Liposomal Formulations of Cyclosporin A: Influence of Lipid Type and Dose on Pharmacokinetics

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Purpose. Liposomal formulations of Cyclosporin A (CyA)[3] have been described in more than 30 publications to substitute Cremophor EL (CrEL), a triricinoleate ester of ethoxylated glycerol, as drug carrier. However, conflicting reports did not allow to draw consistent conclusions about the influence of liposomes on CyA pharmacokinetics (PK) and pharmacodynamics. ***Methods.*** A series of liposomal CyA-formulations with varying liposome composition and lipid dose but constant CyA dose was compared in rats. Data were analysed with a PK-model taking into account the varying volume of distribution with the varying lipid concentration in blood. ***Results.*** Surface properties and lipid type of liposomes are not important PK predictors of liposomal CyA, at least for small dosages of liposomes. Rather, the absolute lipid amount and the lipophilicity of cyclosporins are critical factors influencing the PK of liposomal CyA. The higher the concentration of lipid in blood and the greater the lipophilicity of cyclosporin is, the higher are the concentrations of CyA in blood. ***Conclusions.*** These relations may explain the inconsistent literature results. Together with earlier observations from our group the above findings indicate, that CyA is not caged in the liposomal membranes. Reports in literature, which claim lower clearance and a lower volume of distribution of CyA in obese rats compared to lean rats, support our assumption about the involved mechanisms. A semi-quantitative model of CyA distribution is presented, which points to the variable free fraction of CyA in plasma as the crucial factor for all previously reported phenomena in liposomal CyA formulations.

KEY WORDS: cyclosporins; liposomal membranes; lipid dose; rat; pharmacokinetics.

INTRODUCTION

Cyclosporin A (CyA) is the active component of Sandimmun®, an efficient and clinically well established immunosuppressive agent. It is the drug of choice in transplantation medicine and for treatment of auto-immune diseases. CyA selectively inhibits the interleukin-2 (IL-2) driven proliferation of activated T-lymphocytes. By impairing IL-2 production by T-helper cells, CyA suppresses proliferation and generation of T-cytotoxic lymphocytes, while sparing T-suppressor cell subpopulations [1].

Intravenous liposomal formulations have been used extensively to decrease toxicity—especially nephrotoxicity—of many drugs [2]. In this context, CyA is reported in the literature as an ideally suited drug for encapsulation in liposomes [e.g. 3, 4, 5, 6, 7]. Other publications [e.g. 8] claim an improvement in efficacy from an intravenously administered liposomal CyA formulation or an increased [5] or decreased [9] residence duration of CyA in the blood in comparison to conventional i.v. formulations of CyA.

Besides the proposed benefit of reducing nephrotoxicity—a current side effect of chronic CyA-therapy—which would also increase the therapeutic ratio, is the avoidance of Cremophor EL (CrEL) as it is suspected to cause nephrotoxicity [10]. Improved pharmacodynamics, a decrease in adverse effects of CyA-liposomes or the dependence of CyA pharmacokinetics (PK) on liposome charge, size and composition are subject of ongoing controversial discussions. Some publications claim higher, others lower CyA blood concentrations (c_{CyA}) of liposomal formulations compared to reference formulations. All these conflicting results on the *in vivo* behaviour of liposomal CyA will be shown to be explainable in our new unified model.

MATERIALS AND METHODS

Cyclosporines CyA, IMM125 and dihydroCyA (DHCyA) were obtained from Sandoz Pharma Ltd. Basel. Radioactive isotopes of these cyclosporines were synthesised and kindly provided by the radiochemistry group of Sandoz Pharma Development. Egg phosphatidylcholine (EPC grade) was purchased from Lipoid KG (Germany). 1,2-dipalmitoylphosphatidylcholine (DPPC) was obtained from Avanti Lipids, USA. Soybean phosphatidylcholine (sPC) was obtained from Sigma (Switzerland). All other chemicals used where at least of analytical grade. The lipid marker ^{3}H-cholesteryl-oleoyl-ether (COE) was purchased from Amersham (U.K.) 2-di-stearoyl phosphatidyl ethanolamine-polyethylen glycol (DSPE-PEG) was synthesized inhouse.

Liposome production was done by extrusion using a commercially available device (LiposoFast, Avestin Inc., Canada) to a final size of either 50 nm or 200 nm (measured by dynamic laser light scattering with a ZetaSizer III, Malvern, U.K.). Liposomes with PEG-surfaces contained 95 mol% DPPC and 5% DSPE-PEG 5000. Lipid content was measured by a phospholipase-D/cholinoxidase/PAP-test (WAKO Chemicals GmbH, Neuss, Germany).

Male Wistar rats, (BRL, Füllinsdorf, Switzerland) with a mean body weight of 300 g were used. The animals were housed in a constant humidity-temperature environment in wire-bottom metabolism cages with alternating 12 h light and dark cycles during the experiment.

Administration of Liposomes to Rats. All experiments were performed according to the guidelines of the Swiss Cantonal Agency of Animal Protection. One day prior to the experiments the animals were anaesthetised by a Forene® (Abbott, Cham, Switzerland) inhalation and a permanent catheter was placed into a *vena jugularis* to allow later blood sampling from the conscious animals. Food was withdrawn, but water was available *ad libitum* during the experiment. The next day the liposome formulations (volume: 300 µl to 500 µl) were administered by injection into a femoral vein during a short Forene® anaesthesia. For analysis 100 µl

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[3] General abbreviations: CyA: Cyclosporin A; CrEL: Cremophor EL; c_{CyA}: blood concentration of CyA, c_{Lipid}: blood concentration of lipid marker; AUC: area under the curve from time =0 to last data point, COE: cholesteryl oleoyl ether, b.w.: body weight.

