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Chronopharmacokinetics of Cyclosporine A in the Wistar rat following oral administration

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SUMMARY

The pharmacokinetics of Cyclosporine A (CsA) was studied in male Wistar rats weighing 300 ± 50 g trained to a 12:12 light-dark cycle. Oral administration (40 mg/kg) was performed at 1 of 4 different temporal stages: 09.00 h, 15.00 h, 21.00 h or 03.00 h (local time) i.e. 0200, 0800, 1400 or 2000 HALO (hours after light on). Blood samples were collected over 72–96 h after dosing, plasma was separated by centrifugation at 37°C and stored frozen until assay, using radioimmunoassay (RIA). Two experiments were performed: the first with 4 groups of 48 rats and a non-specific polyclonal antibody (P-RIA); and the second with only 2 groups of 48 rats and a more specific monoclonal antibody (M-RIA). Plasma concentration data were evaluated with model-based linear pharmacokinetic concepts, with apparent zero-order or first-order absorption and n-exponential disposition (n = 1, 2 or 3) : models MN0 or MN1. A compartment-independent approach was also conducted and led to area under the plasma concentration-time curve (AUC) and mean residence time (MRT) determinations. A comparison of the pharmacokinetic profiles across time of administration indicates that absorption, first-pass metabolism and tissue distribution of CsA in the rat are circadian-dosing stage dependent.

INTRODUCTION

Cyclosporine A (CsA: Ciclosporin, Sandoz, France), a cyclic polypeptide of fungal origin has been widely used for the last 10 years as a potent immunosuppressive agent. The drug is indicated for the prophylaxis of organ rejection in allogenic transplantations and for the treatment of some autoimmune diseases. Unfortunately, it poses particular problems in its use, in relation to quite a narrow therapeutic window; overdosage causes renal and, to a lesser extent hepatic toxicity but neither the therapeutic nor the toxic effects correspond well to the dose administered. Moreover, there are extensive inter- and intrapatient variations in pharmacokinetic parameters, whether from absorption, or distribution, metabolism and elimination phases. Variable pharmacokinetics necessitate frequent blood level monitoring and the selection of the best index to avoid the two risks: graft rejection and toxicity. The controversy is intense regarding the choice of matrix, assay technique and kinetic parameters (1). The recognized causes of such a variability are multiple (2): interactions with other drugs, food, liver disease, diarrhoea but also a circadian influence has been reported (3–5). Statistically significant variations can be demonstrated in the kinetics of drugs when the administration time

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is manipulated: the temporal organization of biological functions influences drug behaviour and, reciprocally, the target systems are affected by the drug and to a different extent, depending on the temporal stage of administration (6, 7). A better understanding of periodic, and thus predictable, changes in CsA effects and kinetics is required to optimize both timing(s) and dosage(s). Circadian variations in the kinetics of a drug may be explained by changes in its absorption, tissue distribution, hepatic metabolism and renal excretion.

The present study investigates the chronopharmacokinetics of cyclosporine A in the male Wistar rat. The plasma level profiles were established after a single oral dose (40 mg/kg), at one of 4 equally spaced 24 h stage of treatment. Drug was monitored using radioimmunologic methods. Two series of data are presented: one corresponds to the determination with a non specific antibody, which also precipitates the possible metabolic products of CsA (polyclonal RIA); the second was performed with a more specific antibody (monoclonal RIA).

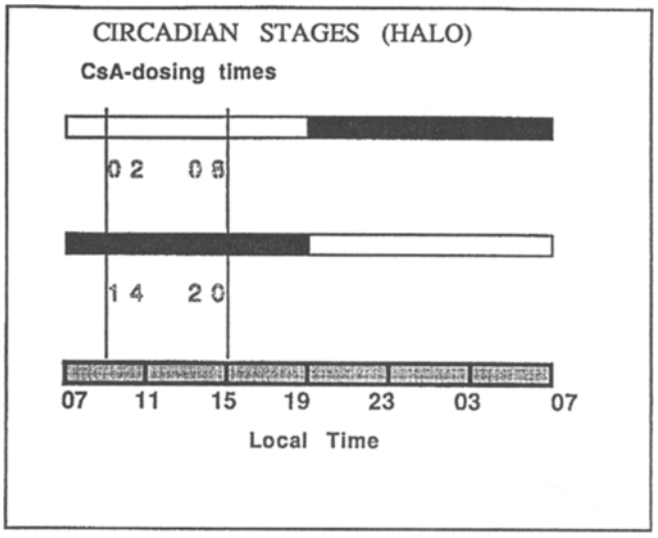

Fig. 1 : Time schedule with inversed light-dark cycles: the vertical bars indicate the 2 CsA dosing times (0900 and 1500 h) and the 4 related different circadian stages expressed in hours after light on (HALO)

MATERIALS AND METHODS

Materials

Cyclosporine A (Ciclosporin, Sandoz, France) powder was dissolved in olive oil-ethanol (90:10 by volume).

