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Contribution of cyclosporin metabolites to immunosuppression in liver-transplanted patients with severe graft dysfunction

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Abstract. The aim of this study was to analyse the immunosuppressive contribution of cyclosporin metabolites in liver-grafted patients. Therefore the immunosuppressive potency of 17 metabolites, alone and in combination, was tested in human mixed lymphocyte cultures, and the results were correlated with metabolite blood levels in liver-grafted patients. Of the 17 metabolites tested only six highly lipophilic metabolites showed a detectable immunosuppressive activity of up to 10% of the activity of cyclosporin; the effect of combining metabolites was additive. For calculation of the in vivo activity, blood levels of seven major cyclosporin metabolites were determined in liver-grafted patients with normal liver function (group A, 43 episodes) and with severe hyperbilirubinaemia (group B, 66 episodes). Both patient groups had comparable levels of parent drug (122.9 ± 17.4 vs. 111.1 ± 23.5 ng/ml by HPLC) and similar blood levels of the highly lipophilic metabolites 17, 1 and 18. By contrast, blood levels of the less lipophilic metabolites 8, 9, 26 and 203–218 were substantially increased in group B ($P < 0.05$). High overall metabolite blood levels in group B were also indicated by a non-specific monoclonal RIA (520 ± 199 ng/ml for group A vs. 1318 ± 407 ng/ml for group B). Despite the very high levels in group B, however, the overall contribution of the metabolites to immunosuppression was similar in both groups (12.6 ± 5.0% for group A vs. 13.8 ± 5.6% for group B). These findings indicate that, despite a marked accumulation of cyclosporin metabolites in patients with severe cholestatic liver dysfunction, their immunosuppressive contribution remains low. This suggests that for assessment of the immunosuppressive potency of cyclosporin therapy monitoring of parent drug levels is necessary and sufficient. Since a variety of non-immunological effects of high metabolite levels cannot be excluded, however, additional non-specific measurements may, nevertheless, be useful in patients with severely disturbed liver graft function.

Key words: Cyclosporin, role of metabolites, liver transplantation – Metabolites of cyclosporin, in liver transplantation

Cyclosporin is one of the most effective drugs for immunosuppression after organ transplantation. It is metabolized in the liver with metabolite excretion into the bile [8, 12, 16]. In liver dysfunction the process of metabolization and metabolite excretion can be severely disturbed with subsequent accumulation of parent drug and metabolites in the body. The effects of liver dysfunction on cyclosporin metabolite levels in liver- and kidney-grafted patients have been described [2, 4, 14, 15]. Since some of the metabolites have been shown to possess reduced but definite immunosuppressive activity in vitro [20–22], the question arises as to what extent increased metabolite levels contribute to immunosuppression during episodes of liver dysfunction.

Usually routine monitoring of cyclosporin therapy is done by trough level determination in haemolysed blood using the specific radioimmunoassay (RIA) selectively detecting unmodified parent drug. In this study we determined the blood levels of cyclosporin and individual metabolites by high performance liquid chromatography (HPLC) [5] and by monoclonal specific and non-specific RIA [18] in patients after liver transplantation. The levels of parent drug and individual metabolites were compared during periods without graft dysfunction and during periods of severely disturbed graft function. The contribution of the metabolites to the overall immunosuppressive effect of the cyclosporin therapy in both groups of patients was calculated on the basis of in vitro studies analysing the inhibitory effect of isolated metabolites in human mixed leucocyte cultures.