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blood samples were taken and subjected to liquid scintillation counting. Two or 24 hours after administration the animals were killed and samples from liver, lung, spleen, kidney and brain were taken for determination of incorporated radioactivity. The tissue samples were solubilised with Biolute-S (Zinsser, Vienna, Austria) and subjected to liquid scintillation counting. Using only two rats for each form tested, it was tried, to keep the number of animals to an absolute minimum. The reproducibility for almost all duplicate experiments was surprisingly high. Therefore, this ethic approach did not invalidate the statistical analysis. In rare cases, where the estimated parameters do not seem to be from a common population, estimated values were tabulated individually.

Outline of the Two Series of Experiments. In the first series of experiments, the influence of charge, fluidity and surface modification (by DSPE-PEG) of the liposomes on the lifetime of CyA after i.v. administration was investigated. Total lipid dose in this part was 40 mg/kg for all liposome types, CyA dose was 1.0 mg/kg for all experiments

For the second series of experiments aiming at the assessment of the influence of lipid dose on the PK of CyA

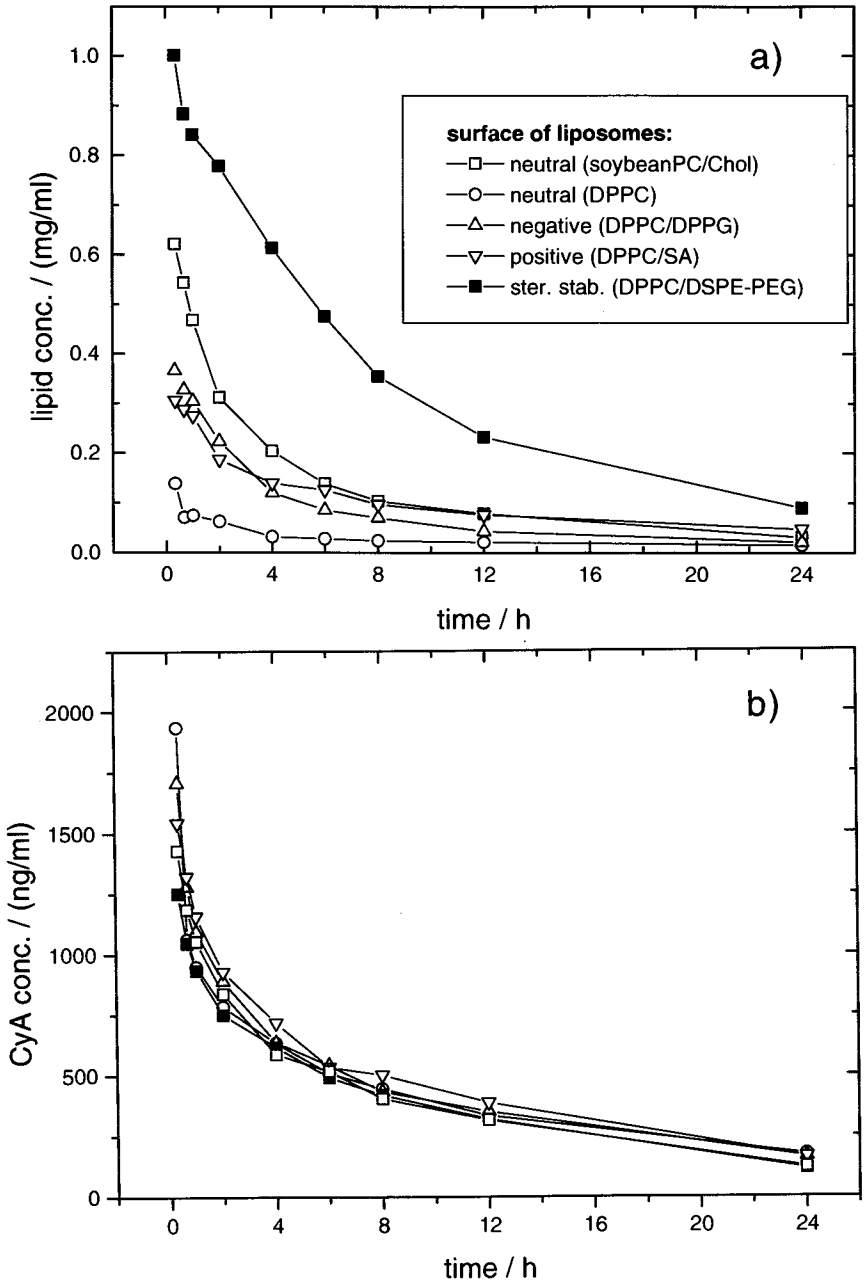


Fig. 1. Time course of lipid (a) and CyA (b) blood concentration for liposomes with different surface charges in rats. Lipid dose 40 mg/kg for all liposome types, CyA dose for all liposome types 1.0 mg/kg. Liposomes were sized to 200 nm by extrusion. Ster. stab. refers to sterically stabilised. SoybeanPC/Chol liposomes have a high fluidity at body temperature, the other liposomal lipids are below the phase transition.

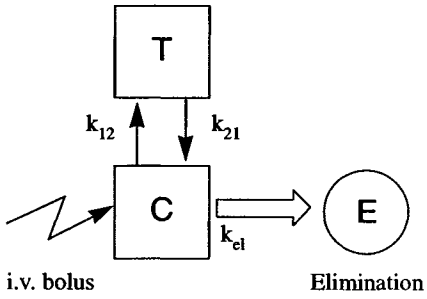
Table I. AUC_0^∞, Clearance and Volume of Distribution of the Different Liposome Types. Values Are Mean Estimates Using Data from Experiments Shown in Fig. 1

Lipid type	AUC/ (mg · h/ml)	Cl/b.w./ (l/h · kg)	V_D/b.w./ (ml/kg)
DPPC	1.22	32.8	780
DPPC/DPPG	2.09	19.1	263
DPPC/SA	3.43	11.7	272
DPPC-PEG	8.91	4.5	54
sPC/Chol	3.28	12.2	160

liposomes consisting of eggPC were used exclusively. Total lipid dose was varied, CyA dose for all experiments in this part was 5 mg/kg.

