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Original article

Ursodeoxycholic acid modulates cyclosporin A oral absorption in liver transplant recipients☆

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Abstract – The aim was to study the ursodeoxycholic acid (UDC) effect on the cyclosporin A (CsA) pharmacokinetics after oral administration of the microemulsion formulation Neoral® (CsA-ME) in liver transplant recipients, and test the potential protective effect of this bile acid on liver and renal CsA-ME-induced toxicity. At entry into the study, 12 patients who underwent orthotopic liver transplantation received CsA-ME, for at least 6 months. They then received a cotreatment CsA-ME plus UDC (13.8 $mg \cdot kg^{-1} \cdot day^{-1}$) for three months. Blood concentrations of CsA were measured using a monoclonal antibody specific for the parent compound. The kinetic data were analysed by a mathematical model incorporating a time dependent rate coefficient for CsA intestinal absorption, before and after UDC treatment. Changes in serum markers of hepatic and renal injury were assessed. Individual serum bile acids were determined by chromatography. Serum levels of UDC increased from 3 to about 45 % of total serum bile acids after UDC treatment. The estimated model parameters indicate that UDC administration modulates CsA intestinal absorption. In the nine non-cholestatic patients, UDC reduced the absorption rate and the bioavailability of CsA without modifying the elimination rate constant of CsA and the CsA pre-drug levels. In contrast, in the three cholestatic patients, the bioavailability tended to be higher and the absorption rate faster when CsA was combined with UDC. UDC significantly decreased elevated γ-glutamyl transferase and creatinine serum levels and induced some clinical improvements such as disappearance of headaches in four patients. In conclusion, a 3-month UDC treatment modifies CsA intestinal absorption without affecting CsA elimination rate constant. On the other hand, UDC supplementation appears to improve CsA tolerability. © 2000 Éditions scientifiques et médicales Elsevier SAS

cyclosporin pharmacokinetics / liver transplantation / ursodeoxycholic acid / mathematical modelling

1. Introduction

Cyclosporin A (CsA) is an essential immunosuppressive drug used in the long-term management of organ transplant recipients. In an attempt to optimize the oral absorption of the traditional oral formulation Sandimmun®, a new formulation Neoral® (CsA-ME) was developed that incorporates the drug in a microemulsion. CsA-ME reduced the dependence of CsA absorption on the physiological state of gastrointestinal tract and decreased inter- and intrapatient variability in CsA pharmacokinetics [1, 2]. CsA-ME provides a better systemic drug exposure and improves the stability of immunosuppression. However, CsA-ME appears to have broadly similar profiles for the type, incidence and severity of

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adverse events [3] as compared with the standard formulation.

Ursodeoxycholic acid (UDC) is a hydrophilic bile acid [4] that has been shown to protect the liver parenchyma in certain forms of chronic cholestatic liver disease [5, 6]. Kallinowski et al. [7] showed that UDC was an effective treatment for heart-transplanted patients who developed cholestatic liver disease while under immunosuppression with a regimen containing CsA. On the other hand, it has been demonstrated [8] that UDC can favourably improve bioavailability of CsA specially in patients with impaired enterohepatic circulation. Our group [9] in rats and Al-Quaiz et al. [10] in humans demonstrated another benefit from UDC which is due to an enhanced biliary excretion of CsA metabolites accumulated during cholestasis. Lastly, it has been shown that the addition of UDC to a CsA-based immunosuppressive regimen significantly reduces multiple episodes of acute cellular rejection [11].

Based on these findings, we conducted a study to investigate whether UDC modulates CsA pharmacokinetics from the CsA-ME formulation and reduces the CsA toxicity in 12 clinically stable liver allograft recipients.

2. Materials and methods

2.1. Patient selection

Twelve adult patients who underwent orthotopic liver transplant at Archet II Hospital (Nice, France), were enrolled in this trial. *Table I* describes the clinical status of patients. The mean delay after transplantation was 6.2 years (range 3–10 years). Patients were required to have stable graft function and to have received the CsA-ME (Novartis Pharma S.A., Paris) for at least 6 months prior to entry into the study. Alcoholic cirrhosis was the most frequent indication (5 of 12) for transplantation. The protocol was approved by local medical ethics committees and the study was performed in accordance with the Declarations of Helsinki and Tokyo. Each patient signed a written informed consent declaration after having been informed of the nature of the investigation.