Polyclonal radioimmunoassay ([^{3}H]-P-RIA) was purchased from Oris France (Ciclokit) (assay sensitivity about 40 µg/l). Monoclonal radioimmunoassay ([^{125}I]-M-RIA) was obtained from Baxter (Cyclo-Trac SP [^{125}I]-RIA kit) (assay sensitivity about 10 µg/l).

All other chemicals were reagent grade and only bi-distilled water was used.

Animals and administration

Male Wistar rats purchased from Cerj France were used in this study. The animals were housed for 4 weeks prior to the experiments in groups of 6, in polycarbonate cages. The cages were kept under constant conditions of temperature and controlled conditions of light/dark cycle (12:12), lights on at 07.00 h (normal cycle) or 19.00 h (reversed cycle). Adaptation to cycle was shown to be effective within the first 3 weeks. Water and standard food were supplied freely.

The drug was administered orally, with an intragastric feeding needle and syringe. When P-RIA was used, 4 groups of 48 animals were constituted, differing in the hour of administration. This temporal-stage will be referred to as time elapsed since the light onset, i.e. 'hours after light on : HALO', so that each group corresponds to a different temporal dosing-stage: 0200, 0800, 1400 or 2000 HALO (Fig. 1). For M-RIA determinations, only 2 groups could be set up, administered either 2 or 14 h after light on (0200 or 1400 HALO).

Sample analysis

Blood samples for drug determination were collected by cardiac puncture under light anaesthesia with ether at 1, 2, 4, 6, 10, 12, 24, 48, 72 and/or 96h following drug administration. CsA concentration measurements may be made using whole blood, serum or plasma. The decision as to whether blood or plasma is most suitable for CsA monitoring is a matter of controversy. Blood is the matrix generally recommended because the distribution of the drug between plasma and blood is temperature dependent. However, some authors believe that the plasma measurements are more relevant (8, 9). In our experiments, blood was drawn into heparinized tubes and, immediately after puncture, plasma was separated by centrifugation at 37°C and then stored frozen until use.

Pharmacokinetic evaluation

Different models with first-order (MN1) or apparent zero-order (MN0) absorption (10) and mono-, bi- or triexponential disposition (n = 1, 2 or 3) (11) were fitted to pharmacokinetic profiles (12). The choice of the better fit was done on the criteria of the minimum residual sum of squares Σd^2 (deviations between the estimated and the mean values for each subgroup).

The pharmacokinetic parameters were calculated assuming linear pharmacokinetics and that the elimination occurred from the central compartment only. As a consequence, the calculated plasma concentration-time curves were described by the following equations:

Model MN1:

$$C(t) = A_o\, e^{-k_a (t - t_{lag})} + \sum_{i=1}^{N} A_i\, e^{-\lambda_i (t - t_{lag})}$$

Model MN0:

$$C(t) = \sum_{i=1}^{N} A_i \frac{[1 - e^{-\lambda_i T}]\ [\theta - e^{-\lambda_i (t - t_{lag})}]}{[\theta - e^{-\lambda_i T}]}$$

where:

t_{lag} = time between drug administration and first $C(t) \neq 0$

T = time corresponding to C_{max} in zero order absorption

k_a = first order rate constant of drug absorption

λ_i = disposition rate constants

θ = 1 during absorption ($t < T$) or 0 at other times

The coefficients A_o, A_i, k_a, and λ_i were computed as well as the corresponding half-lives $t_{1/2}$. A mean absorption time MAT was calculated, which is 0.5 T (zero-order absorption) or k_a^{-1} (first-order absorption) (12).

After a rough estimation of the coefficients on the basis of compartmental analysis with the precedent models using the classical method of residues, log linear or multiple regressions, a non linear regression program developed in the laboratory was used. As much as possible, results are expressed as 95% confidence intervals.

