ELSEVIER

International Journal of Pharmaceutics 174 (1998) 9–18

international journal of pharmaceutics

Age and sex dependent pharmacokinetics of cyclosporine in the rat after a single intravenous dose

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Received 17 February 1998; received in revised form 11 May 1998; accepted 9 June 1998

Abstract

The dependency of cyclosporine (CyA) pharmacokinetics on the age (10 and 40-week-old) and the gender was studied in Wistar rats who were given 10 mg/kg dose of the drug intravenously. CyA levels in whole blood were analyzed by a specific fluorescence polarization immunoassay (Abbot TDx). Blood concentration vs time profiles were characterized by resorting to compartmental and noncompartmental methods. The first approach showed that the best model fitting experimental data was a three-compartment open model with first-order kinetics. It indicated that the drug undergoes extensive distribution in a wide variety of tissues. The mean half-lives corresponding to λ_1 and λ_2 phases coincided in all groups and lasted on average 0.22 and 4.30 h, respectively. The mean volume of distribution at steady-state depended mainly on the rats gender and age, indicating values of 2.64 ± 0.37 l/kg for older males as compared to 2.03 ± 0.29, 1.71 ± 0.12, and 1.87 ± 0.16 l/kg for young males and females and 40-week-old females, respectively. Drug elimination rates ranged from 0.019 to 0.123 h^{-1} and manifested a marked dependency on both, the gender and the age. The bioavailability ($AUC_{0-\infty}$) was higher for males (81.06 ± 8.31 and 139.62 ± 41.34 $\mu g \cdot h$ per ml vs 50.19 ± 2.10 and 49.65 ± 5.68 $\mu g \cdot h$ per ml) while the systemic blood clearance (CL) was significantly lower for males than for females (85 ± 19 and 109 ± 3 ml/h per kg vs 198 ± 9 and 205 ± 29 ml/h per kg). No statistically significant differences were detected between compartmental and noncompartmental parameters by paired *t*-tests. Therefore, the results demonstrate that female rats clear CyA faster than males, probably due to differences in drug metabolism.

Keywords: Age; Sex; Pharmacokinetics; Cyclosporine; Rat; Intravenous

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0378-5173/98/$ - see front matter
PII S0378-5173(98)00198-7

1. Introduction

Cyclosporine is a potent immunosuppressive drug extensively employed to inhibit organ rejection in transplant patients and in the treatment of autoimmune disorders. This drug has a great relevance on achieving successful results in transplantation but it must be carefully monitored due to the associated high risk of nephrotoxicity and hepatotoxicity (Fahr, 1993). The narrow therapeutic window for drug trough levels (100–250 ng CyA/ml blood) (Masri et al., 1992), and its highly variable pharmacokinetic behavior have inspired numerous studies dealing with the identification of factors responsible for such variability in humans. By an oral route, the poor and highly variable bioavailability which has been attributed to physiological factors affecting drug absorption, distribution and metabolism is quite evident (Grevel et al., 1990; Lemaire et al., 1990; Lindholm, 1991). Furthermore, physical, chemical and technological factors have been inferred to exert an influence as well (Ismailos et al., 1991; Vonderscher and Meinzer, 1994; Molpeceres et al., 1996), confirmed by the improved and more predictable pharmacokinetics when the recently marketed micro-emulsion formulation was administered (Mueller et al., 1994).

Many of the controversies concerning CyA pharmacokinetics may be attributed to basic differences when it comes to securing the pharmacokinetic parameters. Most studies carried out on humans correspond to different transplant populations (kidney, bone marrow, liver...), nonetheless, some of them are pre-transplant studies while others deal with the postransplant period. In addition, CyA treatment alters some of the physiological factors involved in drug pharmacokinetics, i.e. lipoprotein levels (Awni et al., 1990; Rödl and Khoshsorur, 1990; Kuster et al., 1994). Finally, specific and non specific methods are used to analyze drug concentrations in blood or plasma. Henceforth, all these factors sum up for a difficult comparison of results when taken from different studies geared at identifying additional variables which might influence drug availability in the human body.

