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Pharmacokinetic interpretation of FK 506 levels in blood and in plasma during a European randomised study in primary liver transplant patients

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The clinical investigators in the European FK 506 Multicentre Liver Study were as follows (listed in alphabetical order of the participating centres): P. Neuhaus (Berlin), P. McMaster (Birmingham), R. Calne (Cambridge), R. Pichlmayr (Hannover), G. Otto (Heidelberg), R. Williams (London), H. Bismuth (Paris), C. Groth (Stockholm)

Abstract The efficacy and safety of FK 506 compared with cyclosporin were evaluated in a European multicentre study with primary liver transplant patients. The daily intravenous doses ranged from 0.15 to 15.9 mg and the daily oral doses from 0.5 to 30 mg. Trough concentrations of FK 506 in blood and plasma were determined by an enzyme immunoassay. Blood concentrations ranged from 0.5 to 391 ng/ml and from 0.5 to 616 ng/ml after intravenous and oral doses, respectively. The corresponding plasma levels ranged from 0.05 to 56 ng/ml and from 0.05 to 104 ng/ml, respectively. In comparison to the parallel US trial, the mean oral doses in this European study were about 20% lower and the mean blood concentrations were 40% lower. However, the efficacy in these two trials was similar. No significant relationship between blood levels and selected adverse events or serum creatinine concentrations were observed in the European study (6-month data). An analysis of plasma protein and albumin concentrations showed an increase to normal ranges 4–8 weeks post-transplantation. The level of both markers remained lower in patients who withdrew due to adverse events.

Key words Liver transplantation
FK 506 · Pharmacokinetics
Blood levels · Plasma levels
Drug monitoring · Adverse events
Serum creatinine · Plasma protein
Plasma albumin

Introduction

FK 506 (tacrolimus) is a macrolide with potent immunosuppressive effects. It has been successfully used as a prophylactic immunosuppressant for solid organ transplantation at the University of Pittsburgh Medical Center [1–4]. FK 506 is highly lipophilic and binds strongly to plasma proteins (> 98.8% in rat, dog, monkey and man) and to erythrocytes [5]. The amount of FK 506 in red blood cells is rapidly available for redistribution.

Two multicentre, open, prospectively randomised, parallel-group clinical studies in primary liver transplantation were initiated in Europe and in the United States of America (US) in 1990. These two studies were similar in design and compared the use of FK 506 and corticosteroids with conventional cyclosporin (CyA) based immunosuppressive regimens (CBIR).

The European multicentre study GHBA-157 [6] was conducted in eight centres in four countries. In this paper, the results of pharmacokinetic evaluation of this study concerning dose and trough drug level data in patients randomised to receive FK 506-based immunosuppressive therapy are presented. The data reported are based on FK 506 dosage for the first 183 days of the study.

The objective of these evaluations was to define:

A. A suitable matrix for drug monitoring

B. A relationship between dose and trough blood concentrations

C. A relationship between blood concentrations and efficacy and adverse events

D. Dosage

Material and methods

Study population

Two hundred and seventy patients received FK 506. Safety and efficacy was evaluated in 267 patients. Three patients were excluded as a result of misrandomisation.

Dosage of FK 506

Intravenous dosing

Of the 267 patients randomised to treatment with FK 506, 245 received an initial intravenous infusion of FK 506, while 17 patients commenced treatment with oral therapy and 5 patients did not receive treatment. The median initial intravenous dose was 0.038 mg/kg (2.40 mg/subject) with a range of 0.003–0.091 mg/kg (0.20–7.00 mg/subject).

Seventy-one patients received their initial intravenous FK 506 infusion over a 4-h period and 107 patients had an initial infusion period of 12 h. Three patients had an infusion time of less than 4 h, 40 patients received their initial intravenous dose over a period of more than 4 but less than 12 h, nine patients over a 12- to 24-h period and 13 patients had an initial infusion time of 24 h. The initial intravenous dose of FK 506 was administered within 6 h following surgery.

Oral dosing

Seventeen patients received an oral dose as their first dose. On day 2, 106 patients in the FK 506 treatment group were receiving oral medication. By day 7, these figures had increased to 212 patients.