On the basis of non-compartmental methods, statistical moments were also determined. The zero order statistical moment (area under the plasma concentration-time curve, AUC) and the area under the first moment curve AUMC were calculated from zero to the final experimental point (tf) using Simpson's method. The mean residence time MRT was derived from the quotient AUMC/AUC.

The variance of AUC was estimated by:

$$S_{AUC}^2 = \frac{1}{4}\left[\frac{SS_f}{n_f (n_f - 1)} (t_f - t_{f-1})^2 + \sum_{i=1}^{f-1} \frac{SS_i}{n_i (n_i - 1)} \left(t_{(i+1)} - t_{(i-1)}\right)^2\right]$$

with:

SS_i = sum of squares for the i^{th} point t_i (size n_i)

SS_f = sum of squares for the final point t_f = (size n_f)

Statistical analysis

Analysis of variance (ANOVA), nonlinear regression analysis and multiple comparison of the parameters were used for the statistical analysis. A probability level of < 0.05 was considered significant.

RESULTS

P-RIA CsA pharmacokinetics

The pool-CsA plasma concentration-time curves of orally administered CsA at 4 different circadian stages are shown in Figure 2 (a–d). Plasma levels correspond to pooled concentrations of parent and metabolic products, since P-RIA is not specific. The pharmacokinetic parameter estimates are given in Table I.

Absorption

The first-order absorption model, MN1, underestimates the peak in sets of data corresponding to the groups of rats administered with CsA on 0200, 1400 and 2200 HALO. The model with apparent zero-order absorption, MN0, gives a better adjustment to these experimental data, with lower residual sum of squares Σd^2. On the contrary, data corresponding to the dosage at 0800 HALO are better described by the first order model MN1.

The time lag giving the best fits lies between 0 and 44 min. The absorption phase appears to be variable too, both in duration and efficacy, as a function of the time of administration. Maxima in plasma concentrations are reached after 4 h or less when the

