Transplant Int (1992) 5 [Suppl 1]: S454–S458

TRANSPLANT
International

Toxicology of FK506 in the cynomolgus monkey: a clinical, biochemical, and histopathological study

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Abstract. We investigated clinical, biochemical, and histopathological parameters in FK506-treated cynomolgus monkeys. Eight monkeys given oral FK506, 1 ($n = 4$) or 10 ($n = 4$) mg/kg daily, survived the 90 days of treatment apparently in good health and without significant changes in biochemical and histopathological parameters, as did 2 control monkeys except one monkey on 10 mg/kg/day FK506 orally, who was found to have a malignant lymphoma. In contrast, monkeys given intramuscular FK506 1 mg/kg daily ($n = 4$) had to be sacrificed at day 20, 25, 32, and 47 because of severe illness. They showed abnormal biochemical parameters (increased serum urea and aspartate aminotransferase activity) and major histopathological changes in the kidney (mesangial cell proliferation and acute tubular necrosis), pancreas (depletion of beta cells), liver (steatosis), and heart (cardiomyopathy). Intramuscular administration of 1 mg/kg daily resulted in serum levels ranging from 10 to 15 ng/ml, while oral administration at a dose of 1 or 10 mg/kg daily resulted in equal or even higher serum levels (range 2–70 ng/ml). Thus, the height of the serum trough level of FK506 using the enzyme immunoassay is not related to the toxicity of FK506 in cynomolgus monkeys.

Key words: FK506 – Fujimycin – Toxicology – Pharmakinetics – Monkeys

FK506 is an immunosuppressive drug isolated from the fermentation of *Streptomyces tsukubaensis* [15]. It is a macrolide with a molecular weight of 822 D_a [29]. Although its working mechanism appears to be similar to that of cyclosporin A (CsA), the efficacy in vitro is compared by dose, about 100 times more potent [14]. The results from in vitro studies indicate that FK506 has a potent inhibitory activity on murine and human lymphocyte proliferative responses and on the generation of human cytotoxic cells [15, 17, 29]. In a variety of animal transplant models FK506 showed the prevention of allograft rejection [1, 9, 11, 13, 18–20, 23, 24, 27, 31–34, 36]. As far as side effects are concerned, early information indicates that these vary among different species. In rats, only a few pathologic effects of FK506 such as weight loss, thymic medullary atrophy (as with CsA), and dose-related elevations in nonfasting blood glucose levels have been reported [21]. Studies in 3 different groups of renal allografted dogs showed two major side effects: anorexia and vasculitis in various organs, particularly in the heart and the gastrointestinal tract [8, 24, 31]. In baboon kidney allograft recipients, anorexia, lethargy, and hyperglycemia have been reported [5]. In this study we investigated the clinical, biochemical, and histological parameters in FK506-treated nontransplanted cynomolgus monkeys, especially the dose, route of administration and serum levels of FK506 in relation to the side effects.

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Materials and methods

Animals. Fourteen cynomolgus monkeys obtained from three different inbred colonies were used for the experiment. Previously these monkeys were involved in other behavioural studies or in programmes for the induction of antisera. Their estimated ages at the start of the study were 1.5–8 years, and their body weights ranged from 2 to 5 kg. The animals were kept in separate cages and fed with standard primate pellets (Hope Farm, Woerden, The Netherlands) and fresh fruit. They had ad libitum access to water.

Drug administration. FK506 was supplied by the Fujisawa Pharmaceutical Company, Osaka, Japan. For intramuscular use, the drug form, containing 27% of FK506 in mannitol and the surfactant HCD-60 (polyoxyl-60-hydrogenated castor oil), was suspended in normal saline solution to a concentration of 4 mg/ml. For oral use, a solid dispersion formulation (SDF) was used; FK506 was dispersed with hydroxypropyl methylcellulose, a water-soluble polymer, to give a content of 20% wt/wt [12]. The dose of FK506 given always refers to the dose of the mass of pure compound. The oral use of the placebo (control group) contained 100% of the water-soluble polymer. For the oral administration of FK506 and the placebo, the powder was rubbed onto the animals' favourite piece of fruit. Complete acceptance of the FK506 was monitored face to face.

Table 1. Summary of clinical findings

Treatment	Mortality	Morbidity		Weight reduction[a] (mean)
		Loss of appetite	Lethargy	
Placebo 1.0 mg/kg daily orally	0/2	–	–	4%
FK506 1.0 mg/kg daily intramuscularly	4/4	+	+	15%
FK506 1.0 mg/kg daily orally	0/4	–	–	4%
FK506 10.0 mg/kg daily orally	0/4	–	–	2%

[a] Mean weight reduction = (pretreatment body weight – body weight at 90 days or at sacrifice/pretreatment body weight) × 100%

Experimental design. The following groups were studied: 1 mg/kg placebo given orally once a day (control group, $n = 2$), 1 mg/kg FK506 given intramuscularly (i.m.) once a day ($n = 4$), 1 mg/kg FK506 given orally once a day ($n = 4$), and 10 mg/jg FK506 given orally once a day ($n = 4$). A body weight reduction of more than 10% was taken as a sign of drug toxicity, and the dose was reduced to 50% of the initial value. After 90 days or when the animals became lethargic, the monkeys were sacrificed by a lethal dose of pentobarbital sodium given intravenously.