Due to the inconvenience associated with the clinical use of CyA, many studies have been conducted whose goal was to identify potential sources of pharmacokinetic variability and toxicity in animal models (Sangalli et al., 1988; Bernareggi and Rowland, 1991; Kolars et al., 1992; Shibata et al., 1993; Malmary et al., 1995; Brunner et al., 1996). While, the development of alternative CyA formulations with improved pharmacokinetic and pharmacodynamic properties is under thorough investigation, particularly those that deal with colloidal carriers (Vadiei et al., 1989; Guzman et al., 1993; Bonduelle et al., 1995; Sanchez and Alonso, 1995). These compounds are normally tried and assayed in animals (rodents) in order to prove their potential advantages over other existing preparations. Albeit many pre-clinical pharmacokinetic studies are carried out in one gender species only, to avoid dispersion but the gender based genetic differences in the expression of certain proteins, such as cytochromes P450 (Smith, 1991), recommend that these studies should be extended to cover both sexes. Animal weight represents yet another parameter usually kept constant in pre-clinical research. However, based on the different growth curves for males and females, the age acquires a significant role.

In recent years, gender associated differences in heart and skin experimental transplantation have proven that female rats reject organ grafts faster than males while under CyA as immunosuppressive treatment (Hirasawa and Kamada, 1992). In addition, CYP450 3A was identified as an isoenzyme responsible for CyA metabolism, whilst, no levels of this protein have been detected in uninduced female rats (Kolars et al., 1992).

Therefore, the aim of this work consisted on studying the intravenous (i.v.) pharmacokinetics of CyA in male and female rats in order to evaluate the potential contribution of age and gender towards the variability as confirmed by previous studies carried out on rats.

2. Materials and methods

2.1. Chemicals

The i.v. solution of CyA was kindly supplied by the Ciudad Sanitaria La Paz, Madrid, Spain. All

other reagents used in the experiments were of analytical grade with the solvents of HPLC grade.

2.2. Experimental design

20 Wistar rats were requested from the Central Stabulary of the University and divided into four groups (five animals each) by age (10 and 40-week-old) and gender. Two additional groups ($n = 5$) of weight matched (250–300 g) males (10-week-old) and females (40-week-old) and not subjected to any drug treatment were used for haematological and biochemical determinations. The remaining four groups were used for pharmacokinetic evaluations. The number of rats in each group was estimated by considering the area under the curve obtained by Bernareggi and Rowland (1991) in order to achieve a minimum 80% statistical power which would allow to detect a minimal difference of 20% or more between groups at the usual 5% level of significance. The animal weights within each group (Table 1) were also matched (C.V. $< 6\%$) to reduce the inherent physiological variability, posteriorly determined for a proper drug dose-administration. The rats were maintained in metabolical cages 24 h before the experiments, with a preserved 12 h dark–light cycle and free access to water. CyA administration (Sandimmun® i.v. solution 50 mg/ml diluted with 0.9% saline NaCl to a final concentration of 12.5 mg/ml) was always carried out within the 09:00–10:00 h period to avoid chronopharmacokinetic effects (Malmary et al., 1995). A bolus dose of 10 mg/kg was punctured into the right jugular vein of lightly isoflurane (Abbott Laboratories) anaesthetized rats. 150 μl whole blood samples were withdrawn from the left jugular vein of animals at 0 (pre-dose), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 32 and 48 h post-administration. Additional time points (10, 26 and 28 h) were obtained from weight matched 10-week-old male and 40-week-old female rats in order to obtain a clearer estimation for the last linear phase of the curve. Samples were collected in polyethylene vials over 20 μl disodium EDTA (22 mg/ml), thoroughly mixed and frozen at -20°C until further analysis within 2 days of the study. Urine and faeces were collected from each cage; the urine volume was recorded and a 1.5 ml aliquot stored at -20°C until drug content assay.

2.3. Biochemical and haematological analysis

Since CyA pharmacokinetics is strongly influenced by several biochemical and haematological parameters, some of them were monitored in

Table 1
Mean ($\pm$S.D.) pharmacokinetic parameters of CyA following a single bolus i.v. administration of 10 mg/kg ($n = 5$)