Pharmacokinetics and Statistical Programmes. PK calculations and simulations were done using the program SimuSolv on a VAX 7100 computer under VMS 5.5. c_{CyA} versus time curves corresponding to the four lipid doses and to the CrEL sample were fitted simultaneously with a two compartment open model in which the inverse volume of distribution (V) was expressed as a linear function of the lipid concentrations:

$$V^{-1} = c_{Lip} \cdot A + B$$



(1)

Since the drug was administered as an i.v. bolus injection, the initial amount of CyA in the central compartment is $m_c(0)$ = Dose = 1.5 mg. There were four different c_{CyA} versus time curves with their simultaneously measured $c_{Lip}(t)$ curves plus one curve where no concomitant lipid concentration measurement could be performed (CyA in CrEL).

From the identity $m_c(t) = m_c(0) + [m_c(t) - m_c(0)]$ it follows for the central compartment:

$$c^i_{CyA}(t) = (c^i_{Lip}(t) \cdot A + B) \cdot \left(m_c(0) + \int_0^t \frac{dm_c(\tau)}{d\tau} \cdot d\tau \right) \quad (2)$$

where the exponent i denotes the different lipid doses and the CrEL solution. This system was integrated simultaneously for all curves with one single set of fit parameters A, B, k_{12}, k_{21} and k_{el}. Equ. 2 can be solved as a bi-exponential function, whose amplitude is a function of lipid concentration in blood [e.g., $c_{CyA} = (c_{Lip}(t) \cdot A + B) \cdot (C_1 \cdot e^{-\alpha \cdot t} + C_1 \cdot e^{-\beta \cdot t})$].

RESULTS

Influence of Lipid Type on Pharmacokinetics. Fig. 1a shows the time course of the liposome lipid concentration in blood (c_{Lip}), Fig. 1b shows the corresponding time course of c_{CyA} for different surface charges and fluidity of the liposomal drug carrier. No statistically significant differences could be found between the fit parameters of the c_{CyA} time courses. Table I compiles estimates for clearance and volume of distribution (V_D) for liposome PK. The great value of V_D in the case of DPPC-liposomes may be due to the very low c_{Lip} after 6 hours (see Fig. 1a), where bound lipid label in blood may feign a very long elimination phase (see discussion).

Table II compiles PK parameters of all c_{Lip} and c_{CyA} profiles. A two-compartment model was sufficient to describe the data sets. As already obvious from Fig. 1b, no statistically significant difference for the PK parameter $t_{1/2\beta}$ of CyA for the different liposomal formulations could be found. However, $t_{1/2\beta}$ of the liposome profile varied significantly between the different formulations, as expected from Fig. 1a.

Most of the liposomes were taken up in the liver (data not shown). The most pronounced liver uptake was visible for DPPC-liposomes, whereas sterically stabilised liposomes exhibited much lower hepatic extraction from the circulation. This behaviour is also seen in the blood concentration profiles, indicating that DPPC-liposomes were cleared much faster from the blood than sterically stabilized liposomes (see Fig. 1a). The relatively large amount of lipid marker in the sterically stabilised liposomes in the kidney after 2 h (data not shown) reflects the amount of liposome, which is present in the blood of this highly vascularised organ. After the administration of liposomal CyA formulations distribution of CyA in different organs of the rat after 2 and 24 hours was not significantly different for the different liposomal formulations (data not shown). The only exceptions were higher liver values for the DPPC/SA and the DPPC formulations.

Influence of Lipid Dose on CyA Pharmacokinetics. Fig. 2a shows the time course of c_{Lip} for different lipid doses. Fig. 2b shows the corresponding time course of c_{CyA}. AUC's from the c_{CyA} *vs.* time profiles are depicted in Fig. 3 as a

Table II. Half-Lives of the Blood-Concentration Profiles for Liposomal CyA-Formulations in Rat. Values Were Obtained by Fitting a Macroscopic Two-Compartment-Model to the Data-Sets Used for Fig. 1. Values are Means of 2 Individual Experiments; Where the Individual Values Do Not Differ More than 30% from the Calculated Mean Value. [a]Denotes Values Which Were Probably Not from the Same Population

Liposome type	Liposome		CyA	
	$t_{1/2}\alpha$/h	$t_{1/2}\beta$/h	$t_{1/2}\alpha$/h	$t_{1/2}\beta$/h
DPPC	0.94	24.2	0.23	10.4
DPPC/DPPG	1.67	18.9 9.84[a]	0.61	10.5
DPPC/SA	1.61	16.9	0.89	10.1
DPPC-PEG	4.25	11.8	1.14	9.0
sPC/Chol	1.25	9.4	0.91	9.3