The lower oral FK 506 dosage on day 2 (mean of 0.135 mg/kg) reflected the fact that some patients received both oral and intravenous dosing on that day. From day 7 (mean of 0.165 mg/kg) to week 4 (mean of 0.159 mg/kg), the total daily dosage remained relatively stable but subsequently, the dosage was progressively reduced to a mean of 0.115 mg/kg at 6–12 months.

Monitoring of blood concentrations of FK 506

Drug level monitoring was accomplished by measuring trough levels of FK 506 in blood and plasma by a validated enzyme immunoassay (EIA) method. The assays were performed at BCO Medical Services BV, Breda, The Netherlands. The lower limit of quantitation (LOQ) of the assay in blood and in plasma were 0.5 ng/ml and 0.05 ng/ml, respectively.

During routine analyses, a calibration curve and three quality controls (QC), spiked with known amounts of FK 506, were concurrently assayed with each batch of unknown samples. Results of the analyses were only accepted if at least two of the three QC samples were within 30% of the nominal spiked concentration. Failure to meet this criterion resulted in repeat analyses of the whole batch.

Pharmacokinetic data evaluation

The relationship between blood concentrations and the corresponding plasma concentrations of FK 506 was examined by performing regression analysis on the data, with blood values as the dependent variable and plasma values as the independent variable. The relationship between concentrations of FK 506 in blood with the total daily oral dose expressed in mg/day was examined by plotting population mean concentrations against the corresponding dose. The population means were calculated as mean concentrations in blood across all patients at a specified dose. A line of best fit and associated 95% confidence intervals were fitted to the data.

Comparison of FK506 dosing and trough levels between the parallel European and US multicentre efficacy studies

An evaluation of FK 506 dose and trough blood concentrations of FK 506 in adult patients who participated in the US and the European studies was performed. Comparisons were based on data from 233 and 267 patients in the US and European studies, respectively.

Analysis of the relationship between selected adverse events and drug concentrations

The relationship between five selected adverse experiences and trough levels of FK 506 in blood was examined. The method described by James and Tsiatis [7] was used to analyse the relationship between the factor and the time to occurrence of the adverse experience. The following adverse experiences were examined: diabetes mellitus, hyperglycaemia, hypertension, infection and tremor. These adverse experiences were considered to be clinically relevant.

Relationship between serum creatinine and FK506 levels in blood

Serum creatinine levels were also analysed in this study as an indicator of renal function. The relationship between these values and FK 506 concentrations in blood was investigated.

Analysis of plasma protein and albumin

Plasma albumin and protein values were considered to be an indicator of liver function. Changes in mean concentrations of plasma protein and albumin during the course of the study were graphically compared daily for the first 14 days, weekly for the following 2 weeks and then monthly thereafter. The mean plasma protein and albumin concentrations versus time curve in patients withdrawing because of adverse experiences were also graphically compared with the curve in patients who did not withdraw as a result of adverse experiences.

Results

Pharmacokinetics

The daily intravenous doses of FK506 in this study ranged from 0.15 to 15.9 mg and the daily oral doses ranged from 0.5 to 30 mg. Blood concentrations ranged from 0.5 to 391 ng/ml and from 0.5 to 616 ng/ml after the intravenous and oral doses, respectively. The corresponding plasma levels ranged from 0.05 to 56 ng/ml and from 0.05 to 104 ng/ml, respectively (Table 1). The overall mean concentrations in blood and plasma were 15.3 ng/ml and 0.42 ng/ml, respectively.