Parameter	Males		Females	
	10 weeks	40 weeks	10 weeks	40 weeks
Rat weight (g)	270.33 ± 13.10	555.6 ± 26.54	206 ± 10.82	248.75 ± 3.09
λ_1-HL (h)	0.20 ± 0.11	0.29 ± 0.21	0.25 ± 0.03	0.17 ± 0.11
λ_2-HL (h)	4.82 ± 2.49	3.93 ± 0.35	4.29 ± 2.15	4.17 ± 0.99
λ_3-HL (h)	13.77 ± 3.01*	33.95 ± 14.81**	6.89 ± 1.51	10.81 ± 5.12
V_1 (l/kg)	0.51 ± 0.29	0.52 ± 0.19	0.84 ± 0.14**	0.40 ± 0.24
V_{ss} (l/kg)	1.98 ± 0.27***	2.68 ± 0.63****	1.72 ± 0.14	1.58 ± 0.21
AUC_c (μg·h per ml)	81.06 ± 8.31	139.62 ± 41.34**	50.19 ± 2.10	49.65 ± 5.68
CL_c (ml/h per kg)	123 ± 13	66 ± 13**	198 ± 8	201 ± 24

* $P < 0.05$ vs 10-week-old females;
** $P < 0.05$ vs 10-week-old males and 40 weeks old females;
*** $P < 0.05$ vs 10-week-old females;
**** $P < 0.05$ vs 40-week-old females.

weight matched (about 250 g) males and females. 6 ml whole blood aliquots were collected from the abdominal aorta and divided within two glass tubes (3 ml each). One of them was immediately taken for the haematological analysis while the other was centrifuged at $3000 \times g$ for 15 min. After spinning, the upper plasma fraction (1.5 ml) was readily pipetted into separate clean tubes and frozen at -20°C until the time of the assays. Among biochemical parameters, plasma levels of glucose, creatinine, total proteins, GPT, triglycerides and total and HDL-cholesterol were determined by standard methods. A complete haematological study was carried out by means of an automatic analyzer (Coulter).

2.4. Drug analysis

CyA concentrations in whole blood and urine were determined by using a monoclonal antibody fluorescence polarization immunoassay (monoclonal TDx, Abbot). The original method was slightly modified to measure drug concentrations in the 0–15 μg/ml range. Calibration curves were obtained each time a set of samples was analyzed. Under these conditions, percent recovery of CyA in the samples ranged from 95.22 to 102.16% and the within-day and between-day coefficients of variation did not exceed 4.66% for the same batch of reagents. The limit of quantification was 25 ng/ml.

2.5. Pharmacokinetic analysis

The pharmacokinetic parameters associated to each animal were estimated by compartmental and noncompartmental methods. Iterative nonlinear regression analysis (NONLIN) showed a three-compartment open model with bolus intravenous input and first-order output from the central compartment fitted to the drug concentration–time profiles with the lowest Akaike's number. The corresponding pharmacokinetic parameters (suffixed with c) were derived according to standard equations (Gibaldi and Perrier, 1982).

On the basis of noncompartmental analysis, statistical moments were determined. The zero-order moment area under the curve (AUC_T), was determined to the last experimentally measured concentration $Cn(t_z)$ at time t_z by the trapezoidal rule and extrapolated to infinity by adding the term $Cn(t_z)/\lambda_z$, where $Cn(t_z)$ denotes the concentration estimated at time t_z from the curve fitting and λ_z is the slope of the terminal phase estimated by log-linear regression of the last four to five experimental blood concentrations. The mean disposition residence time (MDRT) was derived from the ratio $AUMC_T/AUC_T$, where $AUMC_T$ is the area under the curve for the plot of the product of concentration and time vs the time from time zero to infinity. The total body clearance CL, the volume of distribution associated to the terminal phase V_β, and that at steady state V_{ss} were calculated by using the following equations: $CL = Dose/AUC_T$, $V_\beta = CL/\lambda_z$ and $V_{ss} = CL \cdot MDRT$.

CyA concentrations in urine samples were converted to quantities necessary for the determination of renal clearance (CL_r). CL_r was calculated as $X_u^\infty/AUC_{48\,h}$ where X_u^∞ and $AUC_{48\,h}$ are the total amount of drug excreted in urine during 48 h and the area under the curve for the same time period, respectively.

2.6. Statistical evaluation

The harmonic mean values for half-lives and clearance were calculated using the sum of their reciprocal values as previously described (Lum et al., 1992). CyA pharmacokinetic parameters were compared by using analysis of variance (two-way ANOVA) at the 0.05 significance level (EPISTAT software package). In cases where statistical significant differences were found, the least significant difference test (L.S.D.) was applied. The comparison between compartmental and noncompartmental parameters was carried out by paired t-tests.

