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The use of plasma levels for FK 506 dosing in liver-grafted patients

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Abstract FK 506 plasma levels were analyzed in 89 liver-grafted patients under FK 506-based immunosuppression. Plasma levels were found to be influenced by the patients' liver function: compared to patients without major liver dysfunction, those with cholestasis had higher plasma levels and these plasma levels were able to differentiate between rejection and toxicity. In patients with stable liver function, no clear difference was observed with regard to the plasma levels detectable during toxicity or rejection. We conclude that plasma levels can be used to determine the FK 506 dose but only in patients with cholestasis (i. e., during the early post-transplant course, or in patients with cholestatic rejection). In patients with stable liver function, plasma levels are only of limited clinical relevance.

Key words FK 506, liver transplantation, plasma levels · Liver transplantation, FK 506, plasma levels · Plasma levels, FK 506, liver transplantation

Introduction

The macrolide immunosuppressant FK 506 is currently under clinical investigation in patients who have undergone different types of organ transplantation [8, 10]. Its use is associated with side effects such as nephrotoxicity, neurotoxicity, diabetogenicity, and hypertension [2, 7, 11]. There is evidence that these side effects are related to elevated FK 506 plasma levels rather than to dose [1, 5, 11]. We have analyzed the pattern of FK 506 plasma levels measured in 89 liver-grafted patients under FK 506 immunosuppression and we report our findings here.

Patients and methods

Between September 1990 and May 1993, 89 patients receiving liver grafts at our clinic in Hannover were placed on FK 506 immunosuppression for different indications. Forty-six patients were treated with FK 506 for primary treatment after liver transplantation. In these patients on day 1 of treatment, FK 506 was administered as two 4-h intravenous infusions or as a continuous 24-h infusion. The initial intravenous dose was between 0.05 and 0.15 mg/kg body weight per day. Usually, the patients were switched to oral FK 506 on day 2 of treatment. The starting oral dose varied between 0.10 and 0.30 mg/kg body weight per day. In all patients starting dosages were lowered if there were signs of liver or kidney dysfunction. Target plasma levels were 0.5–1.5 ng/ml during the early postoperative course, followed by levels below 0.3 ng/ml in stable, long-term patients.

In patients converted from CyA to FK 506 immunosuppression ($n = 43$), two different dosing schemes were used. In patients switched for treatment of graft rejection, the intravenous starting dose was 0.10 mg/kg body weight on day 1, followed by an oral dose of 0.20 mg/kg body weight per day. Target plasma levels were 1.0–3.0 ng/ml in the early treatment phase, followed by levels below 0.3 ng/ml in stable, long-term patients. In patients switched for CyA toxicity or CyA malabsorption, FK 506 was given orally with a starting dosage of 0.10 mg/kg body weight per day. Target plasma levels were below 0.3 ng/ml.

In patients under primary FK 506 immunosuppression, a methylprednisolone bolus of 10 mg/kg body weight was given after reperfusion. Starting on day 1 after transplantation, prednisolone was administered at a daily dose of 20 mg, and this was tapered to a final dose of 5 mg at 3 months. Forty percent of all patients were withdrawn from steroids, usually at 6 months after initiation of FK 506 treatment. In patients converted from CyA to FK 506, the pre-

Table 1 Frequency of episodes of FK 506 toxicity in liver-grafted patients under plasma level-guided FK 506 immunosuppression (oral starting dose 0.10–0.30 mg/kg body weight per day)

Group	Side effects (incidence[a]/prevalence at month 12)		
	Nephro-toxicity	Hypertension	Diabetes
LTX primary ($n = 46$)	58.7/4.3	32.6/8.7	32.6/4.3
LTX rejection ($n = 29$)	55.1/4.8	20.7/14.3	19.5/14.3
LTX other indication[b] ($n = 14$)	50.0/14.3	21.4/7.1	21.4/14.3

[a] Number of patients with at least one episode of FK 506 toxicity
[b] CyA toxicity (e.g., nephrotoxicity), CyA malabsorption, CyA malmetabolisation

conversion steroid dose was maintained. In selected patients steroids were withdrawn after stabilization of liver function.

In all patients FK 506 nephrotoxicity was defined as an increase in creatinine above 140 μmol/l in adults or as an increase of more than 50 % of baseline levels in children responding to a reduction in the FK 506 dose. FK 506-mediated hypertension was defined as a de novo increase in blood pressure requiring antihypertensive treatment; the definition of an episode of diabetes mellitus was de novo hyperglycemia (excluding posttransplant days 1–7) requiring antidiabetic medication.

For the analysis of FK 506 in plasma (separated at room temperature), a modified enzyme immunoassay (EIA) using a polyvalent mouse monoclonal anti-FK 506 antibody (Fujisawa, Osaka, Japan) was used. This assay is known to crossreact with FK 506 metabolites [12]. The assay was performed as described elsewere [9, 11].

