5.0	20.5	16.7	9.3	6.5
6	5.2	2.9	19.4	—[a]	7.6	—[a]
Mean[b]	3.6	3.2	17.5	13.8	6.4	6.5
Significance (paired *t*-test)	NS		NS[c]		NS[c]	

[a] Insufficient terminal phase data to gain estimate of half-life.
[b] Harmonic mean ($t_{1/2} = 1/k$ where k is the mean terminal rate constant).
[c] Excluding data for subject 6.

Table 2 AUC values and ratios for cyclosporin, metabolite 17 and metabolite X in whole blood following oral administration of cyclosporin, before and after pre-treatment with phenytoin

Subject		*AUC (ng ml^{-1} h)* Cyclosporin Control	Cyclosporin Phenytoin	Metabolite 17 Control	Metabolite 17 Phenytoin	Metabolite X Control	Metabolite X Phenytoin		*AUC(0,∞) Phenytoin / AUC (0,∞) Control* Cys	Met 17	Met X	*AUC(m, 0,∞) / AUC(d, 0,∞)* Metabolite 17 Control	Metabolite 17 Phenytoin	Metabolite X Control	Metabolite X Phenytoin
1	AUC(0,t)[a]	10638	7548	6879	4562	4376	4646								
	AUC(0,∞)[b]	10930	7699	11879	7895	4783	5154		0.704	0.665	1.080	1.090	0.991	0.438	0.669
	% Extrap	2.6	2.0	42	42	8.5	9.9								
2	AUC(0,t)	9331	4804	7571	3872	3748	2046								
	AUC(0,∞)	9620	5150	12966	5622	4029	2497		0.535	0.433	0.620	0.742	1.092	0.419	0.485
	% Extrap	3.0	6.7	42	31	7.0	17.9								
3	AUC(0,t)	7606	3516	4343	2796	2823	2341								
	AUC(0,∞)	7796	3716	8343	4240	3212	2547		0.477	0.508	0.793	1.070	1.140	0.412	0.685
	% Extrap	2.4	5.4	42	34	12.1	7.9								
4	AUC(0,t)	10702	6714	9858	5183	6062	4319								
	AUC(0,∞)	10991	7026	15531	9111	6607	5063		0.639	0.587	0.766	1.413	1.297	0.601	0.721
	%Extrap	2.6	4.4	36.5	43	8.2	14.7								
5	AUC(0,t)	12553	7715	8466	4285	6453	4253								
	AUC(0,∞)	12669	7945	17613	6928	7847	4608		0.629	0.398	0.587	1.390	0.872	0.619	0.580
	% Extrap	0.9	2.9	52	38	17.7	7.7								
6	AUC(0,t)	11371	2851	7929	615	5457	1098								
	AUC(0,∞)	12075	3065	4901	2356[d]	6218	2023		0.254	0.158	0.325	1.234	0.769	0.515	0.663
	% Extrap	5.8	7.0	47	74	12.2	45.8								
									NS (Cys vs Met X)						
Mean	AUC(0,t)	10367	5525	7591	3552	4753	3117	Mean	0.539	0.457	0.695	1.16	1.027	0.500	0.634
	AUC(0,∞)	10680	5769	11872	6019	5350	3642	C.V. (%)	27.3	38.8	36.2	19.6	32.7	16.8	12.4
	% Extrap	2.6	4.7	42.1	43.7	11	14.1		NS (Cys vs Met 17)	NS (Met 17 vs Met X)					
		$P < 0.01$		$P < 0.01$		$P < 0.05$						NS		$P < 0.05$	

[a] Area up to last observation, estimated by linear trapezoid approximation.
[b] Calculated total area = AUC(0,t) + C^*/k, where C^* is the last measured concentration and k is the terminal rate constant.
[c] Percent of total area that is estimated by extrapolation [(C^*/k)/AUC(0,∞) × 100].
[d] Assumed value of k in subject 6 to be the corresponding mean value for the other five subjects, cf. Table 1.
[e] NS—not significant ($P > 0.05$).

on the basis of rate limitations. Freeman *et al.* (1984) had an authentic reference sample for metabolite 17 but not of metabolite 18; they based their identification of metabolite 18 on h.p.l.c. retention time data. The designation of metabolites follows the scheme of Maurer *et al.* (1984), in which metabolite 18 is depicted as the non-enzymatic degradation product of metabolite 17 (the monohydroxylated product of the terminal η-position of the C_9-amino acid). According to precursor-product theory, a product cannot be eliminated any faster than it is formed. That is, a product (in this case metabolite 18) cannot fall with a half-life shorter than its precursor (in this case metabolite 17). Yet, the terminal half-life of the product, designated by Freeman *et al.* (1984) as metabolite 18, is much shorter ($t_{1/2}$ = 6.4 *vs* 17.5 h). Accordingly, it cannot be derived from metabolite 17, and most likely is derived from cyclosporin itself. Perhaps it is metabolite 1 (hydroxycyclosporin) which chromatographs very close to metabolite 18 (Maurer *et al.*, 1984). This metabolite is designated hereafter as metabolite X, although the identity of the metabolite is not crucial to the interpretation of the data.