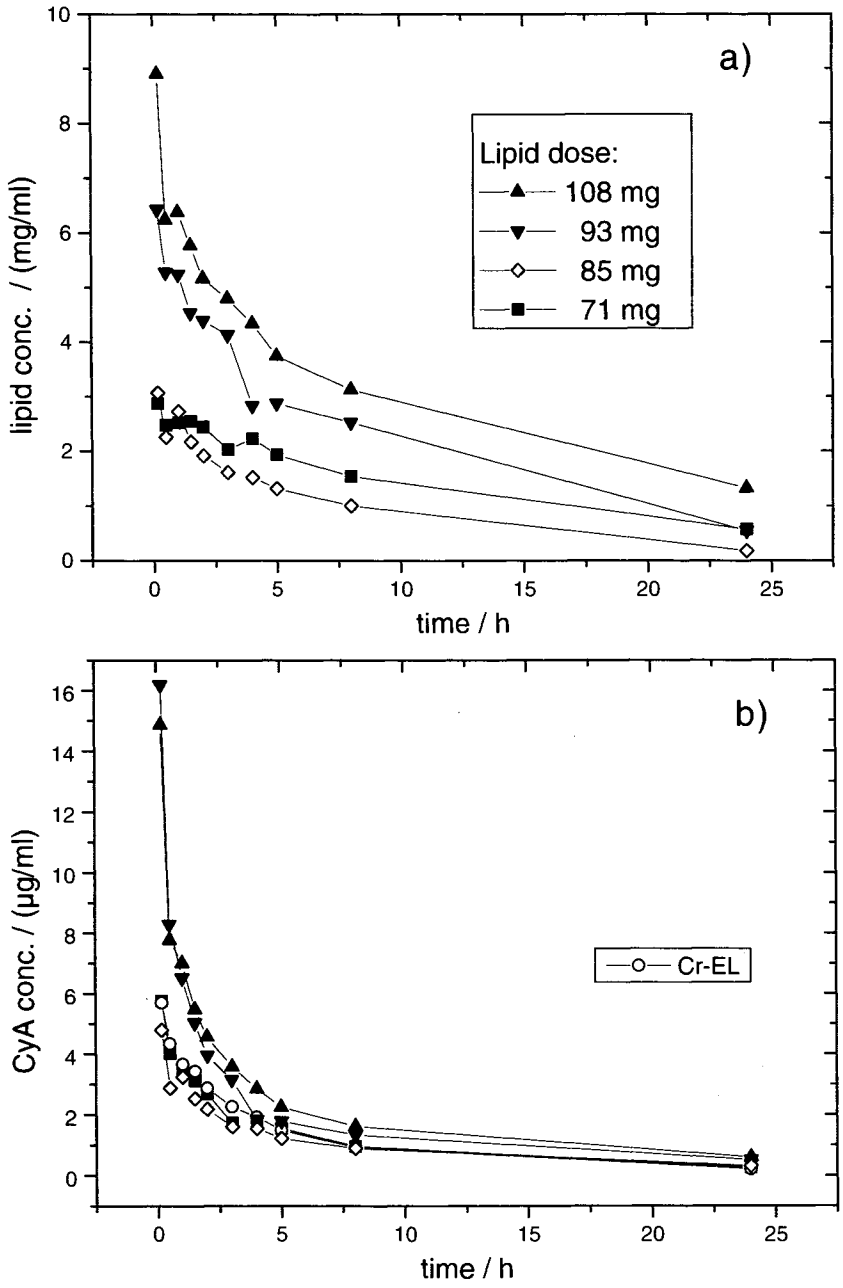


Fig. 2. Liposome (a) and CyA (b) blood concentration in rats after administration of liposomal CyA with different lipid doses at equal CyA doses of 5 mg/kg b.w.. The kinetics of CrEL could not be registered selectively. Liposomes were made of eggPC, diameter 50 nm. Values are from duplicate animal experiments.

function of lipid dose. Noticeable is the sharp rise in CyA-AUC at lipid doses greater than 85 mg per animal.

c_{Lip} and c_{CyA} were first subjected to model-independent PK analysis. Analysis of c_{CyA} (Tab. III) shows significant differences ($p < 0.01$, Mann-Whitney test) for the low lipid-dose group and the high lipid-dose group in all parameters investigated such as clearance, AUC and V_d. The analysis for the liposome data shows a trend to lower clearances at higher lipid doses. V_d seems to be smaller at high lipid doses (Tab. III).

No clear relationship between liposome dose and organ distribution of CyA was visible 2 h after administration (Fig. 4a). After CyA and liposome-exposure of the body for 24 h, a trend to higher CyA-concentrations in all organs except brain for high lipid doses was visible (see Fig. 4b).

Fig. 5a summarizes the observed c_{CyA} and c_{Lip} versus time curves. For easier visual comparison the curve after administration of CyA in CrEL is shown in all curves of this figure (thin lines). It is obvious that higher lipid doses resulted in elevated CyA levels as compared to CrEL. At a dose of 71 mg lipid, the CyA(t) curve is indistinguishable from the curve of c_{CyA} in CrEL, whereas at doses between 85 mg and 108 mg lipid, a clear increase in c_{CyA} can be observed. The $c_{Lip}(t)$ curves show systematic oscillations

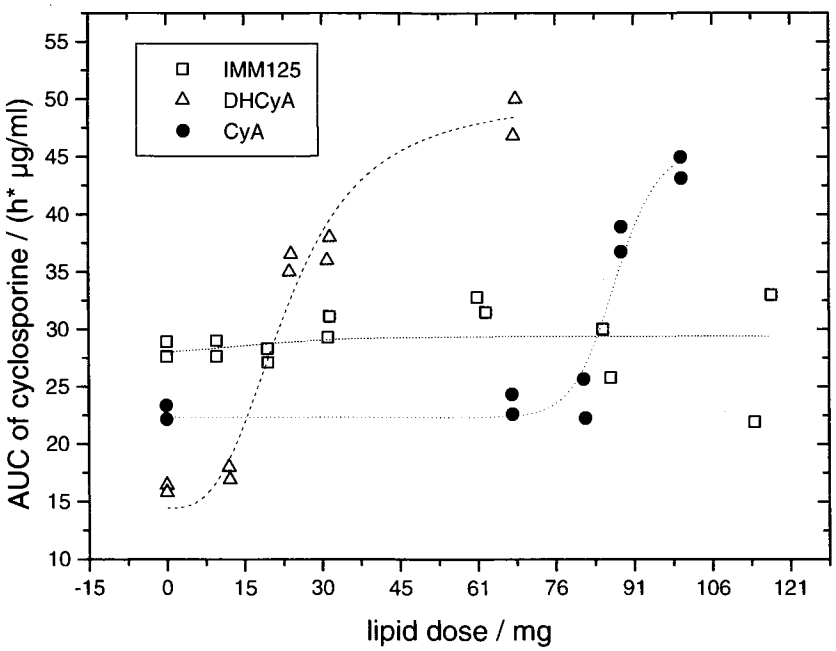


Fig. 3. Increase of AUC for CyA and for other cyclosporines at increasing liposome dosages in rats. Data from experiments described in Fig. 2b. Data values for IMM125 and DHCyA were obtained in the same way as for CyA, but are not shown here in detail. Dose of cyclosporins for all formulations was 5 mg/kg (mean rat weight 300 gm). Lines are fits of an equation from the type of Eq. 3, where f_u is a function of lipid dose.