3. Results

Plots representing the mean concentration–time data after i.v. administration of CyA to all groups are shown in Fig. 1. The blood concentration–

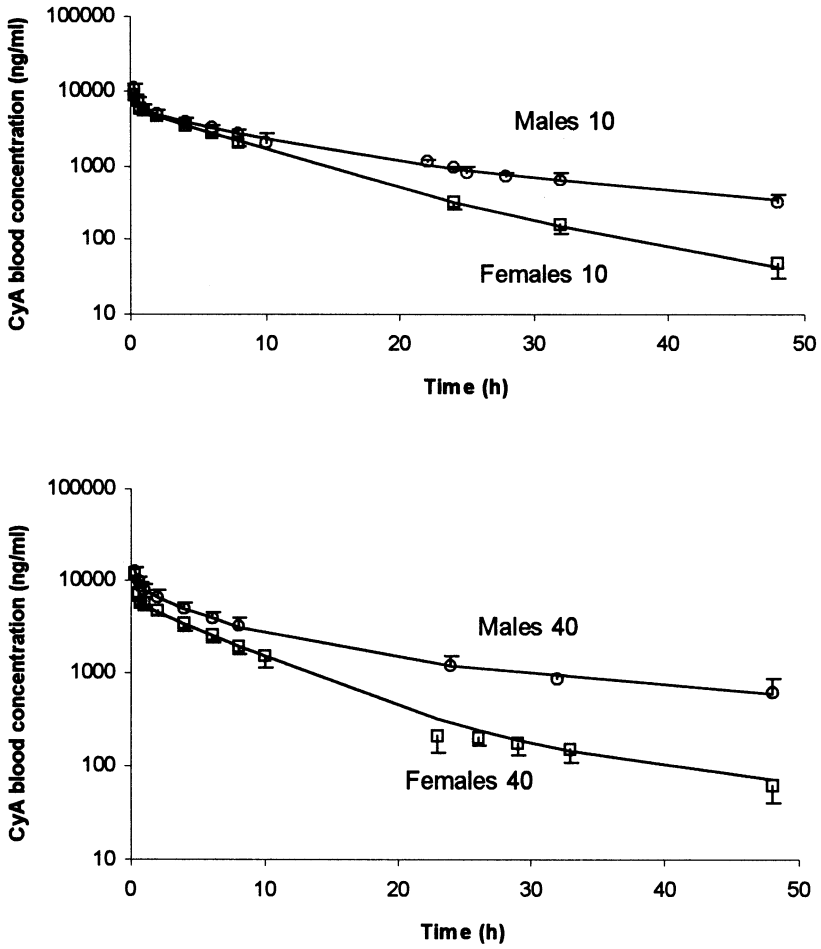

Fig. 1. Whole blood concentration–time profiles of CyA (mean $\pm$ S.D., $n = 5$) following a single 10 mg/kg i.v. dose administration to young adult males and females (10 weeks old) (upper), and adult males and females (40 weeks old) (lower).

time profiles declined multiexponentially in all animals. NONLIN fitting of experimental data to the tri-exponential model generated the pharmacokinetic parameters presented in Table 1. An adequate data fit was evidenced by the high correlation ($r^2 > 0.99$) between computer-calculated and experimental CyA blood concentrations and the plot of residuals. The mean harmonic values for λ_1-HL and λ_2-HL were 0.17 h (range 0.08–0.61 h) and 4.22 h (range 0.99–8.54 h) and showed no dependency on the studied variables. The intercompartmental drug distribution rates (K_{12}, K_{21}, K_{13} and K_{31}) did not show statistical significant differences due to their high variability (results not shown). In a global sense, drug distribution was characterized by a 2-fold higher transfer rate of CyA into compartment 2 (2.28 h^{-1}) against the rate of reversion to the central compartment (1.34 h^{-1}). The distribution of CyA into the third compartment was much slower ($K_{13} = 0.12$ h^{-1}) but within the same range as K_{31} (0.08 h^{-1}). The K_{10} values were not statistically different among groups (mean = 0.22 h^{-1}) but old

females exhibited highly dispersed values for this parameter. The V_{ss} strongly depended on the gender and the age showing higher values for older males when compared to younger males or females. The terminal elimination half-lives (λ_3-HL or λ_z-HL) showed gender and age-associated differences and their values ranged from 5.61 to 36.48 h. Finally, total exposure to the drug (AUC_c) as well as systemic clearance (CL_c) also showed a strong dependency on gender. Concerning the age, only the male rats exhibited significant differences for these two parameters.