2.2. Immunosuppressive therapy

From the start of immunotherapy, patients received CsA as Sandimmun® p.o. (Novartis Pharma S.A., Paris). At least 6 months prior to the commencement of the study, CsA-ME was administered as soft gelatin capsule, and doses determined by clinical requirements ranged from 2.1–4.5 $mg \cdot kg^{-1} \cdot day^{-1}$. Daily doses were divided into two equal doses administered every 12 h. Immunosuppressive therapy consisted of CsA-ME exclusively, except for one patient who received CsA-ME associated with azathioprine.

2.3. Study design

The study was conducted in two stages. In the first leg of the study (D0), patients had received CsA-ME exclusively; then, the same patients received a cotreatment CsA-ME plus UDC for 3 months (D90). UDC was given orally twice a day in the form of 250-mg capsules (Délursan Hoescht-Houdé, Paris) at a mean dose of 13.8 $mg \cdot kg^{-1} \cdot day^{-1}$ (range 11.1–16.7 $mg \cdot kg^{-1} \cdot day^{-1}$). UDC and CsA-ME were administered simultaneously.

Table I. Patient characteristics.[a]

	Sex	Age (years)	Diagnosis	Time (years)	CsA-ME (mg/kg/day)	UDC (mg/kg/day)
AMO	m	54	PVC	10	2.9	14.7
ARM	m	49	BC	5	2.8	13.9
CAR	f	24	M	9	3.7	13.9
GLI	m	53	AC	4	4.3	12.3
LAR	m	51	AC	3	2.2	11.1
MAH	f	54	AC	5	3.1	15.6
MAR	f	58	AC	5	4.5	13.4
MIG	m	59	AC	8	2.1	13.9
ZED	m	40	WD	4	4.2	12.5
HAN	m	27	PVC	9	2.7	13.5
KER	m	24	FTH	7	3.4	16.7
MUR	m	69	SBC	6	2.2	14.5

[a] m: male; f: female; PVC: post viral cirrhosis; BC: Budd-Chiari; M: myofibromatosis; AC: alcoholic cirrhosis; WD: Wilson's disease; FTH: fulminant toxic hepatitis; SBC: secondary biliary cirrhosis; Time: time after transplantation.

At D0, venous blood samples were collected for the determination of CsA. The sampling protocol was designed to supply the highest pharmacokinetic information [12]. It consists of a first pre-drug sample, before the oral administration of drug, and then, in samples at 0.25, 0.5, 0.75, 1, 2, 2.5, 3, 6, 7, 11, and 12 h later. Samples were collected in EDTA-containing tubes and frozen at 4 °C prior to analysis of CsA. At D0, additive blood samples were taken for creatinine, and liver parameter determinations. Blood samples were centrifuged and the resulting serum were frozen at −20 °C for further biochemical analyses. At D90, the same sampling protocol as D0 was followed.

At D0 and D90, CsA-ME or CsA-ME and UDC were administered immediately before consumption of a standardized continental breakfast. At 4 h after CsA dosing, subjects were given standardized meals that were identical on all profiling days.

2.4. Biological parameters

Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase activities, and creatinine concentrations were determined on serum samples by conventional methods. Blood samples for bile acid analysis were taken at 8 a.m. after an overnight fast and 12 hours after the last ingestion of UDC. Serum bile salt concentrations were measured using 3α-hydroxysteroid dehydrogenase (Enzabile, Nyegaard, Oslo, Norway).

Individual serum bile acids were determined by gas liquid chromatography and thin layer chromatography as previously described [13]. A proportion of UDC larger than 30 % of total serum bile acids indicates a good compliance with UDC treatment.

Cholestasic liver dysfunction was judged by a simultaneous elevation of γ-glutamyl transferase (> 50 IU/L), alkaline phosphatase (> 117 IU/L) activities and serum bile salt concentrations > 10 μM [14]. Serum creatinine levels > 124 μM were accepted as signs of nephrotoxicity.

2.5. Measurement of CsA levels

CsA blood levels were measured in duplicate using a monoclonal antibody specific for the parent compound (Enzyme immuno Assay, Emit 2000®, Syva Biomérieux, France). The limit of quantification of the method was 20 $\mu g \cdot L^{-1}$; the intra- and inter-assay coefficients of variation were 7 and 10 %, respectively.