with peaks around 1 h and 4 h. This might be an indication of a recirculation pathway in the lipid or lipid marker elimination. In support of Equation 1, these oscillations also occur to a minor extent in the c_{CyA} curves. Fig. 5b shows that the simultaneous fit of Equation 2 with all c_{CyA} and c_{Lip} data gives a good fit to all curves. The resulting optimal fit parameters were (point estimator ± SEM): k_{12} = (0.48±0.04) h^{-1}, k_{21} = (0.34±0.04) h^{-1}, k_{el} = (0.093±0.011) h^{-1}, A = (1.07±0.02) g^{-1} and B = (0.98±0.15) l^{-1}.

Blood concentrations of CrEL could not be determined experimentally. Setting the CrEL concentration to zero did not yield a good fit. However, a good fit was obtained when the CrEL concentrations were set to the values of the 71 mg lipid dose. This indicates a certain degree of binding of CyA to CrEL, which was already observed experimentally (M. Lemaire, *personal communication*). The same kind of results were obtained for similar liposomal formulations of IMM125 and DHCyA, but to avoid redundancy with CyA-data no details are presented here except the dependence of AUC on the lipid dose (Fig. 3, see discussion for explanation).

DISCUSSION

The investigation of different types of liposome forming lipids in the first part of our study showed that for the sterically stabilised liposomes a very low clearance (4.5 ml/h · kg, see Tab.I), as compared with unmodified DPPC-liposomes (32.8 ml/h · kg, see Tab. I), is in agreement with previous results [11]. The three other liposome types used did not differ very much from each other with respect to clearance and half-life (see Tab. I & II).

Liposomes consisting of sPC/Chol have the shortest half-life with a $t_{1/2\beta}$ of 9.4 h (Tab. II). However, even at very short periods after administration, the blood concentration of DPPC-liposomes is very low (see Fig. 1a) with a very short $t_{1/2\alpha}$ of 0.94 h. Because of the rapid removal of these liposomes from blood, the lipid label COE could reappear

Table III. AUC_0^∞, Clearance and Volume of Distribution of Liposomes and CyA. Values Are Estimates Using Data from Experiments Shown in Fig. 2b. Calculations Are Made by Using Formulas Described in Materials and Methods

	Liposome			CyA		
Lipid dose (eggPC)	AUC/ (mg · h/l)	Cl/b.w./ (l/h · kg)	V_D/b.w./ (l/kg)	AUC/ (mg · h/l)	Cl/b.w./ (l/h · kg)	V_D/b.w./ (l/kg)
108 mg	95.0	3.79	69.3	58.0	0.086	1.50
93 mg	60.6	5.12	63.7	57.0	0.088	1.59
85 mg	24.1	11.74	117.7	27.9	0.179	1.98
71 mg	31.6	7.49	115.9	28.4	0.176	2.13
CrEL	—	—	—	28.2	0.180	2.50

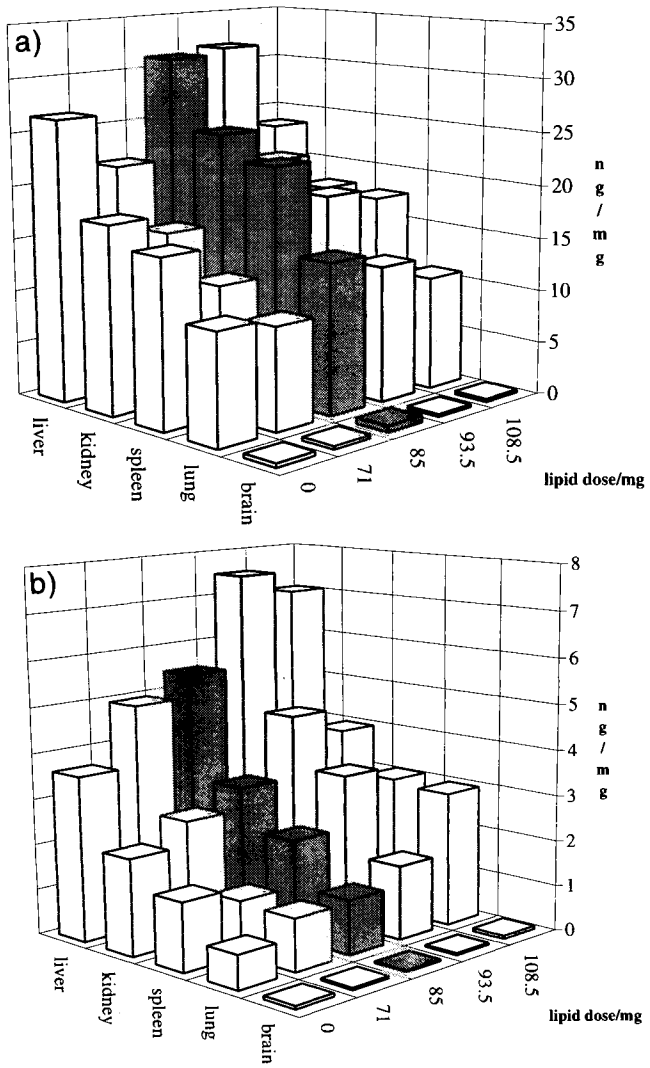


Fig. 4 Organ distribution of CyA after 2 h (a) and 24 h (b) of an i.v. liposomal CyA administration in rats. Plotted values are means from two experiments. Liposomes were made of eggPC, diameter 50 nm. Lipid dose as indicated (lipid dose '0' denotes the Cremophor EL formulation), CyA dose for all liposome types was 5 mg/kg.

from the liver in the blood at later times (bound to lipoproteins). This effect could feign a long elimination phase as calculated in Tab. II for DPPC-liposomes ($\tau_{1/2\beta} = 24.2$ h). Another explanation may be that liposomes disintegrate partly in blood and COE binds to lipoprotein as its natural binding site. This ever-present lipoprotein-bound label plays a measurable role, when liposomes are almost completely cleared in the time period investigated.