Clinical observations. Every day the monkeys were observed. Two times a week the animals were scored systematically for the following clinical observations: weight, appetite (none, mild, moderate, good), and activity (apathetic, slow, normal, hyperactive).

Biochemistry studies. Once a week fasting venous blood samples were taken for the measurement of potassium, sodium, chloride, calcium, serum urea nitrogen (SUN), creatinine (Cr), alkaline phosphatase (AF), γ-glutamyl transferase (GGT), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total bilirubin, amylase, lactodehydrogenase (LDH), fasting blood glucose, leukocytes, hemoglobin (Hb), and hematocrit (Hct).

Histology studies. A complete postmortem examination was performed in all animals with microscopical evaluation of necropsies of the liver, kidney, heart, lung, spleen, pancreas, small bowel, brain and paraaortic lymph nodes. Tissues were fixed in 10% neutral buffered formalin and were paraffin embedded. Sections of 4 µm thick were stained with H & E or with special techniques as indicated. All sections were examined blindly without knowledge of the treatment regimen. The histopathologic changes were graded on a 0–3 scale, recorded as normal (0), minimal (1), moderate (2), and severe (3).

Drug level monitoring. The 24-h trough serum level of FK506 was determined in weekly blood samples which were collected prior to the daily drug administration. The samples were centrifuged at room temperature and stored frozen at −30°C until analysis. FK506 was extracted from the serum samples using Sep-Pak columns. The bound FK506 was eluted with methanol and evaporated to dryness under nitrogen at 37°C. The dried samples were analyzed by a modified enzyme immunoassay (EIA), in which a monoclonal antibody (Fujisawa, Osaka, Japan) directed against FK506 was used [3, 4, 28].

Results

Clinical studies

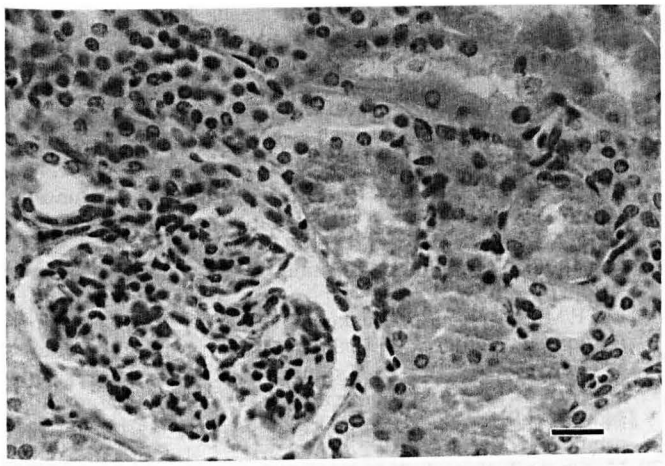
The monkeys given FK506 orally and the 2 placebo monkeys survived the 90 days of the experiment in apparently good health. They showed no clinical signs of side effects, and no significant body weight reduction was observed (Table 1). In contrast, all the monkeys treated with FK506 intramuscularly had to be sacrificed because of severe illness before the end of the experiment on days 20, 25, 32, and 47. From day 18 to 20 they developed a diminished appetite and signs of lethargy; two developed severe diarrhea during the last 3 days before sacrifice. Three of the four monkeys showed a body weight reduction of more than 10%, and accordingly protocol dosages were reduced to 0.5 mg/kg daily on days 14, 19, and 27. None of the animals showed clinical signs of infection.

Biochemistry findings

The biochemistry findings are summarized in Table 2. The most pronounced findings were found in the i.m.-treated animals. In these animals the SUN levels of monkeys MF2, MF3, and MF4 increased. The serum creatinine level of MF2 was elevated. GGT was increased after 90 days in MF3, and ASAT was increased in monkeys MF2 and MF3. One of the 4 monkeys on FK506 given intramuscularly, MF4, had an increased fasting B-glucose (from 2.8 to 11.1 mmol/l). The results concerning the influence of FK506 on glucose metabolism are described in detail elsewere [10]. The monkeys on orally given FK506, both the 1 mg/kg daily and the 10 mg/kg daily dosage, did not show significant changes of the biochemical parameters during the 90 days of the experiment. The other measured biochemical parameters did not show any significant changes.