Materials and methods

Patients

For this study cyclosporin and metabolite blood levels were analysed in adult patients who had undergone liver transplantation at the Medizinische Hochschule, Hannover, between January 1988 and

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July 1989. The immunosuppressive protocol usually consisted of triple or quadruple drug therapy during the first postoperative week, including rabbit ATG (Fresenius AG, Oberursel, FRG) 5 mg/kg per day from day 0 to 6, azathioprine 1 mg/kg per day and tapering doses of prednisolone starting with 1 mg/kg per day. Cyclosporin (Sandimmun, Sandoz Ltd., Basle, Switzerland) was introduced between day 1 and 7 postoperatively at a dose between 0.5 and 2 mg/kg per day i.v. depending on the individual kidney and liver function, and was administered in two daily doses given as a 4–6 h infusion. After the first week most patients were on triple drug therapy, unless azathioprine had to be withdrawn because of an increased risk of infection. With triple therapy, cyclosporin blood trough levels of 100–200 ng/ml measured by monoclonal specific RIA (Sandoz Ltd., Basle, Switzerland) were aimed for. Cyclosporin administration was switched from i.v. to oral administration after removal of the T-tube, usually 3–4 weeks postoperatively.

According to the graft function two sets of blood level data were analysed. Group A consisted of 66 metabolite measurements in 47 patients with total bilirubin blood levels below 50 µmol/l, either stable or decreasing, indicating normal or only slightly disturbed liver function. Group B consisted of 43 measurements in 23 patients with total bilirubin levels above 150 µmol/l, either stable or increasing, indicating severely disturbed cholestatic liver dysfunction. The reasons for cholestatic liver dysfunction included acute rejection, chronic rejection, infection, biliary obstruction, impaired hepatic blood flow, drug toxicity and unknown causes.

For reasons of comparability only patients with cyclosporin HPLC blood levels between 80 and 180 ng/ml were included in the analysis. In order to obtain steady state conditions, measurements were not included if cyclosporin dose changes had been performed up to 2 days prior to the date of analysis. Two or three measurements per patient were included in the study if changes in liver function (e.g. further deterioration in group B) or in cyclosporin dosage (in both groups) had occurred and if the measurements were at least 1 week apart. During the analyses in group A ($n = 66$) cyclosporin was administered orally in 56 instances (8.0 ± 2.8 mg/kg per day) and intravenously in ten instances (2.5 ± 0.6 mg/kg per day). In group B ($n = 43$) the drug was administered orally in only two instances (7 and 8 mg/kg per day, respectively) and intravenously in 41 instances (1.9 ± 1.0 mg/kg per day). The measurements in groups A and B were performed at a median of 30 days (range 7–241 days) and 24 days (range 7–167 days) after transplantation, respectively.

Isolation and measurement of cyclosporin and its metabolites

For in vitro analysis cyclosporin metabolites were isolated from T-tube bile of cyclosporin-treated liver-grafted patients by HPLC as described elsewhere [5, 6]. In brief, cyclosporin and its metabolites were extracted from bile (500 ml) into dichloromethane (500 ml). The dichloromethane layer was evaporated, the residue dissolved into 150 ml 50/50 v/v acetonitrile/water (pH 3.0), washed with 150 ml hexane and again extracted by dichloromethane. The dichloromethane phase was evaporated, the residue dissolved into 2 ml 50/50 v/v acetonitrile/water, and washed with 2 ml hexane. A 250 µl portion of the resulting solution was injected into the HPLC system. Cyclosporin and its metabolites were eluted using a concave acetonitrile/water (pH 3.0) gradient at 75°C column temperature. Three consecutively linked 250.10 mm preparative columns filled with RP-8 7-µm material (Macherey-Nagel, Düren, FRG) were used. The isolated metabolites were identified by HPLC, FAB-MS and ^{1}H-NMR.

The isolated fractions were designated 'H' followed by the retention time of the isolated fraction under the chromatographic conditions used multiplied by 10 [6]. Eleven of the fractions were able to be characterized as cyclosporin metabolites 1 (H380), 8 (H250), 9 (H255), 13 (H320), 16 (H340), 17 (H370), 18 (H400), 21 (H430), 25 (H300), 26 (H270), and 203–218 (H350). Six unidentified fractions were isolated (H230, H235, H310, H315, H355, H410/420), found to be pure in HPLC and showed crossreactivity with cyclosporin in the original Sandoz polyclonal cyclosporin RIA [7].

After solubilization and dilution of the metabolite preparations for cell culture analysis (see below), the concentration and purity of the metabolites in the culture medium were again analysed by HPLC and dose-response curves adjusted to the actual metabolite concentration. The loss of the metabolites during preparation for the cell cultures was between 10 and 85%.