Influence of Lipid Dose. In the second part of this study, the influence of lipid dose on CyA and liposome PK was studied. As can be seen from Tab. III, liposomal AUC's rises overproportionally, as the liposomal dose is increased. This is to be expected, because of the saturability of the reticuloendothelial system (RES) [12].

It can be seen from Fig. 2b that for high lipid dosing a fast decay phase appears for the $c_{Lip}(t)$ profiles. This type of behaviour was recognised by Perrier et al. [13] as typical for product-inhibition kinetics, which is consistent with the saturation phenomenon discussed above. Another compatible explanation is a composite clearance by the rat liver with a high-affinity/low-capacity and low-affinity/high-capacity pathway.

The influence of CyA on the PK of liposome disposition and elimination (data not shown) was not significant, as suggested previously, showing that CyA does not affect RES activity [7, 14].

Non-Compartmental Pharmacokinetics of CyA. Our results on CyA clearance with CrEL (0.16 l/h · kg vs. 0.18 l/h · kg) and V_d (2.7 l/kg vs 2.5 l/kg) are comparable with the results from the group of Rowland [15]. Brunner et al. [16] evaluated PK profiles of CyA in hyperlipidaemic versus lean rats. Clearance and V_d decreased by a factor of two in hyperlipidaemic rats without significant change in elimination. This change in PK parameters is in orientation and amount the same as presented here (Tab. III).

Compartmental Pharmacokinetic Analysis. Detailed PK analysis using a compartment-model of CyA infusion data in rats is rare in the literature. The available data, however, are consistent with the PK analysis performed here. The group of Rowland [15] applied a 3-compartment model to blood concentration data after i.v. infusion of CyA (6 mg/kg b.w.). If our data from Fig. 1b are evaluated with a 3-compartment model, the results are quite comparable: they obtained a $t_{1/2\alpha}$ of 0,15 h (our value 0.09 h), a $t_{1/2\beta}$ of 4 h (2 h) and an $t_{1/2\gamma}$ of 16 h ($\cong$ 10 h). Despite the different blood sampling scheme and the different CyA dosage (6 mg/kg *vs.* 5 mg/kg in our experiments), the values are almost equivalent. The trend towards longer half-lifes in the cited studies can be explained by the longer sampling time (83 h compared to 24 h in our studies). In the light of this fact the AUC_0^∞ of Rowland's studies (31.6 μg · h/ml *vs.* 28.2 μg · h/ml; Tab. III) support our results.

AUC of c_{CyA}: Dependence on Liposome Type. Neither the charge, the surface nor the fluidity of the liposomal membrane seems to influence the c_{CyA} time course (see Fig. 1). These findings, in combination with *in-vitro* transfer experiments [17], show that CyA can rapidly migrate to blood constituents. A hypothesis of liposome caged CyA is therefore obsolete.

AUC of c_{CyA}: Dependence on Liposome Dose. In contrast to the explainable effect of lipid dose on liposome PK, the dependence of CyA-AUC on lipid dose was surprising (see Fig. 3). The values found for AUC are comparable to those in the studies of Vadiei et al. [6]. However, in these studies, despite the large amount of lipid used (about 200 mg/kg), no significant differences between AUC for CrEL and the liposomal formulation were found. The cause for the sudden increase in CyA—AUC in the present study can be explained as follows:

- CyA introduced into the systemic circulation without additional lipid (e.g., with CrEL) binds rapidly to lipoproteins, erythrocytes (cyclophilin) and to some extent to albumin.
- Unbound CyA can migrate to fat, cyclophilin, cell membranes and other specific and unspecific binding sites.
- If high lipid doses are coadministered with CyA, binding of CyA in blood will be enhanced and the fraction unbound in plasma (f_u) thereby lowered. Less CyA