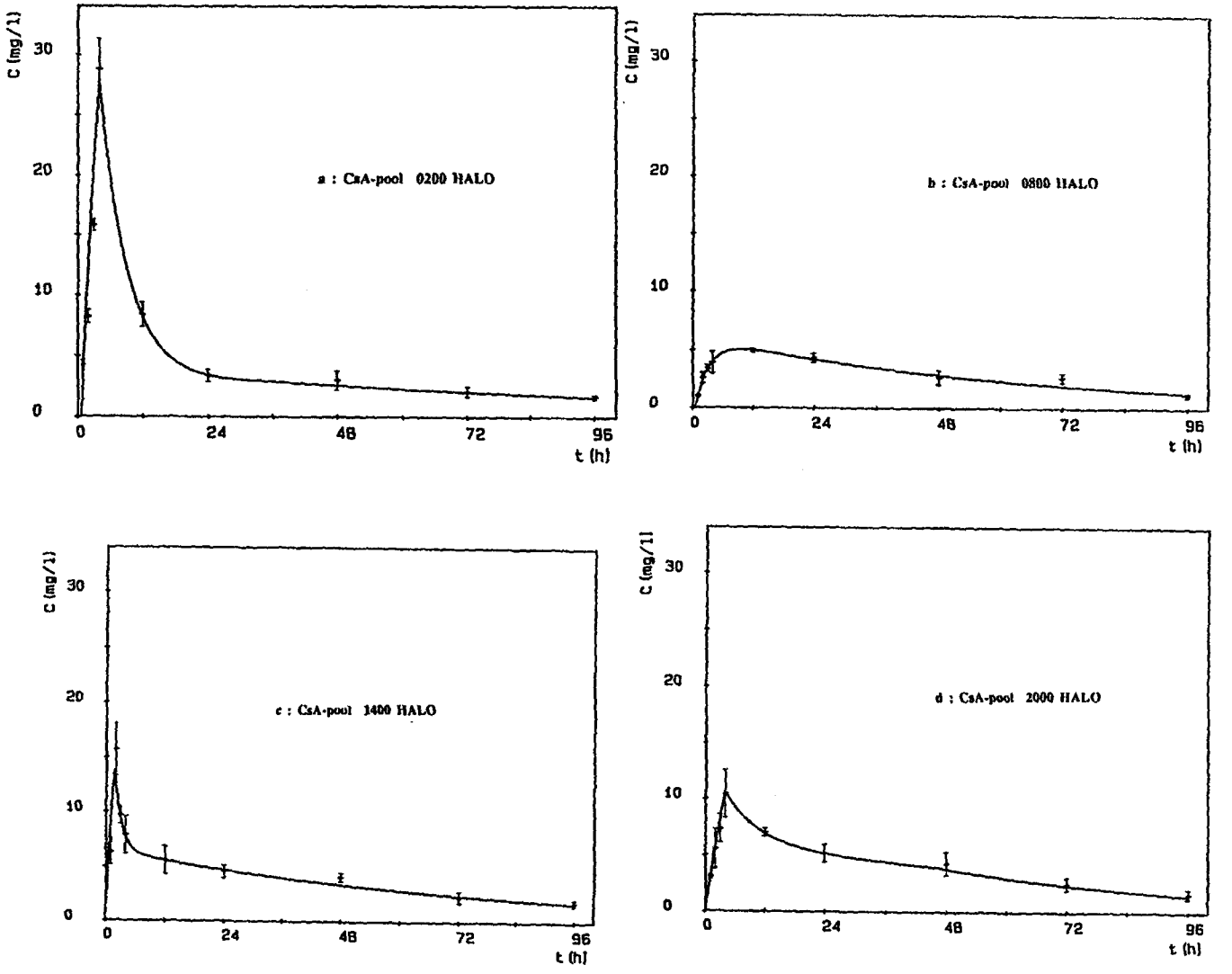

Fig. 2 : Pharmacokinetic profiles for P-RIA determinations of plasma pool CsA concentrations following oral administration at 1 of the 4 dosing times
a – 0200 HALO: b – 0800 HALO: c – 1400 HALO: d – 2000 HALO

drug is administered at any moment except 0800 HALO, whereas near 10 h are necessary when rats are dosed in that time. The mean absorption time MAT is about 2 h when CsA is administered 4 h before or 2 h after the dark to light transition (i.e. in groups dosed on 2000 or 0200 HALO). A lower value (0h52) is obtained from the group 1400 HALO and a higher one (2h48) from the group 0800 HALO.

The maximum plasma concentration (C_{max}) is highly variable between groups: the percentage of variation about an overall mean (14 µg/ml) reaches 198% when the drug is administered in the beginning of the light period (0200 HALO) and falls to 36% when the time of administration is 6 h later (0800 HALO).

Disposition

The plasma concentration-time profiles obtained for the groups 2000 and 0200 HALO were best described by a triexponential disposition model M30. The disposition parameters from these fittings were sometimes unreliable, among others the λ_2's which were not significantly different from zero, as if the occurrence of the terminal phase was simply delayed while the distribution was achieved. In the same 2

Table I: Pharmacokinetic parameters of P-RIA dosed plasma CsA after oral administration to male Wistar rats at one of four circadian stages

Time of oral CsA (local time)	*09.00 h*	*15.00 h*	*21.00 h*	*03.00 h*
Circadian stage (HALO)	*0200*	*0800*	*1400*	*2000*
Model	*M30*	*M11*	*M20*	*M30*
Pharmacokinetic parameter				
t_{lag} (h)	0h44	0h28	0h35	0
T_{max} (h)	4h	10h	1h45	4h
C_{max} ($\mu g.ml^{-1}$)	27.8	5.1	13.9	10.5
k_a (h^{-1})	—	0.356	—	—
$t_{1/2}k_a$ (h)	—	1h57	—	—
MAT (h)	2h	2h50	0h52	2h
λ_1 (h^{-1})	0.197	—	0.722	0.156
$t_{1/2}(\lambda_1)$ (h)	3h34	—	0h58	4h27
λ_2 (h^{-1})	0.0046 (NS $\neq 0$)	—	—	0.011 (NS $\neq 0$)
$t_{1/2}(\lambda_2)$ (h)	∞	—	—	∞
λ_3 (h^{-1})	0.010 ± 0.006	0.016 ± 0.003	0.014 ± 0.002	0.020 ± 0.008
$t_{1/2}(\lambda_3)$ (h)	2d 21h	1d 19h	2d 1h	1d 11h
AUC_{0-96h} ($\mu g.ml^{-1}.h$)	397 ±33	284 ± 20	368 ± 25	384 ± 35
MRT	1d 4h ± 1h	1d 15h ± 2h	1d 12h ± 2h	1d 11h ± 2h20
AUC_{0-12h} ($\mu g.ml^{-1}.h$)	155 ± 15	48 ± 4	81 ± 8	89.4 ± 10
C(12h) ($\mu g.ml^{-1}$)	8.1	5.0	5.6	7

groups, the λ_1's were not statistically different, giving a mean distribution half-time of about 4 hours.