Routine analysis of cyclosporin blood 12-h trough levels was performed by RIA using monoclonal specific and non-specific antibodies from Sandoz. The specific monoclonal antibody detects only unmodified parent drug, whereas the non-specific monoclonal antibody shows broad crossreactivity with many cyclosporin metabolites [18]. Analyses were done daily for the first 2 weeks and at least three times per week thereafter during hospitalization of the patients. Cyclosporin dosages were usually adjusted to achieve blood levels between 100 and 200 ng/ml in the specific assay during triple drug therapy with dosage reduction when blood levels in the non-specific assay exceeded 1200 ng/ml. HPLC analysis was done three times per week also measuring 12-h blood trough levels by a technique described elsewhere [6].

Cell culture assays

Peripheral blood mononuclear cells (PBMC) (10^5) were cultured in U-shaped microtitre plates in a final volume of 200 µl. The culture medium consisted of RPMI-1640 substituted with 4 mM glutamine, 15 mM Hepes buffer, 100 IE/ml penicillin-streptomycin and 10%

Table 1. HPLC and RIA blood levels of cyclosporin and several metabolites in liver-grafted patients with normal liver function and with severe cholestasis. NS, not significant. Statistical analysis by Mann-Whitney U-test

	Cyclosporin and metabolite blood levels (ng/ml) (mean ± SD)		Significance of difference
	Group A ($n = 66$) Good liver function	Group B ($n = 43$) Severe cholestasis	
HPLC			
Ci	122.9 ± 17.4	111.1 ± 23.5	NS
M17	144.9 ± 56.5	140.8 ± 58.4	NS
M1	65.8 ± 48.1	53.5 ± 38.9	NS
M18	18.9 ± 31.3	30.2 ± 43.7	NS
M8	51.7 ± 42.2	370.5 ± 165.5	$P < 0.05$
M9	5.4 ± 16.0	88.6 ± 91.2	$P < 0.05$
M26	3.7 ± 14.4	70.9 ± 60.7	$P < 0.05$
M203–218	5.9 ± 14.2	109.1 ± 90.3	$P < 0.05$
RIA			
specific	152.4 ± 47.5	120.5 ± 35.8	NS
non-specific	520.1 ± 199.6	1318.1 ± 407.2	$P < 0.05$

Table 2. Effect of increasing hyperbilirubinaemia and liver dysfunction on blood levels of cyclosporin and its metabolites

Patient	Day[a]	Bilirubin (μmol/l)	Cy-dosage (i.v.) (mg/kg/day)	RIA[b]		HPLC[b]							
				specific (ng/ml)	non-specific	Cy	M17	M1	M18 (ng/ml)	M8	M9	M26	M203–218
B.P.	14	80	1.5	97	817	114	81	–	–	197	41	36	–
	18	133	1.5	122	1100	138	83	22	–	236	37	40	–
	20	186	1.5	·80	1619	84	85	26	–	416	55	78	50
E.K.	5	124	3	202	802	198	185	30	–	142	12	45	56
	6	135	3	202	1128	239	218	50	82	263	141	–	71
	8	174	3	210	1371	183	195	–	–	360	165	–	91
	9	195	3	184	1559	203	216	52	–	478	157	108	164

[a] Days after transplantation
[b] 12 h blood trough levels of cyclosporin and metabolites by RIA and HPLC

fetal calf serum. Cyclosporin and metabolite preparations were dissolved in absolute ethanol, diluted stepwise with culture medium and finally added to the culture. The final concentration of ethanol in all cultures was 0.2%, which did not inhibit cell proliferation by itself compared to control cultures without ethanol. Cyclosporin, kindly provided by Sandoz Ltd., and a cyclosporin preparation from bile, which was prepared in parallel with the metabolites by HPLC, gave identical dose-response curves (data not shown).