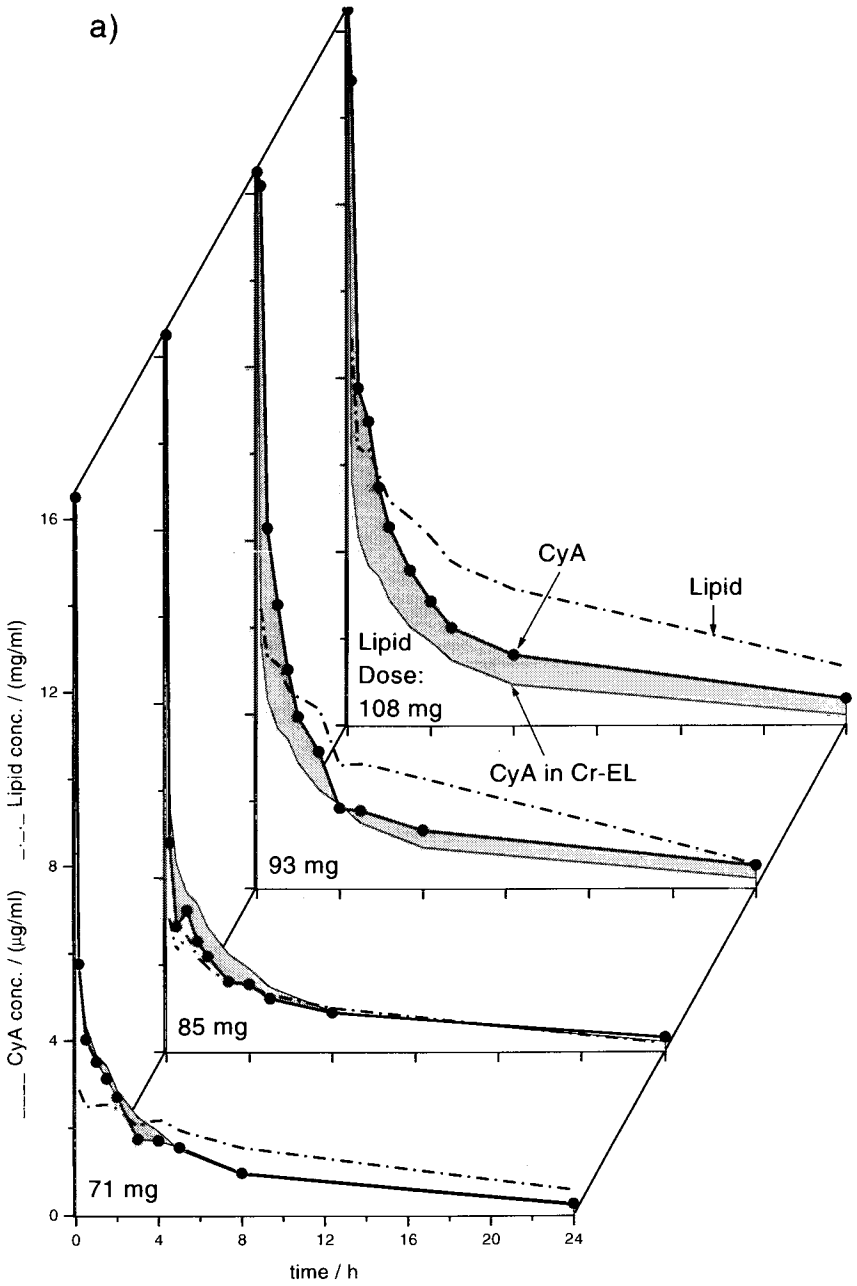


Fig. 5. (a) Waterfall plot for all data sets of CyA and lipid conc. vs. time for the different lipid doses in the CyA formulation (data from Fig. 2, including the theoretical value for c_{CyA} at t=0). The CyA dose was 5 mg/kg b.w. in all experiments. In each plot the curve of CyA with CrEL is added for comparison (thin line). (b) Measured and fitted CyA concentrations vs. time (fit includes data from lipid concentration measurements, see equation 2). The circles mark the measured data while the solid lines correspond to the optimal solutions of equation 2. Fitparameters: $k_{12} = (0.48 \pm 0.04)\ h^{-1}$, $k_{21} = (0.34 \pm 0.04)\ h^{-1}$, $k_{el} = (0.093 \pm 0.011)\ h^{-1}$, A = $(1.07 \pm 0.02) g^{-1}$, B = $(0.98 \pm 0.15) l^{-1}$.

will diffuse to binding sites outside the blood. If the lipid amount exceeds the natural binding sites excessively, the AUC of CyA in blood will increase sharply.

- Caused by the lower f_u, less CyA can be cleared via metabolism and excretion by liver, which also increases AUC.

The increase in AUC and the concomitant decrease in clearance and V_d can also be explained in pure PK terms: CyA is a drug with a low to intermediate extraction ratio [18]. The clearance of CyA will therefore depend on the unbound fraction of CyA. In rabbits, this unbound fraction is reduced from 0.17 to 0.05 by concomitant administration of Intralipid™ (*commercial preparation cont. 20% i.v. fat emulsion for parenteral nutrition*) [5]. This reduction in free fraction may reduce the clearance and explain the higher AUC at the high liposome dose used in our experiments.