Blood samples were drawn immediately before administration of the drug. During the early treatment course, blood was drawn daily and drug levels were determined three times per week. Following discharge from the hospital, measurements were performed weekly and over the long term, monthly.

Results

With the dosages used in this study, FK 506 was effective as an immunosuppressant. In patients under primary immunosuppression the frequency of graft rejection was low (17.4 % of patients with at least one episode of biopsy-proven acute graft rejection; no chronic graft rejection). The incidence and 12-months prevalence of selected FK 506 side effects are shown in Table 1. As can be seen, episodes of FK 506 toxicity were far more frequent in the early postoperative course after transplantation than over the long-term course.

In all patients FK 506 plasma levels were monitored regularly. As shown in Fig. 1, plasma levels depended on liver function ($r = 0.59$; $P < 0.05$). In patients with hyperbilirubinemia, higher plasma levels were detectable. This tendency was not influenced by the initial FK 506 dosage.

The FK 506 plasma levels detectable in different clinical situations such as toxicity, rejection, or during a

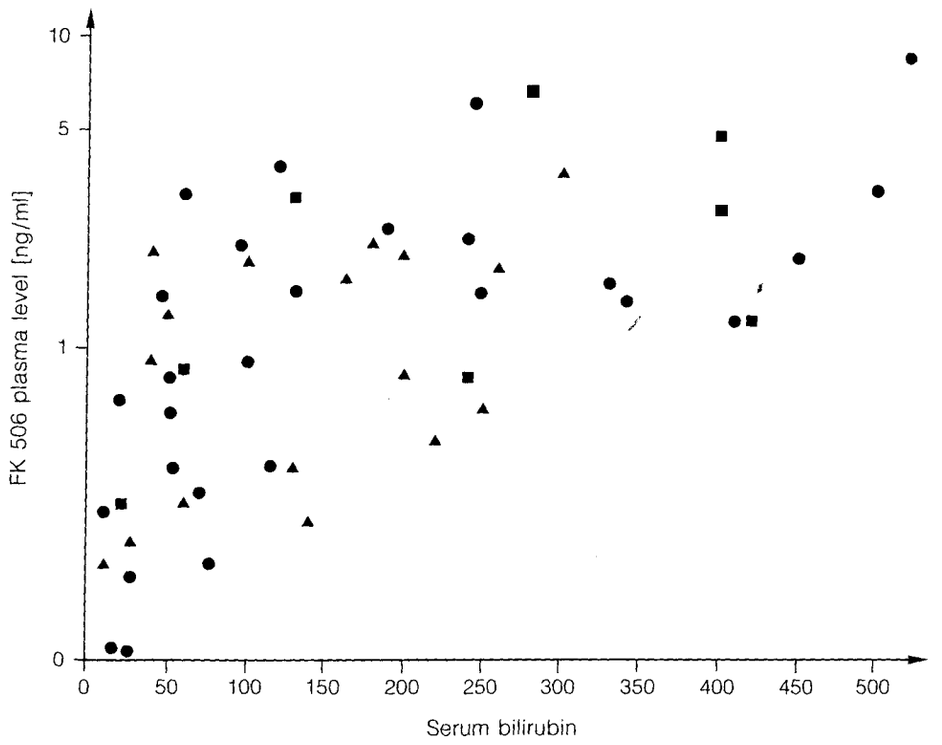

Fig. 1 Correlation of serum bilirubin with FK 506 plasma levels detected on day 5 of FK 506 therapy. Patients with interruptions in FK 506 dosage on days 1–5 were excluded from analysis. The initial oral dosages (mg/kg body weight per day) were: < 0.1 (●), 0.1–0.2 (▲), and > 0.2 (■). Irrespective of the starting oral dose used, plasma levels were higher in patients with liver dysfunction