The plasma concentration-time profile corresponding to group 1400 HALO was adequately described by a biexponential model M20, with a more rapid distribution phase (half-time about 1 h only).

A distribution phase could not be identified from the data obtained after administration at 0800 HALO so that a monoexponential disposition model M11 was adapted.

Elimination

The terminal phase (λ_3) is slow in the four groups since half lives are about 1 day (2000 HALO) to almost 3 days (0200 HALO). The elimination phase of CsA and its metabolites does not depend significantly on the circadian dosing stage: a multiple comparison of the 4 regression coefficients does not give any significant difference between these parameters. The mean value is 0.015 h^{-1}, corresponding to a mean elimination half-life of about 2 days.

Statistical moments

Both the AUC_{0-96} and corresponding MRT values fluctuate as a function of time of administration. The lowest (S_1%) AUC value (284 ± 20 $\mu g.ml^{-1}.h$) is observed in the 0800 HALO group while the three other ones are about 380 ± 31 $\mu g.ml^{-1}.h$. The mean residence time, MRT, is significantly minimal (S_1%) in the 0200 HALO group (28 ± 1 h) and is about 37 ± 2h15 in the three other ones.

M-RIA CsA pharmacokinetics

The M-RIA dosed CsA plasma concentration-time curves of orally administered CsA at 2 different circadian stages are shown in Figure 3. Plasma levels determined using monoclonal antibodies are more representative of parent drug concentrations.

Pharmacokinetics obtained in both cases – oral administration in the beginning of the light (0200 HALO) or dark (1400 HALO) periods – are best described using the model M20, i.e. with biexponential disposition and zero-order absorption. Estimates

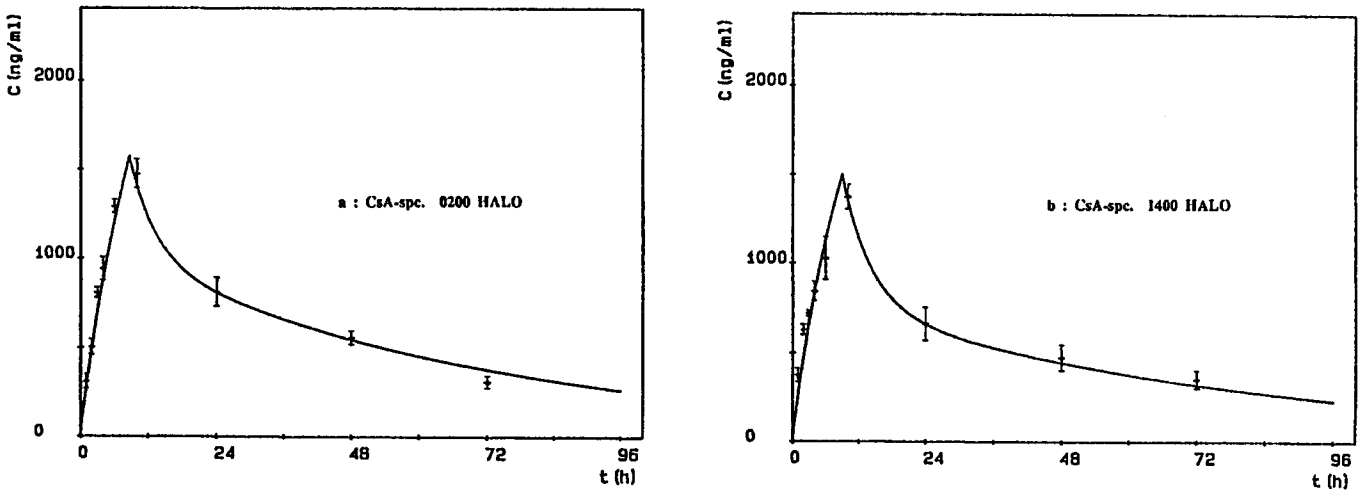

Fig. 3 : Pharmacokinetic profiles for M-RIA determinations of plasma CsA concentrations following oral administration at 1 of the 2 dosing times
a – 0200 HALO: b – 1400 HALO

of pharmacokinetic parameters are given in Table II.

Differences between estimates of pharmacokinetic parameters from the two groups differing in the hour of administration are not statistically significant. The mean absorption time is about 4h30, the distribution half-time is 3h30 ± 0h15 and the elimination half-time is about 36h ± 9 h.