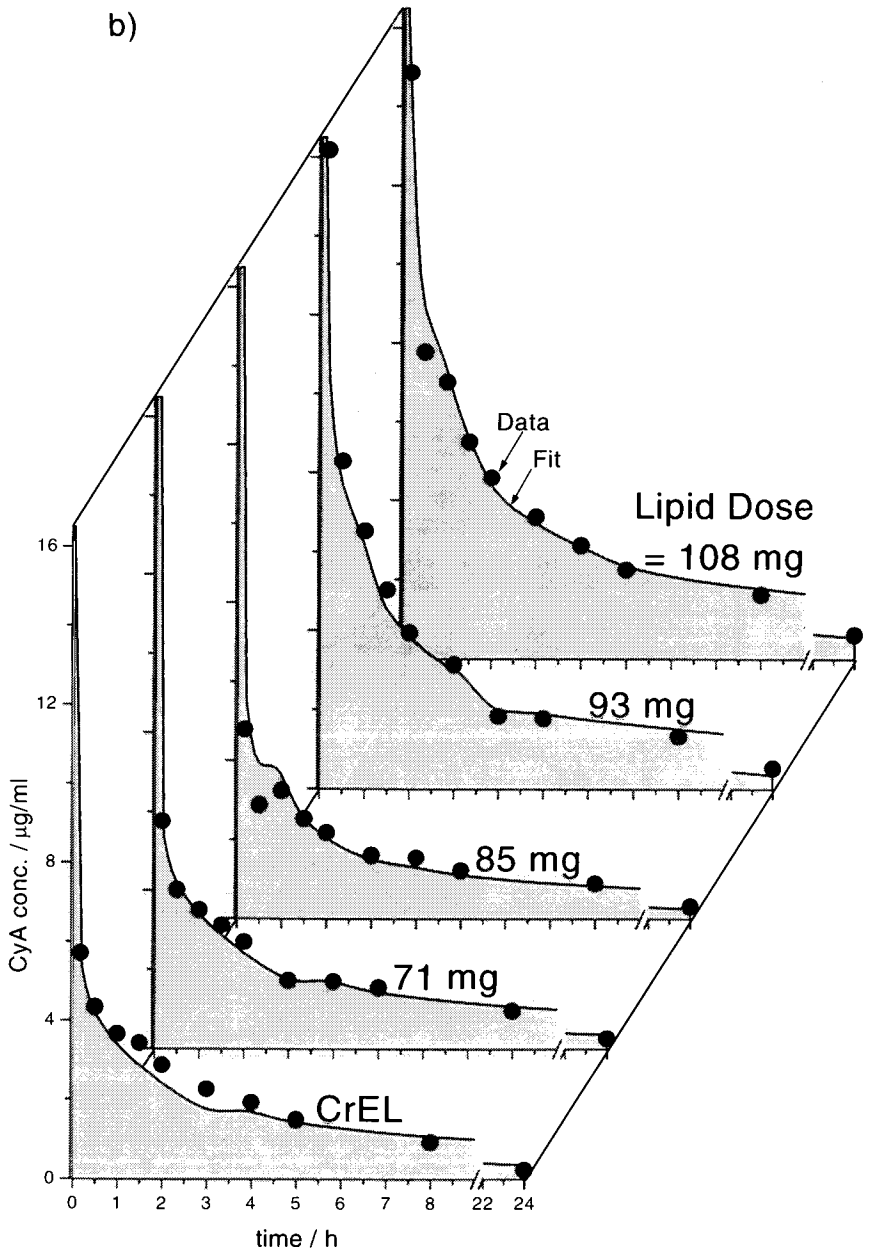


Fig. 5. Continued.

Both the decrease in V_d and clearance and increase in AUC for CyA at increasing lipid doses (Tab. III) can be displayed as a sigmoidal type of curve (Fig. 3). According to Gibaldi and McNamara [19], the apparent V_D depends on the free fraction of drug in blood and in tissue by the following formula: $V = V_P + V_T (f_u)/(f_T)$, where V_P denotes plasma volume, V_T volume of tissue and f_T free fraction of drug in tissue. It is reasonable to assume, that f_u approaches a limiting value for high lipid doses and does not change noticably at low lipid doses because of enough other CyA binding material in blood. f_T of CyA appears to change as well as high lipid dosages (Fig. 4) in different organs.

AUC, k_{el} and volume of distribution at steady state are related by

$$AUC = \frac{D}{k_{el} \cdot \left(V_P + V_T \frac{f_u\ (lipid)}{f_T\ (lipid)} \right)} \qquad (3)$$

This relationship can reasonably explain our AUC curves for CyA (Fig. 3). The functional relationship between f_u and lipid dose was not investigated here, but is expected to be complex. For example, action (and binding) of CyA on liposomal membranes show a cooperative behaviour with a Hill coefficient of 3 [20]. A crucial and simple test of the hypothesis however involves the dependence of AUC on the lipophilicity of the cyclosporine used. If the above described assumptions are valid, increased lipophilicity of the cyclosporine will cause the curve to shift to the left. This is clearly

seen in Fig. 3 where the more lipophilic DHCyA (partition coefficient P.C. = 11 000 [20]) shows an earlier onset of increasing AUC's as the lipid dose increases compared to CyA (P.C. = 4025). For IMM125 (P.C. = 550) an AUC increase was not observed up to 400 mg/kg, indicating a strong shift in the point of inflection to a very high lipid dose.

Findings in the literature support our hypothesis, that the presence of lipidic matter increases CyA blood concentration:

- An uptake study of cyclosporin A in human blood showed, that transport of cyclosporin A into erythrocytes was retarded at higher lipid levels [21].
- CyA concentrations in blood were greater in obese rats than in lean rats [22].
- PK analysis of single i.v. doses of CyA in hyperlipidaemic and normal rats revealed a decrease in V_d and clearance in the obese rat by a factor of two [16].
- CyA uptake into isolated rat hepatocytes is dependent on the amount of lipid in the medium [23], 1 μM LDL caused the highest reduction (49%) of CyA uptake.
- AUC increases by a factor 1.28 in dogs [4] and by a factor of 3 in rabbits [5], when CyA was given in Intralipid™ compared to other formulations.

Clinical Pharmacokinetics of CyA and Lipid Interaction. CyA is extensively bound to plasma lipoproteins [1]. It was shown [24], that PK changes in transplant patients are caused by a change in binding of CyA to lipoproteins. All lipoproteins exhibit a non-saturable, low affinity, high capacity uptake for CyA, whereas binding of CyA to erythrocytes is saturated above 500 ng/ml. Patients with high lipoprotein content show higher AUC and lower clearances than normal [24].

Publications suggest that low serum cholesterol levels predispose patients to CyA side effects [25]. On the other hand, there is evidence that CyA by itself increases lipid levels in patients [26]. A recent case-report [27] finds that administration of a drug in Intralipid™ together with CyA increases c_{CyA} from 250 ng/ml to 997 ng/ml.

Reduction of renal side effects may be explained by retention of CyA in the blood. This may hinder CyA from diffusing excessively to kidney tubules, thereby reducing the effect of CyA on the kidney especially at high c_{CyA} and c_{Lipid} immediately after i.v. administration.

CONCLUSIONS

Most of the partly contradictory results from previous studies concerning CyA and lipid can be conclusively explained by our experimentally based model, that CyA is attracted by a high lipid amount in blood, but is not caged in liposomal membranes. This effect is greater at higher lipophilicity of the drug. There is literature support that high lipid doses bind CyA in blood, thereby minimising the amount of drug available in sensitive organs like the kidney. Effects on PK and reduced side effects of liposomal CyA are based on bare physical properties.

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