FK506 levels in serum

The monkeys given the drug i.m. had after 1 week of daily treatment serum levels between 10 and 15 ng/ml. During the following weeks, the levels increased slowly in spite of dose reduction at days 14, 19, and 27. The serum levels in the animals given 1 mg/kg daily orally, ranged between 0.2 and 5.0 ng/ml, except for 3 peaks of 8 ng/ml in 1 monkey. The FK506 serum levels of the monkeys on 10 mg/kg oral dosage had levels ranging between 2.0 and 70 ng/ml. In none of these 4 monkeys were the serum trough levels stable; they differed from week to week and from monkey to monkey. Further details are described elsewere [35].



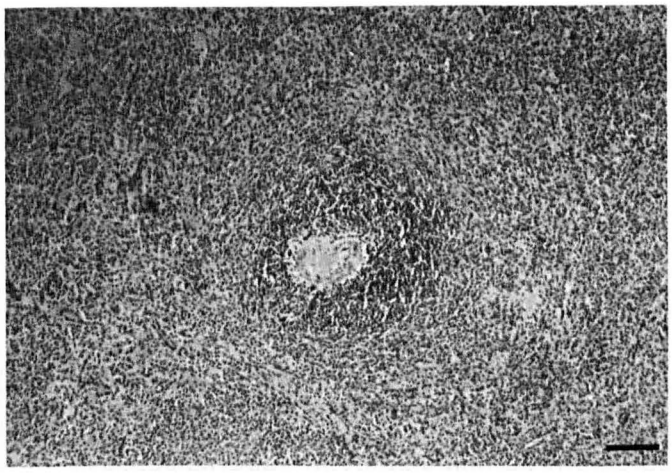


Fig. 2. Depletion of germinal centers in the periarteriolar lymphocytic areas in the spleen of monkey on orally given FK506. *Bar* indicates magnification in microns

The elevated SUN levels of MF3 and MF4 can be explained by the upper gastrointestinal tract bleeding due to hemorrhagic gastritis in these 2 monkeys. Nephrotoxicity was only seen in one of the i.m.-treated monkeys (MF2). Both the biochemical and the histopathological data showed these nephrotoxic changes. The fibrinoid necrosis of medium-sized arteries frequently observed in dogs in various organs, notably the heart and the gastrointestinal tract, was not noticed in any of our monkeys, indicating that monkeys do not become afflicted with this lesion. This is consistent with findings in other studies [5, 30, 33].

A diabetogenic effect in baboons has been described after i.m. administration of the drug and was thought to be due to peripheral insulin resistance [7]. The present study suggests, however, that there may be a direct toxic effect on the pancreatic islets, resulting in selective B-cell depletion, at least after i.m. administration of 1 mg/kg daily. After 3 month's oral administration of FK506 10 mg/kg daily, no direct histological or biochemical effect on the endocrine pancreas was found.

Thiru et al. has previously reported on one FK506-treated baboon which was found to have a malignant lymphoma [30], just as we saw in the animal receiving 10 mg/kg daily orally (FM15). It is well-known that lymphoproliferative disorders and lymphoid tumors occur with an increased frequency in the immunocompromised individual. A few patients treated with FK506 have also developed such lesions [22]. It remains to be seen, however, whether or not FK506 will cause any change in the frequency or the characteristics of transplant-associated lymphomas [2, 26].

One of our objectives was to look for a relation between FK506 serum level and toxicity. The height of the trough serum level using the EIA did not appear to be re-

Fig. 1a–d. Histopathological changes of monkeys treated with FK506 1 mg/kg daily intramuscularly: **a** acute tubular necrosis of the kidney (MF2), **b** hyalinization of pancreatic islets, **c** cardiomyopathy (MF3), **d** malignant lymphoma, diffuse centroblastic form after 90 days at 10 mg/kg daily orally (MF15). *Bar* indicates magnification in microns

lated to the toxicity in cynomolgus monkeys. The measuring of metabolites by the assay might explain the discrepancy observed between the serum levels and toxicity. Several studies indicate the importance of the liver in the metabolism of the drug, and in in vitro studies, metabolites have been found in human liver microsomes [6]. The first pass through the liver after oral treatment could lead to the formation of metabolites which are probably detected by the assay, and levels not related to toxicity will be measured. Another explanation could be the rapid accumulation of the unmetabolized FK506 in the tissues or extremely high peak levels of FK506 after parenteral administration, events which could both lead to tissue damage. Such high peak levels may lead to tissue damage without the trough levels being increased. Therefore, oral administration of the drug appears to be most important in order to avoid severe toxicity.

In conclusion, our results show that FK506 may cause severe side-effects when given i.m., while oral administration is well tolerated. No relation between toxicity and the drug serum levels was found using the EIA. Attempts should be made to elucidate the pharmacokinetics of FK506, with emphasis on the specific methods for detection of the original compound and eventual metabolites, of which some might be toxic and others nontoxic, eventually with species difference as well.

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