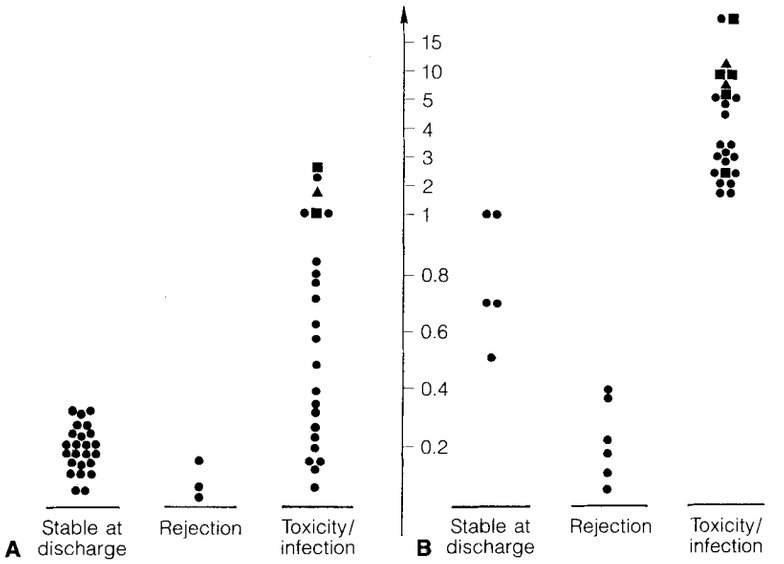

Fig. 2 A, B FK 506 plasma levels in different clinical situations in liver-grafted patients. The clinical episodes were classified as to the actual liver function of the respective patient: **A** patients with stable liver function (bilirubin < 50 μmol/l); **B** patients with hyperbilirubinemia (> 50 μmol/l). In patients with hyperbilirubinemia, plasma levels were able to discriminate between toxicity and rejection. In contrast, in patients with stable graft function, there was no clear difference as to the plasma levels detectable during episodes of toxicity or rejection (● nephrotoxicity, ■ neurotoxicity, ▲ opportunistic infection)

stable course are shown in Fig. 2. In patients with stable liver function (Bilirubin < 50 μmol/l), no clear difference as to the plasma levels detectable during episodes of toxicity or rejection was observed. There was, however, a tendency to higher levels in patients with toxicity. In contrast to patients without major liver dysfunction, in patients with hyperbilirubinemia (> 50 μmol/l) plasma levels correlated with episodes of toxicity or rejection.

In Fig. 3 the clinical course of a group of six patients with early liver dysfunction (ELD) is compared with the clinical course of a group of ten patients without major graft dysfunction. Despite the lower FK 506 dose given to patients with ELD, in this patient group the FK 506 plasma levels were higher than those in patients with stable initial liver function. The mean plasma levels measured in the two patient groups differed significantly from each other; however, no difference in the frequency of rejection episodes (one episode in each group) or in the frequency of FK 506 side effects was observed. The patients' kidney function is shown in the figure.

Discussion

While in stable patients only a weak correlation could be established between FK 506 levels and clinical events in patients with cholestatic liver dysfunction plasma levels were much more conclusive (Fig. 2). The sensitivity of the FK 506 plasma EIA (0.05 ng/ml) is known to be low; this low assay sensitivity at least partly compromises the clinical usefulness of FK 506 plasma levels in stable patients. In cholestatic patients, however, the accumulation of FK 506 metabolites crossreacting with the monoclonal antibody used in the EIA [6, 12] might lead to a shift in the overall concentration of FK 506 (and its metabolites) into a range that eliminates problems of assay sensitivity. High concentrations of these metabolites might induce clinically relevant FK 506 toxicity. This would indicate a causative toxic effect of FK 506 metabolites, at least in patients with cholestasis, and represent (in addition to a FK 506 parent drug-mediated toxicity) a second mechanism of FK 506 toxicity.

Like FK 506, cyclosporin A (CyA) is extensively metabolized by the liver and mainly excreted via the bile. Interestingly, when analyzing CyA parent drug and metabolite levels in liver-grafted patients, two pattern of toxicity have been described: a parent drug-associated pattern and a metabolite-associated pattern [14]. An answer to the question of whether FK 506 metabolites are, in fact, toxic should come from the analysis of FK 506 metabolite levels by assays capable of detecting individual metabolites, such as the HPLC-MS [4].

The observed heterogeneity in FK 506 levels over the long-term course might also be influenced by an inter-individual variation in susceptibility to lowered or elevated plasma levels. While in the majority of patients with nondetectable plasma levels (10 %–30 % of all patients) no graft rejection was observed, in a few patients biopsy-proven rejection was diagnosed. This variation might indicate a higher immunization status in some patients or might reflect differences in MHC incompatibilities between graft and recipient.

In all patients with nondetectable FK 506 in plasma, FK 506 was easily detectable in whole blood with levels in some patients even indicating over-immunosuppression (> 20.0 ng/ml). Therefore, a combination of whole blood EIA and (IMX) has been established in our laboratory. The clinical relevance of whole blood as a ma-