However, detailed frequency analysis of the blood concentrations of FK506 showed that 93% of the

Table 1 Ranges of blood and plasma concentrations and dosage

Route of administration	Variable	Minimum	Maximum
Intravenous	Dosage (mg/day)	0.15	15.90
	Blood (ng/ml)	0.50	397.0
	Plasma (ng/ml)	0.05	56.00
Oral	Dosage (mg/day)	0.50	30.00
	Blood (ng/ml)	0.05	616.0
	Plasma (ng/ml)	0.50	104.0

Table 2a Frequency distribution of FK506 concentrations in blood

Concentration range (ng/ml)	Total number of observations	Percentage of total	Cumulative percentage
0.5–5	1495	28.7	28.7
5–10	1561	30.0	58.7
10–15	838	16.1	74.8
15–20	427	8.2	83.0
20–25	321	6.2	89.2
25–30	206	4.0	93.2
30–35	59	1.1	94.3
35–40	40	0.8	95.1
40–45	41	0.8	95.9
45–50	28	0.5	96.4
>50	194	3.7	100
0.5–616	5210	100	–

Table 2b Relative distribution of FK506 concentrations in blood: days 29–183 (oral dosing only)

Total number of observations	Range (ng/ml)	Percentage of total
1660	0.5–124.1	100
1585	0.5–20	95.5
75	20 –124	4.5

Table 3a Mean and standard deviations of FK506 concentrations in blood by time window after the intravenous doses

Time window (days)	Mean blood concentration (ng/ml)	Standard deviation	Number of samples
0	9.2	9.0	11
1	17.2	9.5	120
2	22.9	12.1	97
3	20.7	8.0	56
4	22.3	10.0	33
5	23.8	12.3	24
6	23.3	12.1	18
7	19.0	11.7	14
8	23.7	12.4	10
9	15.4	10.9	10
10	15.7	9.4	8
11	16.0	11.1	4
12	14.2	10.4	4
13	20.5	15.2	8
14	10.6	4.6	5

Table 3b Mean and standard deviations of FK506 concentrations in blood by time window after the oral doses

Time window (days)	Mean blood concentration (ng/ml)	Standard deviation	Number of samples
1	16.0	8.4	17
2	19.0	12.2	61
3	18.8	10.6	99
4	17.0	9.7	122
5	16.3	10.3	150
6	14.0	8.6	151
7	12.4	7.7	151
8	12.2	8.2	144
9	12.1	8.7	154
10	10.7	7.4	151
11	10.0	6.9	149
12	9.8	7.7	145
13	9.4	7.4	140
14	9.6	7.5	131
15–21	9.1	6.7	491
22–28	9.0	6.7	407
29–35	7.2	5.5	253
36–42	7.2	5.6	161
43–49	7.1	5.1	134
50–56	7.7	5.8	95
57–63	7.8	6.3	110
64–70	7.1	4.8	63
71–77	7.6	6.9	45
78–84	8.1	6.3	49
85–91	9.2	5.9	58
92–98	7.7	4.5	58
99–105	6.8	3.8	29
106–112	7.6	4.9	25
113–119	7.7	5.6	47
120–150	7.4	4.3	155
151–183	7.5	5.7	132

concentrations were in the range 0.5–30 ng/ml – the operating range of the assay (Table 2a). During the initial 4-week period post-transplantation, there was considerable intra-subject variability in daily trough blood levels. Thereafter, the blood levels tended to be more stable, with 95.5% of the values in the range 0.5–20 ng/ml (Table 2b).

In Table 3 are listed the overall mean blood concentrations and their associated standard deviations after the intravenous and oral administrations. The mean concentrations during the first 14 days of intravenous dosing ranged from 9.2 to 23.8 ng/ml (Table 3a). The mean concentrations during the first 4 weeks of oral dosing ranged from 9.0 to 19.0 ng/ml (Table 3b). There was a decrease in the mean oral dose from 9.9 mg (0.159 mg/kg) in week 4 to 7.3 mg (0.115 mg/kg) at 6 months [8]. However, the mean blood concentrations of FK 506 remained relatively stable, with ranges of 6.8 to 9.2 ng/ml throughout this period.