For mixed leucocyte cultures (MLC), 10^5 irradiated (40 Gy) allogeneic stimulator cells were added to the cultures after addition of cyclosporin or metabolites. Cell cultures were set up in triplicate, incubated for 120 h at 37°C in a humidified atmosphere, then 1 μCi ^{3}H-thymidine was added to each well for another 16 h. Finally cells were harvested on glass fibre filters and ^{3}H-thymidine incorporation was measured using a scintillation counter (Betaplate, LKB, Freiburg, FRG). Results are given as mean counts per minute; standard deviation was always less then 7%. For analysis of the activity of individual metabolites three different MLC assays with different stimulator-responder cell combinations were used.

Calculations

The 50% inhibitory concentrations (IC_{50}) were determined by interpolation from the concentration-response curves of the metabolites in the cell culture assays depicted on a semilogarithmic scale. The relative immunosuppressive activity (%) of a metabolite was calculated by the formula:

$$\text{Relative immunosuppressive activity (\%)} = 100 \times \frac{IC_{50}(\text{metabolite})}{IC_{50}(\text{cyclosporin})}$$

The immunosuppressive contribution of the metabolites in vivo was then calculated from their relative activity in vitro and the blood trough levels of cyclosporin and metabolites measured by HPLC, using the formula:

$$\text{Relative additional immunosuppressive effect (\%)} = 100 \times \frac{0.08 \times M17 + 0.05 \times M1 + 0.03 \times M18}{Cy}$$

where M17, M1, M18, and Cy indicate the blood trough levels of the metabolites and cyclosporin, respectively; the multiplication factors of the individual metabolites indicate their relative immunosuppressive activity as determined in the cell culture assays (see Table 3). The result indicates the percentage by which the immunosuppressive effect of cyclosporin is increased by the metabolites present. The immunosuppressive contribution of the metabolites was calculated individually for each measurement. The calculation was restricted to these three metabolites because they were the only ones which were inhibitory in the lymphocyte culture and were also detectable in patient blood. Although levels of metabolite 21 could not be determined in our study for technical reasons, the levels reported in the literature are very low. Taking this together with its very weak inhibitory activity indicates that its contribution to immunosuppression in vivo is extremely low.

Statistical analyses were done by the Mann-Whitney *U*-test, because the data were not normally distributed.

Results

Cyclosporin metabolite blood levels in liver-grafted patients

For this study the results of cyclosporin and metabolite measurements in two different clinical situations were analysed. Group A consisted of 66 determinations performed during periods of normal or only slightly disturbed liver-graft function and group B of 43 determinations during episodes of severely deranged liver function with hyperbilirubinaemia. In both groups only patients with specific cyclosporin blood levels between 80 and 180 ng/ml were included. Thus, in both groups cyclosporin blood levels determined by HPLC and specific RIA were similar. Most interestingly, HPLC blood levels of the highly lipophilic first generation metabolites 17, 1 and 18 were not significantly different between groups, showing only a slight trend towards increased concentrations of metabolite 18 in individual patients. By contrast, blood levels of the less lipophilic second generation metabolites 8, 9, 26 and 203–218 were considerably elevated in patients with hyperbilirubinaemia; these metabolites were usually undetectable during normal liver function (Table 1).

Elevated overall metabolite levels in patients with cholestatic liver-graft dysfunction were also reflected by the high blood levels detected by the monoclonal non-specific RIA; in these patients the mean ratio between levels in non-specific and specific RIA was 10.9 as compared with 3.4 in patients with good liver function (Table 1). Thus, a marked accumulation of metabolites occurred with severe liver dysfunction. Remarkably, the accumulation of metabolites seemed to affect almost exclusively the less lipophilic second generation compounds. In individual patients the evolution of this metabolite pattern could be observed within a few days under constant cyclosporin dosage. An increase in bilirubin was closely paralleled by an increase in blood levels of second generation metabolites, while blood levels of parent drug and first generation