The mean pharmacokinetic parameters derived from a noncompartmental analysis are presented in Table 2. The extrapolated AUC contributed on average of $1.6 \pm 0.9\%$ for females and $14.11 \pm 7.74\%$ for males to the total AUC_T indicating an appropriate sampling period. Females showed greater total body clearances (198 ± 9 and 205 ± 29) for 10 and 40-week-old rats than males (109 ± 3 and 85 ± 19 ml/h per kg) and shorter MDRT 8.64 ± 0.78 and 9.21 ± 0.92 h vs 17.71 ± 3.08 and 32.39 ± 5.10 h (Table 2).

The parameter V_{ss} was used to evaluate the differences in drug distribution, since, changes in V_β were also associated with changes in λ_z values. In accordance with the results obtained by compartmental analysis, noncompartmental methods evidenced CyA, distributed significantly more abundantly in old male rats than in the other groups. Cumulative quantities of CyA recovered from the urine were all around or below 1% of the administered dose (Table 2). The CL_r were 1.21 ± 0.21 and 0.61 ± 0.23 ml/h per kg for young and old male rats and 1.04 ± 0.18 and 2.32 ± 0.56 ml/h per kg for 10 and 40-week-old female rats, respectively.

The coincidence between the two pharmacokinetic analysis was assessed by comparing the AUC, Vss and CL estimates from the noncompartmental analysis with those derived from the compartmental model. The lines of identity and regression showed a slope = 0.90, 0.80 and 0.94 and $r_2 = 0.99$, 0.96 and 0.98 for AUC, V_{ss} and CL, respectively. Paired t-tests did not show any statistically significant difference between the model derived pharmacokinetic parameters and those calculated by noncompartmental analysis.

The power achieved by statistical comparisons of pharmacokinetic parameters was higher when the results of noncompartmental methods were considered. Among them, only V_{ss}, AUC_T and CL comparisons had a statistical power around 80% and therefore these were considered in the discussion.

Table 2
Noncompartmental mean ($\pm$ S.D.) pharmacokinetics parameters of CyA following a single bolus i.v. injection of 10 mg/kg ($n = 5$)

Parameter	Males		Females	
	10 weeks	40 weeks	10 weeks	40 weeks
λ_z (h^{-1})	0.046 ± 0.006*	0.021 ± 0.002**	0.098 ± 0.016**	0.051 ± 0.004
λ_z-HL (h)	15.86 ± 2.28*	32.03 ± 3.77**	7.16 ± 1.19**	13.50 ± 1.08
MDRT(h)	17.71 ± 3.08*	32.39 ± 5.10**	8.64 ± 0.78	9.21 ± 0.92
V_β (l/kg)	2.63 ± 0.32***	3.77 ± 0.52	2.04 ± 0.32**	3.98 ± 0.62
V_{ss} (l/kg)	2.03 ± 0.29*	2.64 ± 0.37**	1.71 ± 0.12	1.87 ± 0.16
AUC_T (μg·h per ml)	91.22 ± 2.30*	131.81 ± 20.67**	50.54 ± 2.29	49.56 ± 7.05
CL (ml/h per kg)	109 ± 3****	85 ± 19**	198 ± 9	205 ± 29
X_n^{∞}[a] (% of dose)	1.05 ± 0.07	0.74 ± 0.11	0.55 ± 0.03	1.18 ± 0.19
CL_r (ml/h per kg)	1.33 ± 0.21***	0.80 ± 0.27**	1.12 ± 0.12**	2.42 ± 0.56

[a] Amount of unchanged drug excreted in urine.
* $P < 0.05$ vs 40-week-old males and 10 weeks old females;
** $P < 0.05$ vs 40-week-old females;
*** $P < 0.05$ vs 40-week-old males;
**** $P < 0.05$ vs 10 weeks old females.