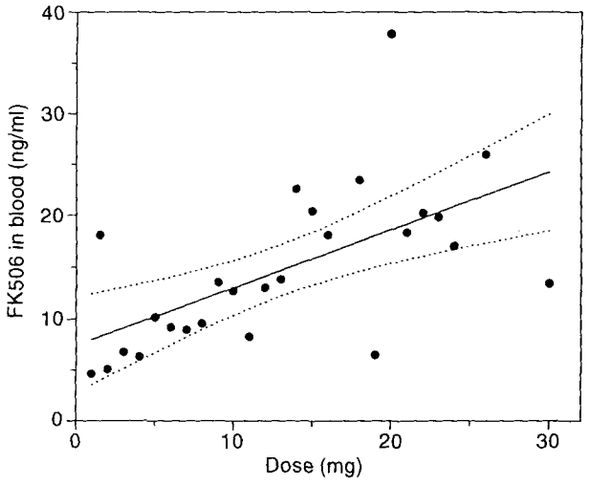


Fig. 1 Mean blood concentrations of FK 506 after oral dosing. Each data point is a mean of all FK 506 blood concentrations across all patients at the specified dose level. The solid line shows the line of best fit and the dotted line, the associated 95% confidence intervals

Table 4 Regression of plasma versus blood concentrations of FK 506. Analysis of variance

Source	*df*	Sum of squares	Mean sum of squares	*F* value	*P* value
Model	1	86517	86517	1594	0.0001
	4330	234874	54		
	4331	321391			

Regression analysis of the blood versus plasma concentrations of FK 506 showed that there was a poor correlation between blood and its corresponding plasma values. The blood to plasma concentration ratio ranged from about 1.1 to 1014 (mean of 56), the variation being largely due to a much greater spread of plasma concentrations, with mean values 0.42 ng/ml (CV = 445%) in plasma and 15.3 ng/ml (CV = 157%) in blood.

Regression of blood values as the dependent variable against plasma as the independent variable gave a significant intercept of 8.2 ng/ml ($P < 0.001$). This indicated that when no measurable concentrations of FK 506 were found in plasma, measurable levels could be found in blood (Tables 4, 5).

The population mean concentrations of FK 506 in blood plotted against the corresponding oral dose are presented in Fig. 1. A line of best fit and its associated 95% confidence intervals showed that most of the data lay within the confidence intervals.

Comparison of FK506 dosing and trough levels between the two parallel (European the US) multicentre efficacy studies

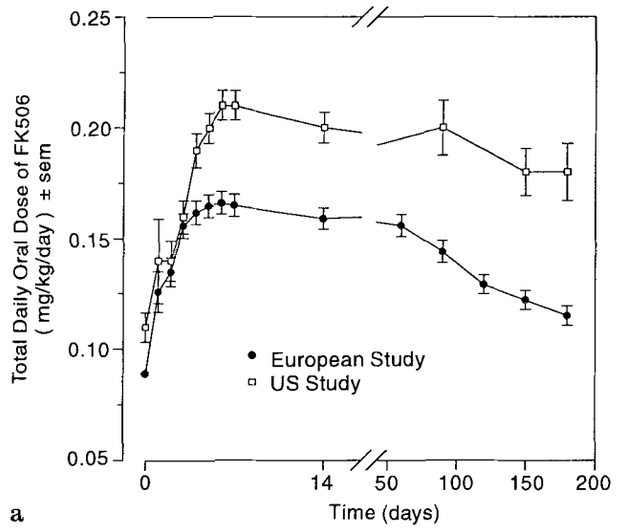
The relationship of FK 506 dosing and trough levels versus days after transplantation is shown in Fig. 2. From day 3 to month 6 post-transplantation, the ratios of oral doses in the European study in comparison to the US study ranged from 0.81 to 0.93 (Fig. 2a). The corresponding trough level concentration ratios ranged from 0.39 to 0.58 (Fig. 2b). Though the dose and concentrations in the European study were lower than in the US study, the efficacy in the two studies was comparable. This indicated that within the overall range of blood levels in these studies, higher blood levels may not necessarily lead to better efficacy.