2.6. Pharmacokinetic study and statistical analysis

Kinetic data were analysed by mathematical modelling. Modelling is recommended instead of the standard non-compartmental analysis when data are gathered from complex experiments like repeated dosages. According to the experimental protocol, it is rational to assume a time delay, t_0 (units, h), between the pre-drug sampling time and the beginning of absorption process. Individual time–concentration profiles of CsA were fitted to a one-compartment model described by the following differential equations:

$$\frac{dq}{dt} = -k_a \cdot t^{\alpha} \cdot q \qquad q(t_0) = D$$

$$\frac{dy}{dt} = k_a \cdot t^{\alpha} \cdot \frac{q \cdot f}{V} - k_e \cdot y \qquad y(0) = y_0$$

These equations describe the kinetics of CsA at the absorption site, q (amount), and in the blood, y (concentration); the associated initial conditions are D at time t_0 and y_0 at time 0, respectively. D is the administered amount, V the volume of distribution (units, L), f the bioavailability, and y_0 the pre-drug level. In the above equations, only the ratio V/f is structurally identifiable. It expresses the apparent volume of distribution $\bar{V}$. For the absorption process, we propose a time dependent rate coefficient, $k_a \cdot t^{\alpha}$, instead of the commonly used first- and zero-order rate constants [15]. Coefficient k_a (units, $h^{-(\alpha+1)}$) and exponent α (dimensionless) define the irregular absorption process of CsA. The first order elimination process is characterized by the elimination constant k_e (units, h^{-1}).

The six model parameters $\bar{V}$, k_e, k_a, α, y_0, t_0 will be compiled in the vector form $\underline{x}$. Estimates of $\underline{x}$, $\hat{\underline{x}}$, were obtained by fitting the model to the observed data according to the maximum likelihood principle [16]. For each patient and from the data at D0 and D90, we obtain two sets of parameter estimates $\hat{\underline{x}}_1$ and $\hat{\underline{x}}_2$, respectively.

It is important to note that $\bar{V}$ over-estimates f-times the corresponding parameter V. Hence, although V does not depend on the absorption process, the estimated $\bar{V}$ is inversely proportional to f. Moreover, if we assume no influence of UDC administration on V, the discrepancies reported between $\bar{V}_1$ and $\bar{V}_2$ are related inversely to the discrepancies between f_1 and f_2.

To statistically characterize the data, non-parametric measures were supplied: median values, interquartile ranges and their ratios as a non-parametric

Table II. Serum laboratory tests of patients before (D0) and after 90 days (D90) of UDC treatment.[a]

		Cr (μM)		ASAT (IU/L)		ALAT (IU/L)		GGT (IU/L)		AP (IU/L)		BS (μM)	
		D0	D90	D0	D90	D0	D90	D0	D90	D0	D90	D0	D90
	AMO	164	144	54	26	31	17	20	15	109	107	9	18
	ARM	134	128	36	23	51	19	237	38	129	104	3	8
	CAR	105	106	28	38	26	44	26	20	95	105	4	7
	GLI	120	119	21	15	27	6	305	15	108	73	3	6
G1[b]	LAR	110	108	37	68	42	72	36	23	127	115	4	8
	MAH	142	142	25	20	12	8	35	21	257	140	10	11
	MAR	177	155	21	14	12	8	87	20	99	75	6	13
	MIG	331	299	21	26	13	15	26	27	84	98	5	12
	ZED	166	147	37	22	34	15	38	32	86	74	10	8
	P	0.0209		0.4065		0.3135		0.0078		0.0661		0.0117	
	HAN	104	108	52	37	48	32	111	50	120	111	46	17
G2[c]	KER	124	120	40	27	39	23	82	27	152	137	41	41
	MUR	192	229	26	21	24	15	62	40	148	138	18	18

[a] Normal values: creatinine (Cr) < 124 μM, aspartate aminotransferase (ASAT) < 37 IU/L, alanine aminotransferase (ALAT) < 41 IU/L, γ-glutamyl transferase (GGT) < 50 IU/L for men and < 32 IU/L for women, alkaline phosphatase (AP) < 117 IU/L, bile salts (BS) < 10 μM.
[b] G1: group of non-cholestatic patients.
[c] G2: group of cholestatic patients.

coefficient of variation (in %). The influence of UDC on the CsA pharmacokinetics was assessed by paired statistical comparisons (Wilcoxon test) between $\hat{x}_1$ and $\hat{x}_2$. Biological parameters were compared before and after UDC treatment by means of the same test. Statistical significance was set to $P < 0.05$.