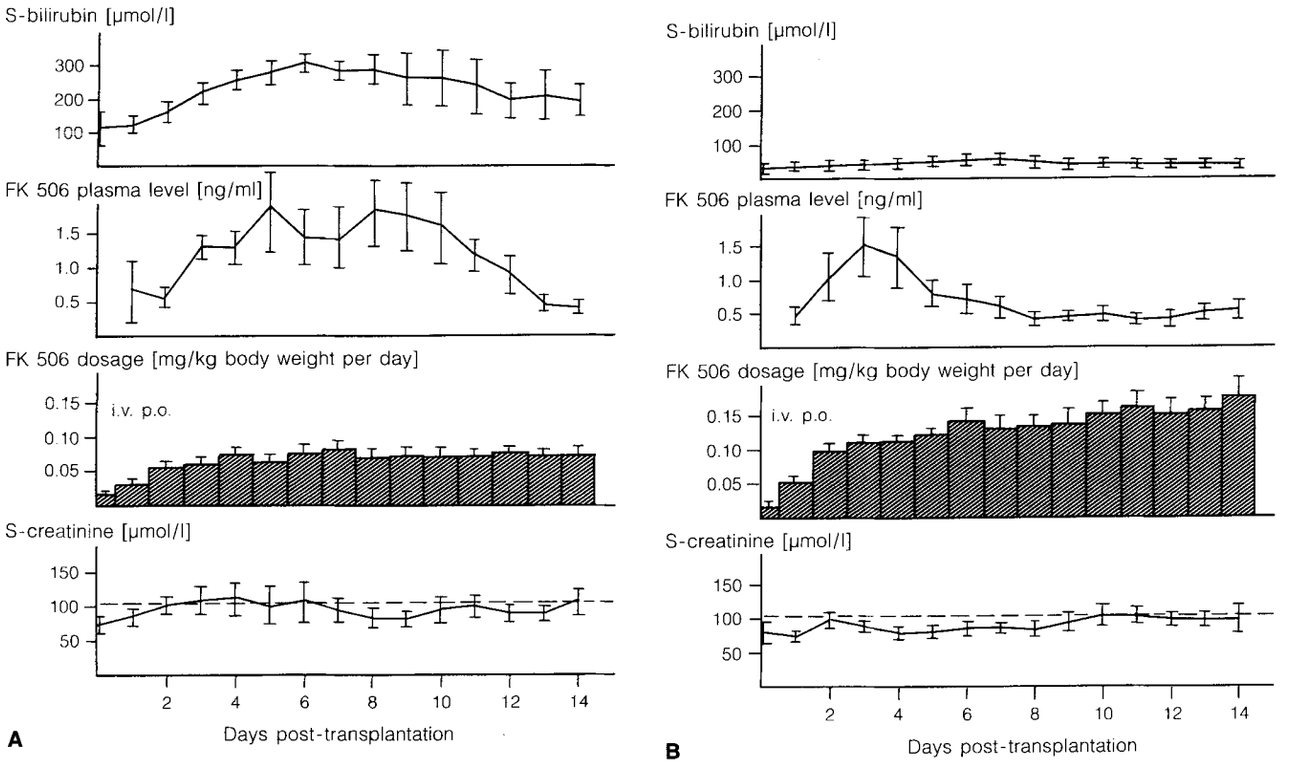

Fig. 3 A, B Comparison of the post-transplant course (days 1–7) of **A** six patients with early liver dysfunction (*ELD;* cholestasis with serum bilirubin > 200 μmol/l) with that of **B** ten patients without evidence of major ischemic graft injury (serum bilirubin < 100 μmol/l). Despite the lower FK 506 dosage given to patients with ELD, these patients had higher FK 506 plasma levels than patients with stable initial graft function

trix for FK 506 therapeutic drug monitoring is currently under investigation in our clinic.

In patients with early liver dysfunction (ELD), an elevated concentration of FK 506 metabolites has been demonstrated by HPLC-MS [4]. FK 506 EIA levels measured in these patients might, therefore, also indicate disproportionate concentrations of FK 506 metabolites. As these metabolites are known to be less immunosuppressive than the parent drug [3, 6], patients with ELD should require somewhat higher FK 506 EIA levels. In fact, compared to patients with stable liver function, patients with ELD showed higher plasma levels despite the fact that they were receiving lower doses, while there was no difference in the frequency of rejection and toxicity (Fig. 3).

As shown by our group and others, therapeutic drug monitoring is mandatory in patients under FK 506 immunosuppression [5, 11, 13]. We have shown here that FK 506 therapeutic drug monitoring is particularly helpful in patients with liver dysfunction. In general, a given plasma EIA level should only be interpreted in accordance with the actual liver function of the patient. First, operational plasma target levels should be between 0.5 and 1.5 ng/ml in the early post-transplant course and below 0.3 ng/ml over the stable, long-term course. Second, compared to patients with stable liver function, those with liver dysfunction should, in general, aim for somewhat higher plasma levels. Third, over the long-term course, it is not possible to define a lower target plasma level.

Due to the low sensitivity and specificity of the plasma EIA, other assays, such as the whole blood EIA or the whole blood IMX, should be tested as to their usefulness in FK 506 therapeutic drug monitoring. The question of whether the clinical relevance of FK 506 levels generated by these whole blood-based assays is greater than those of the plasma EIA is currently under investigation in our laboratory.

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