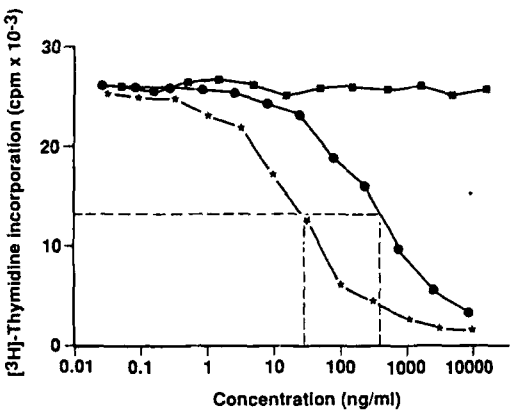

Fig. 1. Immunosuppressive activity of cyclosporin and metabolites 17 and 8 in MLC. Mixed lymphocyte cultures were performed with 10^5 responder PBMC and 10^5 irradiated allogeneic PBMC in a 5-day assay in the presence of different concentrations of cyclosporin (*—*), metabolite 17 (●—●), or metabolite 8 (■—■). Proliferation was assessed by ^{3}H-thymidine incorporation over 8 h. The figure shows the dose-effect curves; the *dashed lines* indicate 50% inhibitory concentrations

metabolites remained constant (Table 2). This suggests that cholestatic liver dysfunction predominantly affects metabolite excretion rather than metabolization itself.

Immunosuppressive effect of cyclosporin metabolites in vitro

Of all metabolites tested in concentrations up to 10 µg/ml, only the highly lipophilic metabolites 17, 1, 18, 21 and two other metabolites characterized by their HPLC retention times of 41/42 min (H410/420) and 35.5 min (H355) had some activity. Figure 1 shows the inhibitory effect of cyclosporin, metabolite 17 and metabolite 26 on a MLC. IC_{50} for MLC of the most important metabolites tested is demonstrated in Table 3; mean values of three different cultures are shown. Compared to the inhibitory potency of the parent drug, the effect of the metabolites was always less than 10%. Remarkably, all active metabolites had a long retention time on the HPLC column indicating high lipophilicity, whereas all tested metabolites with retention times less than 35 min were found to be inactive

Table 3. IC_{50} for mixed leucocyte cultures and relative immunosuppressive effect of cyclosporin and eight metabolites

	IC_{50}[a] (ng/ml)	Relative effect[b] (%)
Cy	26 ± 6	100
M17	530 ± 80	~8
M1	390 ± 80	~5
M18	870 ± 110	~3
M21	900 ± 100	~3
M8	> 10000	< 0.2
M9	> 10000	< 0.2
M26	> 10000	< 0.2
M203–218	> 10000	< 0.2

[a] IC_{50}, mean ± SD of three experiments in different individuals
[b] Metabolites compared to cyclosporin

(Fig. 2). Metabolites H410/420 and H355 had an extrapolated IC_{50} of 500 ng/ml and 12500 ng/ml, respectively. Since neither metabolite could be detected in blood of patients, they were not included in the further analysis.

Combinations of several metabolites showed an additive immunosuppressive effect in vitro, but no potentiation of the inhibitory properties. In the presence of a 50% inhibitory concentration of cyclosporin the IC_{50} of metabolite 17 was unchanged (Fig. 3 A). The same was observed for the combination of metabolites 17 and 1, both of which possess some activity (Fig. 3 B). The inactive metabolite 8 did not change the inhibitory profile of the active metabolite 17, even when metabolite 8 was added in excessively high concentrations (10000 ng/ml, which is ten times higher than the highest blood concentrations detected in vivo) (Fig. 3 C).