All pharmacokinetic calculations were done using the Matlab programming environment [17]. Statistical calculations were done using the statistical package BMDP [18].

3. Results

3.1. Clinical observations

UDC was well tolerated by the 12 patients studied. Some clinical improvements were in fact observed. Four patients who had had headaches (MIG, MAH, MAR, LAR) since liver transplantation were asymptomatic after 3 months of UDC therapy. One of them (LAR) who was hypertensive had normalization of his blood pressure.

3.2. Biological parameters

Serum liver and renal parameters at D0 and at D90 are shown in *table II*. In all patients, baseline values on D0 were identical to those found in the months prior to D0. Patients are divided into two groups depending on the absence or the presence of a cholestasis at entry: a first group of nine non-cholestatic patients (G1), and a second group of three cholestatic patients (G2) as illustrated by concomitant elevations of bile salt, γ-glutamyl transferase and alkaline phosphatase serum concentrations. In the first group after UDC treatment, γ-glutamyl transferase serum levels were significantly decreased ($P = 0.0078$) and elevated γ-glutamyl transferase levels were normalized in all patients. Alkaline phosphatase levels were normal in all patients except one (MAH), and total serum bile salt concentrations slightly increased. Elevated creatinine levels were significantly decreased ($P = 0.043$) in five out of six patients by about 20 μM, and transaminases were normal in all patients except one (LAR). In the second group, UDC treatment normalized γ-glutamyl transferase and decreased alkaline phosphatase levels in all patients. Total serum bile salt concentration decreased in one patient (HAN) and was unchanged in the two others. Creatinine remained elevated in the patient with renal failure (MUR) and normal in the others. Aminotransferases were in the normal range.

3.3. Compliance with treatment

Compliance was assessed by measuring the amount of serum UDC relative to the total level of bile acids in serum after an overnight fast. Serum bile acids before and after 3-month UDC treatment are shown in *table III*. In the two groups of patients, at inclusion, cholic and chenodeoxycholic acids were the predominant bile acids, whereas UDC repre-

Table III. Serum bile salts before (D0) and after 90 days (D90) of UDC treatment.[a]

		C		DC		CDC		UDC	
		D0	D90	D0	D90	D0	D90	D0	D90
	AMO	25.1	16.2	19.8	15.4	51.2	15.7	3.9	53.0
	ARM	44.5	16.2	23.3	18.6	29.7	18.2	2.5	47.0
	CAR	27.2	25.3	47.3	13.6	23.0	19.7	2.5	41.0
	GLI	36.8	29.2	22.1	21.8	38.1	14.7	3.0	34.0
G1[b]	LAR	31.0	21.8	27.0	20.6	39.0	14.3	3.0	43.0
	MAH	29.7	21.7	38.6	16.2	28.4	16.6	3.3	45.0
	MAR	34.2	14.1	29.0	13.3	33.5	18.1	3.3	54.0
	MIG	20.8	24.3	39.2	17.2	35.8	17.1	4.2	42.0
	ZED	23.7	19.0	20.0	15.1	54.0	29.5	2.3	36.0
	min	20.8	14.1	19.8	13.3	23.0	14.3	2.3	34.0
	max	44.5	29.2	47.3	21.8	54.0	29.5	4.2	54.0
	med	29.7	21.7	27.0	16.2	35.8	17.1	3.0	43.0
	iqr	11.10	8.60	17.85	5.25	16.05	3.75	1.10	11.50
	CV	37.37	39.63	66.11	32.41	44.83	21.93	36.67	26.74
	P	0.0117		0.0039		0.0039		0.0039	
	HAN	34.7	18.1	18.7	10.8	45.8	24.4	0.8	47.0
G2[c]	KER	35.6	25.5	12.4	5.7	50.7	23.0	1.3	46.0
	MUR	38.9	18.4	15.2	10.9	43.4	15.1	2.5	56.0

[a] Data are expressed as molar percentage of total serum bile salts. C: cholate; DC: deoxycholate; CDC: chenodeoxycholate; UDC: ursodeoxycholate.
[b] G1: group of non-cholestatic patients.
[c] G2: group of cholestatic patients.

sented only about 3 % of total bile acids. After UDC treatment, the median percentage of UDC was 43 and 47 % in non-cholestatic and cholestatic patients, respectively. UDC became the major serum bile acid species in each patient of the two groups. A significant decrease ($P = 0.02$ Wilcoxon test) in cholic, chenodeoxycholic and deoxycholic acid percentages occurred in non-cholestatic patients. Note that in the three cholestatic patients, the serum concentrations of endogenous bile salts were strongly decreased (cf. *tables II* and *III*).