Table II : Pharmacokinetic parameters of M-RIA dosed plasma CsA after oral administration to male Wistar rats at one of two circadian stages

Time of oral CsA (local time)	*09.00 h*	*21.00 h*
Circadian stage (HALO)	*0200*	*1400*
Model	*M20*	*M20*
Pharmacokinetic parameters		
t_{lag} (h)	0	0
T_{max} (h)	8h40	9h00
C_{max} ($\mu g.ml^{-1}$)	1.6	1.5
MAT (h)	4h20	4h30
λ_1 (h^{-1})	0.21	0.19
$t_{1/2}(\lambda_1)$ (h)	3h17	3h50
λ_2 (h^{-1})	0.015 ± 0.003	0.013 ± 0.004
$t_{1/2}(\lambda_2)$ (h)	1d 22h	2d 2h
AUC_{0-72h} ($\mu g.ml^{-1}.h$)	51 ± 2	43.5 ± 2.6
MRT	1d 12h ± 1h20	2d 23h ± 1h50
AUC_{0-12h} ($\mu g.ml^{-1}.h$)	12.5 ± 2	11.6 ± 0.8
C(12h) ($\mu g.ml^{-1}$)	1.48	1.34

Statistical moments from the two profiles are significantly different ($S_{1\%}$): AUC is higher in the group dosed in the beginning of the resting period: 51 ± 2 $\mu g.ml^{-1}.h$ (0200 HALO) vs 43.5 ± 2.6 $\mu g.ml^{-1}.h$ (2000 HALO), while MRT is higher in the group dosed in the beginning of the active light span: 61 ± 2.6h (2000 HALO) vs 36 ± 1h20 (0200 HALO).

Comparison of pooled and specific determinations

The comparison of the P-RIA and M-RIA determinations corresponding to the same two circadian stages of administration could permit, to a first approximation, an estimate of the degree of metabolism of the drug. This could be carried out by calculating the ratio of K = Cpool/Cspc as a function of time on the basis of the adjusted plasma concentrations following oral dosing at 0200 and 1400 HALO (Fig. 4).

Very rapidly after drug ingestion, this ratio exhibits values as high as 35 (0200 HALO) or 30 (1400 HALO). Then it diminishes during the next 12 h and finally reaches an equilibrium level about 5. Moreover, all these fluctuations are slightly delayed in groups dosed in the beginning of the light period (0200 HALO) compared with those injected 12 hours later (1400 HALO).

The difference between the AUC of the non specific assay (P-RIA) and the specific monoclonal assay (M-RIA) could be regarded too as a rough

representation of metabolite participation (13). When AUCs are calculated from zero to the last point, this difference is slightly greater (but not statistically significant) following a dosage at 0200 HALO compared to a dosage at 1400 HALO (338 ± 34 $\mu g.ml^{-1}.h$ vs 317 ± 25 $\mu g.ml^{-1}.h$). When AUCs are calculated on the basis of the first 12 hours after drug ingestion, the deviation is much more obvious (142 ± 15 $\mu g.ml^{-1}.h$ vs 75 ± 10 $\mu g.ml^{-1}.h$).

DISCUSSION

The present study confirms the circadian dosing-stage dependence of the fate of Cyclosporine A after oral administration to the rat. There are no significant differences in elimination rate constants between groups. The variations observed in the plasma concentration profiles are essentially linked to processes occurring during the first 12 h following drug ingestion. Moreover, these differences are essentially seen when CsA plus its metabolites are measured together, with the non-specific assay P-RIA.

Absorption

The bioavailability of CsA is known to be low, incomplete and highly variable, in part due to time- and dose-dependent changes in the gastrointestinal mucosa which is responsible for its absorption (14). The intestinal absorption of a drug is described by the rate and the extent of drug transfer from the intestinal lumen to the systemic circulation. A variety of factors may influence the absorption processes: gastric and intestinal motility, intestinal barrier to absorption, physicochemical properties of the drug.