Table 3
Biochemical and haematological parameters of male and female Wistar rats

Parameter	Males	Females
Haematocrit (%)	41.16 ± 1.47	40.22 ± 2.29
RBC ($\times 10^{-6}$)	7.42 ± 0.33	7.48 ± 0.32
Plasma proteins (g/l)	60 ± 2.50	60.8 ± 1.90
Total cholesterol (mg/dl)	71.2 ± 4.66	73.8 ± 8.96
HDL–cholesterol (mg/dl)	56.60 ± 3.78	60.0 ± 7.00
Triglycerides (mg/dl)	46.60 ± 5.55	45.40 ± 10.14
Creatinine (mg/dl)	0.53 ± 0.04	0.61 ± 0.06
GPT (UI/l)	25.40 ± 4.22	21.40 ± 4.51
Glucose (mg/dl)	104.60 ± 18.12	103.20 ± 5.02

The haematological and biochemical parameters (Table 3) corresponding to males and females were comprised within the normal range and did not show statistically significant differences among groups (IFFA-CREDO, 1988).

4. Discussion

The therapeutic properties of CyA to forestall graft rejection and graft vs host disease are well known as well as its dose dependent hepatic- and nephrotoxicity. The need to minimize the adverse effects and to optimize drug therapy compels monitoring of drug concentrations since CyA pharmacokinetics show high inter and intra subject variability.

When CyA is administered to rats by the i.v. route in peripheral veins the overall dispersion throughout takes place within blood components and extravascular sites. On most recent dates, triglycerides, total cholesterol, hematocrit, and creatinine plasma levels were identified as crucial factors which allow predicting the CyA erythrocyte to plasma ratio in humans (Shibata et al., 1994). This ratio conditions the distribution of the drug to extravascular sites. Assuming that this model may be qualitatively applied to rats and considering that no significant differences were detected for these parameters (Table 3) in our study, we have predicted a similar intravascular distribution pattern for male and female rats. In rats, two of the main body tissues that retain large amounts of drug are fat and muscles because of the drug lipophilicity and extension of muscle compartment within the body, respectively (Bernareggi and Rowland, 1991). Therefore, the differences found in V_{ss} are more likely the result of age and gender-associated changes brought about by the relative contribution of fat and muscle tissues to total body weight in each group. Shibata et al. (1993) have also found a significant increase in V_{ss} for aged rats and suggested it might be due to an age-related increase in adipose tissue. Indeed, V_{ss} differences achieved statistical significance only then when 40-week-old male rats were compared with the rest of groups. This group clearly outweighs the rest but the liver to body weight ratio was significantly lower (data not shown) and consequently, for a drug administered on a body weight basis and cleared by hepatic metabolism such as CyA, the systemic clearance was reduced.

The metabolic capacity for scantly cleared drugs, (such as CyA), is usually very low compared with the hepatic blood flow, hence the CyA elimination is mainly dependent on drug liver uptake and metabolism. Recently, total body clearance was reported to be dose dependent in male normal rats, with mean values 204.36 ± 39.24 and 237.23 ± 66.3 ml/h per kg for intravenous doses of 5 and 10 mg/kg, respectively (Shibata et al., 1993). In the same study, drug clearance in 35 to 50 weeks aged normal male rats given 5 mg/kg intravenously was 237.72 ± 29.88 ml/h per kg. Our clearance results are quite different for the male groups constituting about one-half of the values as reported in the above mentioned study. These differences are probably due to the different sampling period (48 h in our study vs 8 h) since the mean λ_3-HL reported for normal rats was 4.38 h, sufficiently close to the intermediate mean disposition half-life obtained by other authors (Bernareggi and Rowland, 1991; Malmary et al., 1995) and our team (4.22 h). Mraz et al. (1992) also studied i.v. (2.6 mg/kg) CyA pharmacokinetics in rats during 8 h, reporting bi-exponential profiles with λ_z-HL about 5.52 h. Malmary et al. (1995) found CyA plasma clearance in Wistar rats was 149 ml/h per kg but

considering the normal ratio between CyA blood and plasma levels, drug blood clearance would be close to our values.