3.4. Pharmacokinetics

For the nine non-cholestatic patients, *figure 1* shows the inter-quartile range of data at D0 and D90. We note that UDC association smoothes the CsA peaks without affecting pre-drug levels.

Up to ten candidate models were investigated before selecting the presented one expressing a non-homogeneous absorption process. The best model discrimination was done by using the Akaike's information criterion [19]. The fitting of the retained model was satisfactory: the goodness of fit, expressed by the root mean squared error, has a median value of 16.7 and 12 % at D0 and D90, respectively. As an example, *figure 2* shows for the patient ARM the fitted data by the model.

Table IV presents the estimated model parameters for all patients in both treatments at D0 and D90. At D0, k_a is often estimated at the fixed upper boundary ($5\ h^{-(\alpha+1)}$). On the contrary at D90, k_a is estimated at lower levels (median $0.762\ h^{-(\alpha+1)}$). We note also that k_e was one of the less dispersed parameters (coefficient of variation equals to 22.1 and 23.52 % at D0 and D90 respectively).

For all non-cholestatic patients, we have $\bar{V}_1 < \bar{V}_2$ (i.e., $f_1 > f_2$), and $k_{a1} > k_{a2}$ indicating that the bioavailability is higher and the absorption rate

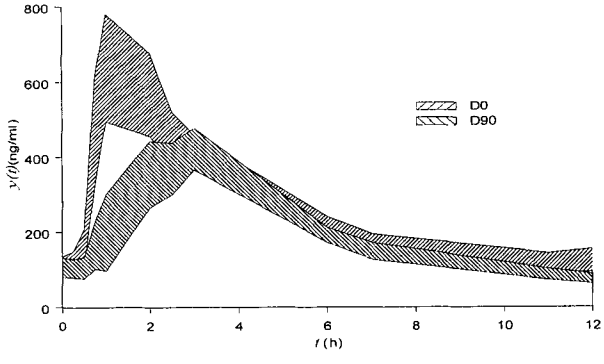

Figure 1. For the nine non-cholestatic patients, the inter-quartile range of CsA blood concentrations at D0 and D90.

Table IV. Estimated model parameters before (D0) and after 90 days (D90) of UDC treatment.[a]

		RMSE (%)		$\bar{V}$ (L)		k_e (h^{-1})		k_a ($h^{-(\alpha+1)}$)		α		y_0 ($\mu g\ mL^{-1}$)		t_0 (h)	
		D0	D90	D0	D90	D0	D90	D0	D90	D0	D90	D0	D90	D0	D90
	AMO	18.3	12.9	162.2	278.1	0.191	0.155	4.993	2.879	1.713	2.000	0.096	0.094	0.242	0.249
	ARM	7.9	9.6	171.5	210.6	0.152	0.162	5.000	0.214	1.834	2.500	0.171	0.134	0.250	0.100
	CAR	15.8	10.8	182.2	255.9	0.156	0.177	0.873	0.762	1.145	1.056	0.125	0.123	0.250	0.181
	GLI	21.0	16.5	154.8	233.9	0.224	0.222	5.000	0.160	1.842	2.500	0.138	0.118	0.237	0.141
G1[b]	LAR	16.7	7.9	184.5	205.6	0.170	0.205	5.000	0.500	2.446	2.500	0.113	0.074	0.181	0.225
	MAH	17.9	12.0	135.1	170.6	0.188	0.197	5.000	3.123	1.924	2.169	0.150	0.117	0.001	0.250
	MAR	21.7	17.5	132.8	150.0	0.182	0.187	5.000	3.280	0.460	2.500	0.226	0.152	0.231	0.141
	MIG	16.3	12.1	207.5	251.2	0.189	0.181	4.983	2.433	1.459	1.213	0.076	0.080	0.223	0.192
	ZED	11.3	10.6	225.1	299.4	0.218	0.227	2.726	0.630	2.079	0.000	0.045	0.050	0.001	0.239
	min	7.9	7.9	132.8	150.0	0.152	0.155	0.873	0.160	0.460	0.000	0.045	0.050	0.001	0.100
	max	21.7	17.5	225.1	299.4	0.224	0.227	5.000	3.280	2.446	2.500	0.226	0.152	0.250	0.250
	med	16.7	12.0	171.5	233.9	0.188	0.187	5.000	0.762	1.834	2.169	0.125	0.117	0.231	0.192
	iqr	6.1	4.6	51.050	78.900	0.042	0.044	1.146	2.644	0.700	1.366	0.075	0.052	0.155	0.103
	CV	36.58	38.26	29.77	33.73	22.10	23.52	22.91	346.80	38.15	62.96	59.76	43.93	67.00	53.63
	P			0.0039		0.2600		0.0039		0.3743		0.0663		0.8590	
	HAN	13.9	18.4	199.0	201.0	0.185	0.174	2.101	4.998	2.500	1.630	0.111	0.128	0.250	0.247
G2[c]	KER	5.1	9.3	250.0	219.9	0.137	0.152	0.307	1.937	1.917	2.500	0.144	0.088	0.114	0.201
	MUR	11.9	16.3	145.4	118.1	0.262	0.234	2.774	5.000	2.500	1.841	0.051	0.053	0.250	0.121