Circadian variability in gastrointestinal motor activity may explain, at least in part, the circadian variability in the pharmacokinetics of some orally administered drugs that require delivery from the stomach to the small intestine before absorption takes place (15). Following a meal, gastric emptying, small intestine transit and mucosal absorption occur in an ordered sequential pattern. Under a 12–12h light–dark cycle, the feeding pattern of a rat consists of a maximum and almost constant rate of intake during the dark period, followed by a cessation when lights went on, and a progressive and slight increase during the light period. In the dark phase, stomach content accumulates in response to the fast increase of food intake rate. Gastric emptying lags by as much as 3 h behind intake (16). This could explain the prolonged T_{max} and lower C_{max} and AUC values obtained in our experiments in the group of rats administered in the middle of the light phase (0800 HALO), compared with the corresponding values in the three other groups, dosed during the dark (1400 and 2000 HALO) or in the beginning of the light phase (0200 HALO). But this effect linked to gastric emptying does not justify the differences between parameters inside these three groups: a shorter T_{max} in group 1400 HALO (1h45 vs 4h) and a higher C_{max} in group 0200 HALO (27.8 vs 10.5 and 13.9 $ng.ml^{-1}$). Now it must be noticed that these differences are not seen in the kinetic profiles resulting from specific determination (M-RIA). Moreover, T_{max}s are, in these last cases, much larger (about 10 h compared to 4 h or less for P-RIA determinations).

Metabolism

One reason for limited oral bioavailability of CsA may be first-pass metabolism. Biotransformation of parent drug in the gut may make an important contribution to the poor systemic bioavailability of CsA (17). CsA has been shown to be metabolized in vivo by cytochrome P-450IIIA in rat jejunal mucosa. A large proportion of the metabolites produced are pumped back into the gut lumen (18, 19) but some experiments in the dog have shown that metabolites were found in the portal vein (20). First-pass metabolism involving the same enzyme system occurs in the liver, too: as much as 50% of a dose of CsA may be extracted from the blood during its first passage through the liver (1). Some experiments have shown important day-night variations in the activity and lipid composition of the

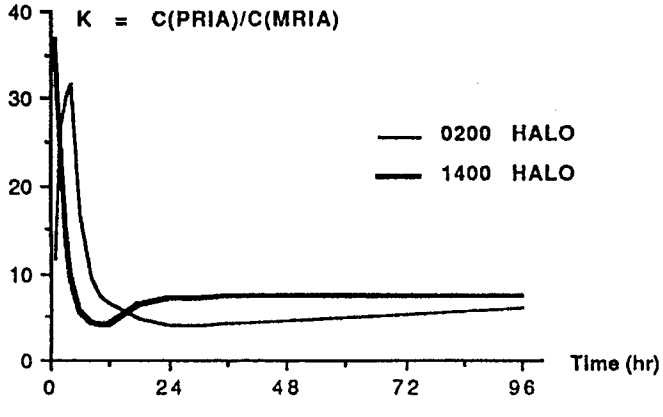

Fig. 4 : Variations as a function of time of the ratio of the adjusted plasma concentrations of P-RIA (pool parent drug + metabolites) and M-RIA (parent drug) determinations

microsomal mixed function oxidase of rat liver (21). Rhythms in intestinal drug metabolism exist also: in rabbit – which has nocturnal activity, like the rat – peak activity of intestinal microsomal enzymes including cytochrome P-450 were observed at approximately 06.00h and nadir between 12–15 h (22).

The temporal variability in the hepatic cytochrome P-450 monooxidase systems has been investigated by many laboratories and it can be concluded that the activity of most oxidases in rat liver are higher during the light phase, i.e. the resting period (23, 24). So day-night variations in the activity and lipid composition of the microsomal mixed function oxidase of rat intestinal mucosa and liver could explain day-night variations in first-pass metabolism of CsA. These could in turn be the reason for the observed differences in the maximum levels of the plasma P-RIA determined concentrations (parent compound plus metabolites), the higher C_{max} being obtained in groups dosed in the beginning of the light period (0200 HALO). In man, similar changes in CsA metabolism were observed. The CsA/M17 ratio was decreased during the night (resting period) suggesting an enhancement of metabolism (25).

Other factors could be evoked, which demonstrate day-night variability, such as bile composition and secretion, or blood flow. The results of studies on the effect of food on CsA absorption are inconclusive (26–28). The role of feeding in the genesis of circadian rhythms is controversial with regard to the interpretation of chronobiological experiments. Rats, which are nocturnal feeders, have been shown to have some enzyme rhythms closely tied to feeding (like sucrase, lactase, acidic and alkaline dehydrogenases) and, conversely, some other enzyme rhythms that persist in fasted animals (like hepatic oxidases).