CyA is converted to its major metabolites by the enzymes belonging to the cytochrome P450-3A subfamily (3A1 and 3A2 in rats). Gender differences in the expression of these proteins in rat liver have been previously reported (Smith, 1991). Only P450-3A2 is considered to be constitutively present in uninduced mature (more than 7-week-old) male rats, however, no statistically significant differences or faster in vitro CyA metabolism have been previously found in males as compared to female rats (Kolars et al., 1992; Prueksaritanont et al., 1993). CYP3A enzymes are also contemplated to be responsible for the biotransformation of other immunosuppressive drugs such as FK506 (Perotti et al., 1994). The maximum in vitro metabolic rates of FK506 in male rats doubled those obtained in females while K_m values remained unchanged. On the contrary, Sadrieh and Thomas (1994) reported female rat liver microsomes metabolized CyA to covalently bound products more efficiently than hepatic microsomes from male rats. Furthermore, Brunner et al. (1996) found CyA blood levels in male rats were 2-fold higher than females after 28 days of subcutaneous drug administration. Since no 3A isoforms have been characterized in uninduced adult female rats, it is possible that another isoenzyme is responsible for a significant quantities of metabolized CyA. Therefore, the sex-associated differences we encountered reflected in drug blood clearance, seem to be the result of distinct drug hepatic metabolism.

Unchanged CyA was also excreted in the urine, although in very low amounts. The age and sex-associated changes in renal clearance must be weighed carefully since the power of the statistical analysis was very low (22%).

The new trends to drug dosage adjustment in clinical practice are based on a number of parameters where CyA trough levels and AUC are included as indicators of total body drug exposure (Plant et al., 1994). A clear relationship between CyA trough levels and drug clearance to rejection episodes have been previously reported (Lindholm et al., 1993; Schroeder et al., 1995). However, many physiological factors have shown to alter drug disposition in humans (Lindholm, 1991). Yee (1990) was able to prove that CL and Vss decreased with patient's age (none of them pediatric patients) when CyA levels were determined in plasma. However, no changes were detected when whole blood was used. Other studies (Grevel et al., 1989; Humbert et al., 1994) have reported no changes in V_{ss} due to the age differences. Yee (1990) suggested that age-related changes in V_{ss} might be associated with changes in lipoprotein concentrations. Further more, because of the lipophilic nature of the drug, CyA pharmacokinetics ought to depend on body weight and even on sex, since aging is expected to induce an increase in adipose tissue deposits (Wagner, 1979). The results from different clinical studies indicate that these relationships still remain unclear (Lemaire et al., 1990; Lindholm, 1991; Humbert et al., 1994). The inconsistencies found in the literature dealing with factors affecting CyA pharmacokinetics in humans, may be also a consequence of the tendency that many of these clinical studies are carried out on stable transplant patients treated with CyA for a long period of time. The treatment can induce changes, specially in lipoproteins profile or cholesterol levels, which constantly modify CyA distribution and clearance (Kuster et al., 1994). Therefore, we face many difficulties in relating factors such as patient's age or sex to CyA pharmacokinetics during clinical studies.

In conclusion, CyA has a low clearance and a high volume of distribution because of extensive distribution into many tissues. The values for several pharmacokinetics parameters presented here, correlate with previous findings in rats (Sangalli et al., 1988; Vadiei et al., 1989; Bernareggi and Rowland, 1991; Mraz et al., 1992; Shibata et al., 1993; Malmary et al., 1995). Considering drug distribution, males showed larger V_{ss} but lower drug clearances indicating slower intrinsic metabolic capacity. Therefore, our results support that the sex-associated changes and relative to the survival time of the grafts after experimental transplantation to rats (Hirasawa and Kamada, 1992) may be due to differences in CyA pharmacokinetics.

Our results in rats partially coincide with previous studies on humans (Lindholm, 1991; Harris et al., 1995; Gleiter and Gundert-Remy, 1996) who reported a faster elimination of drugs metabolized by CYP3A4 in females than in males. Therefore, it is tempting to speculate that age (minus pediatric patients) and gender might actually influence CyA human pharmacokinetics in a significant way. However, this speculation needs further experimental assessment.

Studies are in progress to identify the logic behind the age and gender effects on the CyA distribution and metabolism in rats. Hopefully, these results will establish whether the differences found in rats can be extrapolated to humans.

Acknowledgements

This work was financed by grants from the U.A., CICYT and CAM. The authors are grateful to the staff of the Hospital Militar Gomez Ulla for their technical assistance in measuring CyA concentrations. Language was revised by Jerry Keller (MD), Traducción Científica, ICE/AU.

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