[a] For abbreviations, see text.
[b] G1: group of non-cholestatic patients.
[c] G2: group of cholestatic patients.

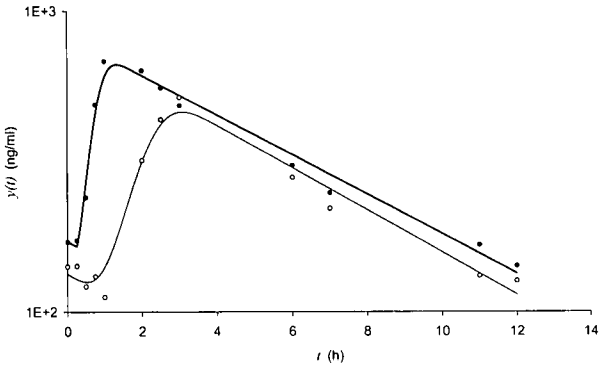

Figure 2. CsA blood concentrations vs time of one patient (ARM) before (D0, ●) and after 3 months of co-treatment with UDC (D90, ○). The thick line (D0) and the thin line (D90) represent the predicted concentrations calculated by the pharmacokinetic model.

faster at D0 than at D90. Wilcoxon test significantly confirms the above relations ($P = 0.004$ only for $\bar{V}$ and k_a).

In the three cholestatic patients, we note inverse relations with respect to the previous ones: the bioavailability is higher ($\bar{V}_1 > \bar{V}_2$, i.e., $f_1 < f_2$) and the absorption rate faster ($k_{a1} < k_{a2}$) when the CsA is associated with UDC. Unfortunately, the low number of cholestatic patients does not allow a statistical confirmation of this observation.

4. Discussion

It is known that CsA intestinal absorption is poor, partially due to low CsA aqueous solubility [20]. CsA is a highly lipophilic drug whose solubility in the gut lumen depends on mixed micelles of bile salts, monoolein, and fatty acids [21]. CsA-ME micro-emulsion has been developed to overcome problems associated with the poor and unpredictable absorption of the standard oral formulation of the drug [1, 2]. However, CsA absorption from CsA-ME is not completely independent of bile secretion as demonstrated in liver transplant recipients undergoing biliary diversion. In these patients, a statistically significant correlation was observed between the volume of externally drained bile and the reciprocal of CsA bioavailability [22]. These concepts are the basis for the use of a bile salt to improve CsA absorption in the gut.

In this investigation, we have supplemented patients with UDC, a bile salt used in chronic cholestatic liver disease [5, 6]. Due to the complex CsA absorption, a new mathematical model was used to describe the absorption kinetics. The model involves a power-law term, t^{α}, which controls the non-linear increase ($\alpha > 0$) or decrease ($\alpha < 0$) of absorption rate [15, 23]. As a matter of fact, first-order kinetics is a limiting case ($\alpha = 0$) of this more general model. Indeed, the classical first-order kinetics were justified in only one set of data (patient ZED, treatment D90) of the 24 analysed (*table IV*). Our study shows that chronic administration of UDC modulates some CsA pharmacokinetic parameters, in liver transplant patients with stable liver function tests. The major activity of UDC is to modify CsA intestinal absorption, whereas it does not affect the CsA elimination rate constant and the CsA pre-drug levels. Note that a simple reduction of the CsA dose, without UDC supplementation, should result in a decrease of all blood CsA concentrations. In this case, pre-drug levels may be too low to ensure effective immunosuppression.