Disposition

Once absorbed, the drug and its many metabolites have to be distributed into the organism and eliminated. The disposition of CsA has been described as both bi- and tri-exponential in man (11, 28). Longer terminal half-lives have been reported from studies in which sampling was extended over more than 24 h, than with a shorter duration of sampling. In our study, the adjustment to a n-exponential disposition with n = 1, 2 or 3 depending on time of administration, even if intermediate rate constant λ_2 estimates were very uncertain, led to much more comparable terminal rate constants λ_3 (about 0.015 h^{-1}). The distribution rate constants λ_1 and the corresponding half-lives vary substantially between groups. This distribution phase is not apparent in the plasma concentration–time profiles obtained from the 0800 HALO-dosed group with the P-RIA determinations. In the 3 other groups, the distribution half-time varies between less than 1 h (1400 HALO) and approximately 4 h (2000 and 0200 HALO). Considering the specific assay determinations, similar times are obtained (3–4 h for both 2000 and 0200 HALO groups).

Several factors could be involved in the dosing-time dependent changes of the distribution phase: plasma protein concentration exhibits, in both human and rodent, decreased levels during the rest period. The penetration of the drug in red blood cells and the permeability of the capillary wall are susceptible to variability, too (29).

Both CsA itself and its many metabolites undergo widespread tissue distribution, with higher concentrations in pancreas, adipose tissue and liver (30). The metabolites are less lipid soluble than parent drug. Because of the lipophilic nature of CsA, the drug binds to multiple blood components or macromolecules such as erythrocytes, lipoproteins and albumin. The molecular weight of CsA (1203) is high and it has a rigid structure which may be a further factor accounting for poor penetration, but it is suggested that part of the protein-bound CsA could be transferred from the vascular space to the tissues. Furthermore, in a lipid environment, CsA acquires internal hydrogen bonds resulting in a more hydrophilic complex (31).

Compartmental dependent or independent approaches

In this study, two kinds of models have been used to describe the oral pharmacokinetics of Cyclosporine A. In the compartmental approach, the models were chosen on the basis of previous reports: different authors (10, 28, 32) supposed that CsA is absorbed in the upper part of the small intestine with a clear cut termination (absorption window). The end of absorption being indicated by a sharp drop in plasma level, the kinetics were well described by apparent zero-order process. Gupta et al. (33) have more recently shown that this could not be true; absorption should be better described as a function of at least 2 first-order processes: the rate of absorption initially increases and later decreases. Unfortunately, it was not possible to verify this hypothesis with our data, so we speak of 'apparent' zero-order absorption. What's more, it would probably be better to use a single

model with dosing time-dependent rate constants to adjust the observed data rather than to adapt 2 different models (zero-order or first-order absorption) depending upon the temporal stage of oral administration.

The compartment-independent approach, based on fitting the data regardless of any physical meaning, leads to pharmacokinetics called 'robust', because they are less sensitive to small changes in the data. As chronopharmacokinetics introduce non-linearity into the pharmacokinetic concepts, the parameters calculated independently of defined procedures, like AUC, are probably the best candidate for chronopharmacological evaluation (34). Moreover, the measurement of AUC was introduced in the practice of clinical CsA therapy as an alternative to trough level monitoring (35).

However, knowing that peak concentrations of CsA in excess are associated with a decline in renal function (36), C_{max} variations associated with different times of administration would be taken into account in view of an optimization of the clinical use of this drug.

CONCLUSION

From our experiments and considering the pool determinations (parent drug plus metabolites), it appears that significantly higher C_{max}, AUC and C(12h) are obtained when the drug is administered in the beginning of the light period, i.e. resting time for the rats. The more specific determinations (with M-RIA) show the same tendency, however, with non significant differences between morning and evening dosing-stage kinetics. High levels are noticed too when the ingestion takes place during the dark active period while the lower plasma concentrations are observed when the drug is given in the middle of the resting period, i.e. far from the usual feeding periods of the rats.

Summarizing the present results, it can be concluded that CsA administered orally to the rat undergoes biological processes highly variable on a 24 h scale, including intestinal absorption, first-pass metabolism and tissue distribution. These complex temporal changes observed in the rat have to be further investigated: some experiments are now being conducted in our laboratory in which drug measurement is performed using HPLC to follow blood and tissue concentrations of the parent CsA and/or its metabolites and to demonstrate possible correlations with some dosing-time-dependent adverse effects (37, 38).

The clinical relevance of such findings for reducing the part of empiricism of the schedule of dosing regimen and thus for optimizing CsA therapy should not be neglected.

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