Calculation of the immunosuppressive effect of cyclosporin metabolites in vivo based on in vitro results

The immunosuppressive effect of cyclosporin metabolites in vivo was calculated from their blood concentrations and the inhibitory effects observed in the mixed lymphocyte culture. This was done under the assumption that the immunosuppressive activity of cyclosporin and its metabolites in vivo may follow a concentration-response relation similar to the inhibitory effect in vitro. The range of the calculated relative additional effects of the metabolites to the immunosuppressive effect of the parent drug was remarkably similar in both study groups; the means were very close (12.6% in group A and 13.8% in group B). This result is explained by the comparable levels of active metabolites in both groups and a selective increase in inactive second generation metabolites in group B. The large inter-individual variations within each group (4.4–31.4%

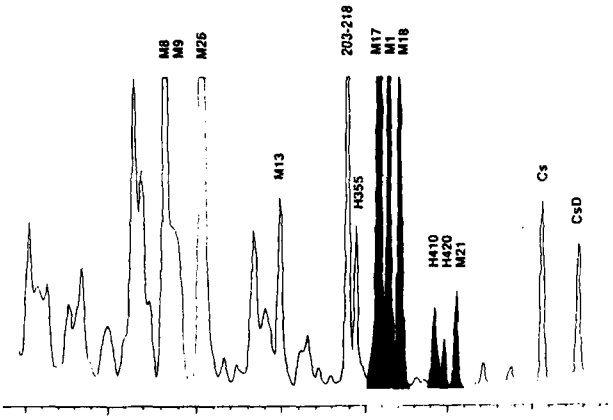

Fig. 2. HPLC chromatogram of urine from a cyclosporin-treated liver-graft recipient. The chromatogram illustrates the high relative concentrations of cyclosporin metabolites compared with the parent compound. On the right side are the substances with the longest retention time on the HPLC column, indicating high lipophilicity; the more hydrophilic metabolites are located to the left of the chromatogram. Only metabolites with retention times longer than metabolite 203–218 possess some immunosuppressive potency *(black peaks)*. Cs, Cyclosporin, CsD, Cyclosporin D (internal standard)

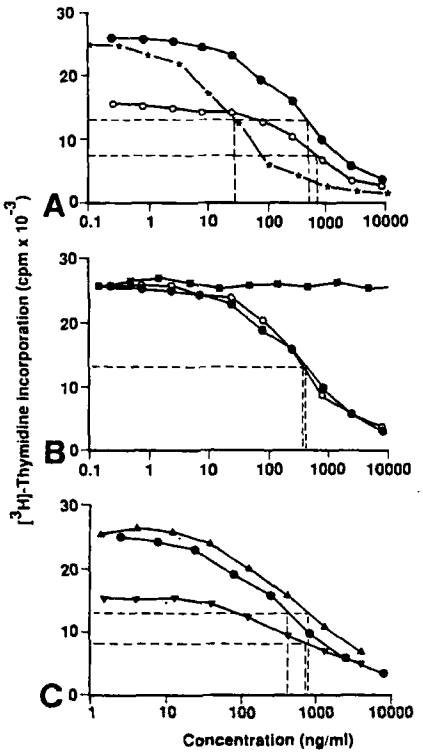

Fig. 3 A–C. Immunosuppressive activity of cyclosporin/metabolite combinations in MLC. Mixed lymphocyte cultures were performed with 10^5 responder PBMC and 10^5 irradiated allogeneic PBMC in a 5-day assay. Proliferation was assessed by ^{3}H-thymidine incorporation over 8 h. **A** The dose-response curves for cyclosporin (*—*) metabolite 17 (●—●), and metabolite 17 in the presence of 20 ng/ml cyclosporin (○—○); **B** for metabolite 8 (■—■), metabolite 17 (●—●), and metabolite 17 in the presence of 10000 ng/ml metabolite 8 (○—○); and **C** for metabolite 17 (●—●), metabolite 1 (▲—▲), and metabolite 1 in the presence of 300 ng/ml metabolite 17 (▼—▼). *Dashed lines* indicate 50% inhibitory concentrations

in group A and 2.7–25% in group B) correlates with a remarkable variation in the concentrations of metabolites 17 and 1 which was independent of liver function. Since unusually high or low concentrations of these metabolites in most cases were observed only sporadically, the relevance of this finding is not clear. In general, however, the immunosuppressive contribution of the metabolites was rather stable and not affected by liver dysfunction.