Of the two metabolites, there is greater confidence in interpretation of metabolite X data. With metabolite 17 the estimated terminal half-life of around 17 h is approaching the 24 h total blood collection period, after cyclosporin administration, of the study thereby giving relatively little confidence in the estimate of both half-life itself and total AUC, with the area under the concentration-time curve beyond the 24 h observation comprising approximately 50 percent of the total area. With metabolite X, the terminal half-life of 6.5 h is relatively short compared to the 24 h collection period, so that the extrapolated area contributes little to the total AUC. Nonetheless, all metabolite data point to phenytoin pre-treatment causing a reduced absorption of cyclosporin, rather than induction of cyclosporin metabolism. This conclusion is based on the failure to find any significant change either in the AUC ratio for each compound between the two phases (Table 2, cf. equation 4) or in the ratio of AUC of metabolite 17 to that of drug between the phenytoin pre-treatment and control phases (Table 2, cf. equation 5). Theoretically, the same result would also be seen had both the formation clearance of metabolite (CL_m) and total clearance of metabolite ($CL(m)$) increased, due to induction, by exactly the same degree. However, this is extremely unlikely as there was no change in the half-life of either cyclosporin or metabolites, which is the usual consequence of enzyme induction. There was a statistically significant increase in the ratio of AUC of metabolite X to that of drug following phenytoin pre-treatment, consistent with the enzyme induction, but the absolute increase in the AUC ratio is small (0.634 *vs* 0.500).

Cyclosporin is sparingly soluble in water and is administered orally in a vegetable oil base. Griseofulvin and dicoumarol are also sparingly soluble drugs and their absorption is reduced by phenobarbitone and heptabarbitone (Riegelman *et al.*, 1970; Putcha *et al.*, 1978; Crow *et al.*, 1979). The griseofulvin–phenobarbitone interaction has been shown to be a formulation-dependent phenomenon, the effect disappearing if griseofulvin is dissolved in polyethylene glycol, which ensures rapid dissolution and absorption of griseofulvin (Jamali & Axelson, 1978). The mechanism for such interactions is not fully understood but has been suggested to be due to a reduction in bile salt concentration (Klaassen, 1971), with bile salts increasing the aqueous solubility and rate of dissolution of griseofulvin. Perhaps phenytoin reduces the absorption of cyclosporin through the same mechanism. In this regard the recent observation of a reduced plasma trough concentration of cyclosporin in a patient receiving phenobarbitone (Carstensen *et al.*, 1986), which was said to be due to enzyme induction, may in fact be caused by an induced malabsorption of cyclosporin.

In the study by Freeman *et al.* (1984) the second dose of cyclosporin was given one day after the last phenytoin dose. The observed interaction would therefore suggest either that there is sufficient phenytoin still in the body to produce its effect directly or that phenytoin exerts an indirect effect which persists after much of the phenytoin has left the body. A cyclosporin-phenytoin interaction study, in which the dose of phenytoin and duration between stopping phenytoin administration and giving cyclosporin were varied, would help to distinguish between the two hypotheses.

In their study, Freeman *et al.* (1984) verified the enzyme inducing ability of phenytoin by showing that the clearance of antipyrine, a frequently used test marker of hepatic drug metabolising capacity, was increased after phenytoin pre-treatment. This observation is not inconsistent with the conclusions of the current analysis. There are numerous hepatic isozymes which exhibit differential sensitivity to inducing agents. Accordingly, demonstrating induction with one substrate (e.g. antipyrine) does not imply that induction occurs with another substrate (e.g. cyclosporin).

Caution should be exercised in interpreting too precisely the cyclosporin and metabolite

whole blood concentration data. The present pharmacokinetic analysis assumes linearity (concentration independence) in the various processes, yet the binding of cyclosporin to red blood cells is a saturable process (Lemaire & Tillement, 1982) and it is likely that at least metabolite 17, which also binds to red blood cells, competes with cyclosporin for the binding sites. Nonetheless, the analysis does suggest that information on metabolites is a useful adjunct to unchanged drug data, in interpreting the mechanism responsible for altered pharmacokinetics. A combined intravenous/oral study should provide a definitive answer.

We wish to thank Drs D. Freeman and G. Carruthers for supplying the original data, which formed the basis of the data analysis and for their helpful comments. One of us (SG) thanks the University of Manchester for a Frederick Craven Moore Scholarship.

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(Received 27 November 1986, accepted 11 May 1987)