We show that UDC has a variable effect on parameters associated with the absorption process depending on the presence or the absence of cholestasis.

In patients with normal biliary secretion, we show that UDC decreases significantly both the CsA bioavailability (median values are equal to 171.5 and 233.9 L for D0 and D90, respectively), and the rate of intestinal absorption (median k_a values are equal to 5 and 0.762 $h^{-(\alpha+1)}$ for D0 and D90, respectively). These effects might be due to increased intestinal permeability to CsA or more probably to physico-chemical modifications of the intestinal aqueous phase when bile is enriched with UDC. It is known that in healthy humans, active ileal transport of bile salts is saturated or nearly saturated. During chronic oral ingestion of an unconjugated bile salt, there is little increase in ileal transport, based on measures of hepatic secretion rate [24]. After a first enterohepatic cycle, conjugates formed in the liver from the newly administered bile salt compete successfully for ileal transport with conjugated endogenous bile salts. Accordingly, the present data show that UDC chronic treatment converts the serum bile salt to a median percentage of 43 % UDC, whereas this bile salt amounted to only 3 % of total bile salts before treatment. UDC became the major bile acid and this increase occurred mainly at the expense of chenodeoxycholic acid which decreased from 36 to 17 %. Similarly, deoxycholic acid decreased from 27 to 16 %. As a result, a new hydrophilic–hydrophobic balance in favour of hydrophilic bile salts occurs. On the other hand, knowing that UDC has a lower

capacity to dissolve lipids than hydrophobic bile salts [4, 25], it is highly probable that UDC solubilizes less CsA in the mixed micellar phase than common bile salts do. As a consequence, CsA absorption is lower during UDC treatment than before.

On the contrary, in the three cholestatic patients, UDC treatment tends to enhance CsA bioavailability and intestinal absorption rate ($f_1 < f_2$ and $k_{a1} < k_{a2}$). In this situation of biliary secretion deficiency, the input of exogenous UDC increases the concentration of biodetergents in the intestinal lumen and thus improves the drug solubility. Consequently, CsA absorption rate tended to be faster and bioavailability higher. These data are in accordance with results of Gutzler et al. [8] who showed that prolonged UDC administration increases CsA absorption in patients with reduced intestinal absorptive surface and with results of Sharobeem et al. [26]. Maboundou et al. [27] reported an increase in the area under the concentration versus time curve of CsA in only one of the three patients with cholestasis, but the weak effect noted in this study was obtained during administration of a single dose of UDC.

Relationships between pharmacokinetic profiles and incidence of side effects have not been established with CsA-ME. However, high peak concentrations may be suspected to lead to adverse events since C_{MAX} values have been correlated with hypertension when CsA was delivered via the standard formulation [28]. UDC treatment which decreases CsA absorption and smoothes CsA blood peaks (see *figure 1*) in non-cholestatic patients may contribute to improve CsA tolerability. In the present study, we note that UDC decreases elevated serum creatinine levels by about 20 μM in five of six patients and normalizes serum γ-glutamyl transferase in all cases. In addition, clinical benefits, e.g., disappearance of headaches and decrease of hypertension, were observed in some patients after UDC therapy. Such beneficial effect persisted in patients who continued the UDC treatment beyond D90 (unpublished results).

In the three cholestatic patients liver tests were ameliorated by UDC treatment with a decrease of γ-glutamyl transferase, alkaline phosphatase and endogenous bile salts. These results are in full agreement with our previously published data [9] and the data of Kallinowski et al. [7] who showed an improvement of cyclosporine-induced cholestasis in four heart-transplanted patients treated with UDC. Therefore, UDC has positive effects in both cholestatic and non-cholestatic liver transplant patients. These results, however, need to be confirmed by a large placebo-controlled trial.

In conclusion, from the selected pharmacokinetic model, UDC treatment appears to modulate the CsA intestinal absorption. In non-cholestatic patients, UDC decreased the absorption rate and bioavailability of CsA without modifying the CsA elimination rate constant and the CsA pre-drug levels. On the contrary, the CsA absorption rate was increased in the three cholestatic patients. On the other hand, UDC supplementation led to CsA improved tolerability.

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