Discussion

The inhibitory effect of cyclosporin metabolites on in vitro lymphocyte functions is rather low compared with that of the parent compound. More than 25 metabolites have hitherto been isolated [8, 24], but immunosuppressive activity was found only for the highly lipophilic first-generation metabolites 17, 1, 18, 21, H410/420 and H355; all others which are less lipophilic are inactive in vitro [11, 13, 20–22, 25]. Of the active metabolites only the first three are usually detectable in blood of cyclosporin-treated patients. Although almost all authors agree on which of the metabolites are active, there are considerable variations in the immunosuppressive potency attributed to individual metabolites. Results similar to those reported here have been published by Ryffel et al. [21] and Hartmann et al. [13]. According to these data, which were all obtained using fresh human peripheral blood mononuclear cells, the activity of the most potent metabolite 17 ranges between 8 and 20% compared to cyclosporin. Discrepant findings have been reported by Freed et al. [11], but in this study unusually high concentrations of parent drug were required to inhibit lymphocyte proliferation and IL-2 production. Moreover, variable sensitivity to cyclosporin and metabolites has also been demonstrated for T-cell clones [27, 28].

The variability in the immunosuppressive action ascribed to the metabolites may have two major causes: (a) difficulties with isolation of the metabolites and their solubilization in culture medium; and (b) the different assay systems used. Both the type of cells used and the means of stimulation have a marked influence on the observed inhibitory effect. In the experiments reported here the metabolite concentrations were measured directly in the cultures using HPLC, and the concentration-response curves were adjusted accordingly. The test system used in this study was a mixed lymphocyte reaction, which represents a complex system of T-cell activation, and is regarded as an in vitro model for the immune response against an allogeneic graft. It is known that different functions of the immune system differ in their sensitivity to inhibition by cyclosporin. Previously we were able to demonstrate that stimulation of PBMC by CD3 monoclonal antibodies and by mixed lymphocyte reaction differs not only in the sensitivity to the parent drug, but also in the relative effect of the metabolites [22].

Since the immune response in vivo depends on a cascade of cellular interactions, the extrapolation of in vitro results to the in vivo situation represents an unresolved

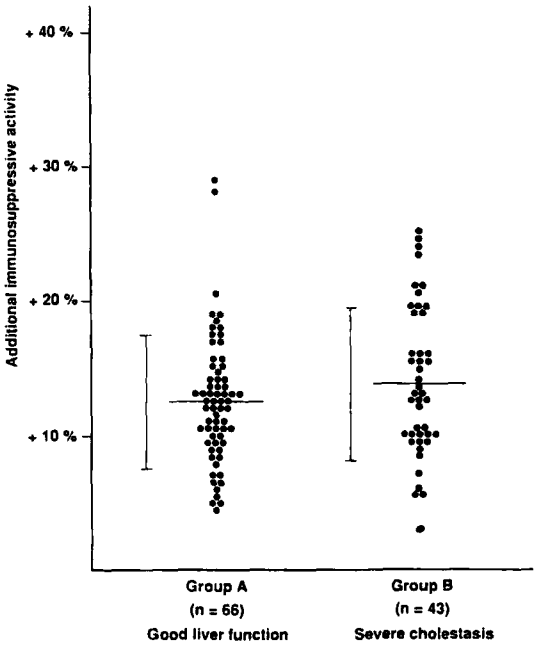

Fig. 4. Calculated immunosuppressive contribution of cyclosporin metabolites in patients with and without severe cholestatic liver-graft dysfunction. The *additional immunosuppressive activity* of the metabolites was calculated from their mean in vitro activity and their blood trough concentrations in individual patients with good liver function *(group A)* and with liver dysfunction *(group B)*

problem. Different cells with different functional profiles contribute to the alloresponse in vivo. Thus, the clinical relevance of in vitro findings obtained with individual long-term cultured T-cell clones [28], or with antibody-stimulated lymphocytes, is not completely clear. Even the mixed lymphocyte reaction, although it may be a particularly realistic model, reflects only some of the events occurring in vivo. Therefore, extrapolations of in vitro results to the in vivo situation have to be interpreted with great caution. Nevertheless, they represent the only way of obtaining at least a general impression on the in vivo immunosuppressive contribution of the metabolites.