Analysis of selected adverse events

Statistical examination of five key adverse experiences (hyperglycaemia, diabetes mellitus, tremor, hypertension

Table 5 Regression of plasma versus blood concentrations of FK 506. Parameter estimates

Variable	*df*	Parameter estimates	Standard error	T for HO: parameter = 0	*P* value
Intercept	1	8.2	0.14	57.1	0.0001
Slope	1	11.2	0.28	30.9	



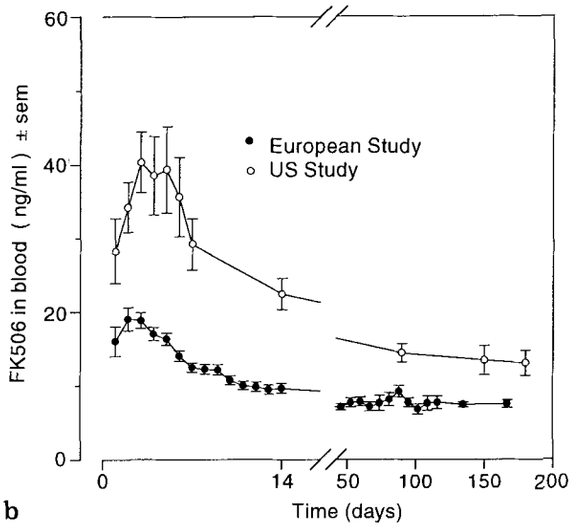
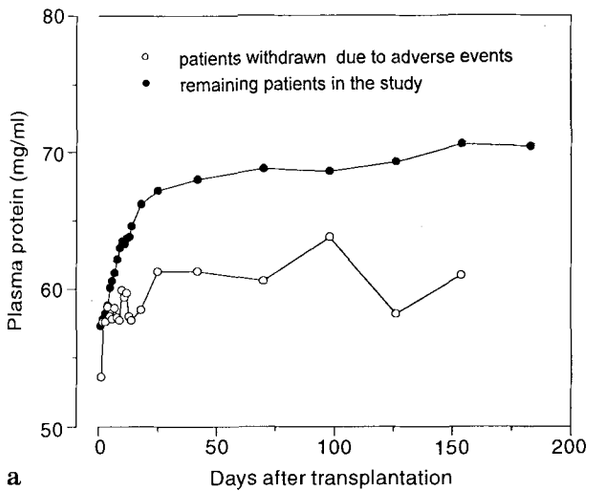


Fig. 2 **a** Mean (±sem) of total daily oral dose of FK 506 following liver transplantation (LTx) in adult patients versus days after transplantation. Timepoints represent the midpoint of the range of days over which the drug was administered. **b** Mean (±sem) of FK 506 trough blood levels following LTx in adult patients versus days after transplantation. Timepoints represent the midpoint of the range of days over which trough samples were taken



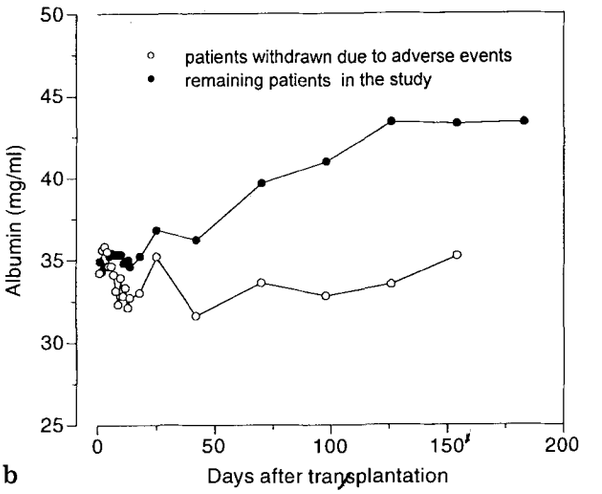


Fig. 3 **a** Mean plasma protein levels in LTx patients receiving oral FK 506 therapy versus days after transplantation. **b** Mean albumin levels in LTx patients receiving oral FK 506 therapy versus days after transplantation

Table 6 Summary of safety analyses by blood levels (days 1–183)

Adverse experience	Mean blood level for those with the adverse experience	Mean difference [a]	Test statistic	*P* value
Hyperglycaemia	15.97	−1.85	−0.59	0.5584
Tremor	12.70	1.72	1.01	0.3121
Diabetes mellitus	14.53	0.35	0.11	0.9163
Infection	15.21	−1.99	−1.26	0.2070
Hypertension	15.08	−0.83	−0.33	0.7450

[a] Mean of the difference between the whole blood value for each patient who experienced the adverse event and the average value for those who were still at risk of experiencing the adverse event at that time

and infection) with blood concentration data showed no significant association between FK 506 concentrations and adverse experiences (Table 6).