A predominantly intracellular site of action of cyclosporin has been well established. Within the cells several proteins have been described as having affinity for cyclosporin and might be involved in its action. Probably the most important is cyclophilin [10, 19], which can reversibly bind cyclosporin and some of the metabolites. By competition for binding to this protein or to other intracellular binding sites even less active or inactive metabolites might gain biological importance, i.e. by blocking or enhancing the effect of cyclosporin. Our in vitro experiments using combinations of cyclosporin with active and inactive metabolites, however, demonstrate only additive immunosuppressive effects without evidence of synergistic or antagonistic action in the test system used. However, in other test systems (rat lymphocytes, human T-cell clones) supra-additive effects have been reported [23, 28]. Thus, such effects remain possible for some specialized T-cell functions, but their relevance for the in vivo situation is questionable.

On the basis of the additive immunosuppressive effects of cyclosporin and its metabolites found in this study, and under the assumption of comparable in vitro and in vivo activity, calculation of the in vivo immunosuppressive contribution of the metabolites was performed. Metabolite blood trough levels in liver-grafted patients with normal or only slightly impaired liver function were similar to those reported for kidney-grafted patients [1], whereas excessively high blood levels of metabolites were observed in patients with severe cholestatic liver-graft dysfunction. Increasing hyperbilirubinaemia in these patients seems to be associated mainly with disturbances of excretion of hydrophilic metabolites into bile or disturbances of late steps of metabolization. Early steps of metabolization are obviously less affected under these conditions. In the presence of constant levels of cyclosporin and first generation metabolites the levels of second generation compounds increased strongly under these conditions. Patients with good and bad liver function, therefore, differ not only in the amount, but also in the pattern, of metabolites in the blood.

The immunosuppressive contribution of cyclosporin metabolites was similarly low (10–20%) in patients with good and bad liver function. This is due to (1) the weak immunosuppressive activity of few highly lipophilic metabolites, and (2) a selective increase primarily of inactive second generation metabolites in patients with impaired liver function. An overproportional increase in blood levels of metabolite 17 leading to a relative increase in its immunosuppressive contribution was sporadically observed independent of liver function; usually levels returned to normal within a few days. Even in these situations, however, the immunosuppressive contribution of the metabolites remained below 35%.

Routine monitoring of cyclosporin therapy in the described patients was done by specific and non-specific monoclonal RIA giving immediate information about parent drug levels and a general impression on cyclosporin metabolite levels; cyclosporin dosage was adjusted according to these results. To avoid potential nephrotoxicity which had been observed in liver-grafted patients in association with high metabolite concentrations [15, 17, 23, 26], cyclosporin dosage was usually adjusted for non-specific RIA levels below 1200 ng/ml. Most patients in both study groups were on triple (or initially quadruple) drug therapy, and usually had parent drug blood levels between 80 and 180 ng/ml. For reasons of comparability, only episodes with cyclosporin HPLC blood trough levels in that range were included in this study. Thus, this study gives information only about patients with parent drug levels within the described range. However, there is no clear evidence for different metabolization patterns for cyclosporin blood levels outside that range.

The implication of the presented results for clinical routine is that the immunosuppressive effect of cyclosporin therapy is largely due to the parent drug. Therefore, the selective measurement of parent drug levels, which can be done either by the monoclonal specific RIA or by HPLC, is necessary and sufficient to determine the immunosuppressive effect of treatment. It should be emphasized, however, that these studies do not permit conclusions about the toxic potential of the metabolites. Although experiments with individual metabolites did not show evidence of nephrotoxicity in the rat model [9, 21], recent clinical studies indicate that deranged cyclosporin metabolism with excessively high metabolite blood levels is associated with episodes of nephrotoxicity [23, 26]. In addition, some metabolites have been shown to be toxic for cultured renal cells [3]. Thus, despite neglectable immunosuppressive contribution, monitoring of metabolite blood levels and appropriate cyclosporin dosage adjustment could be useful in reducing toxic complications in liver-grafted patients.

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