Relationship between serum creatinine and FK506 in blood

Mean values of serum creatinine were elevated (5–25%) compared to the upper limit of the normal range [8]. There was generally no clear relationship between blood concentrations and serum creatinine between days 1 and 183 (data not shown).

Analysis of plasma protein and albumin

Figure 3a and b represents mean plasma protein and albumin concentrations. Mean plasma protein and albumin levels increased steadily to reach normal levels over a period of 4–8 weeks post-transplantation. The normal range for protein is 60–80 mg/ml and for albumin, 40–60 mg/ml [8]. However, the levels in patients withdrawing owing to adverse experiences remained considerably lower throughout their participation in the study. The increase in albumin levels were more gradual than total protein levels. This indicated that it probably takes several weeks for liver function to stabilise.

Discussion

The primary objectives of this multicentre study were the efficacy and safety of FK 506 in liver transplant patients. Pre-dose blood and plasma samples were collected and assayed for FK 506, for drug monitoring and to enable limited pharmacokinetic and pharmacodynamic evaluations to be performed.

The data in this study suggested that in these patients, blood concentrations of FK 506 were a measure of systemic availability of the drug, in contrast to the corresponding plasma concentrations. This was manifest in a three-fold greater variability in plasma levels in comparison with the corrresponding blood levels.

The blood concentrations measured in this study ranged from 0.5 to 616 ng/ml. However, more than 90% of the samples were within the quantification range of the EIA assay (0.5–30 ng/ml), suggesting that this method was adequate for monitoring trough concentrations of FK 506 in this study.

During the initial 4-week period post-transplantation, there was considerable intra-subject variability in daily trough blood levels. Several factors such as changes in liver function, haemodynamics, absorption and metabolism are likely to lead to greater variability in blood levels during this early period post-transplantation. It was not possible to predict the concentrations that could be attained on the basis of administered dose alone, even if the dose was administered intravenously. This suggested that frequent blood level monitoring may be necessary to guage patients exposure to the drug and to maintain the blood levels within a therapeutic range.

During this early post-transplant period, the mean blood concentrations ranged from 9.2 to 23.8 ng/ml and from 9.0 to 19.0 ng/ml after intravenous and oral doses, respectively. Concentrations in these ranges could be considered as initial trough blood level targets to be aimed at during the early post-transplant period. After this early period, the trough blood levels tended to be relatively stable, with 95% of the blood levels in the range 0.5 to 20 ng/ml. Concentrations in this range could be considered as targets for maintenance therapy. The blood levels need to be optimised for each individual patient using dose adjustments, on the basis of clinical evidence of toxicity and efficacy.

Visual examination of the population mean concentrations of FK 506 in blood showed that up to 20 ng/ml, they tended to increase more linearly with increasing daily oral dose (mg/day) than the values greater than 20 ng/ml. Furthermore, the concentrations in the range 0.5 to 20 ng/ml appeared to be more linearly related to the oral daily dose of FK 506 than the concentrations above this range (Fig. 1).

There was no significant association between the incidence of hyperglycaemia, diabetes mellitus, hypertension, infection or tremor and the concentration of FK 506 in blood.

A more detailed analysis of serum creatinine was done in individual patients and overall there was no correlation between serum creatinine and blood levels of FK 506. This suggested that, in these patients, concentrations of FK 506 in blood did not compromise renal function, as measured by serum creatinine.

The mean concentrations of protein and albumin increased steadily to reach stable normal levels 4–8 weeks post-transplantation. Unlike cycloporin, FK 506 binds predominantly to plasma albumin and α1-acid glycoprotein [9]. This suggests that a higher fraction of FK 506 was likely to be in the unbound fraction during the first few weeks post-transplantation. This could explain the higher clearance of FK 506 and a greater incidence of adverse experiences considered to be drug related during this period.

Thus, during this early period, blood levels may need to be maintained towards the lower end of the therapeutic range using lower initial doses without compromising the efficacy. Thereafter, doses need to be adjusted according to individual patient's requirements, using blood level monitoring as a guide.

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