STUDIES OF REPEATED ADMINISTRATION OF FK-506 ON MYOCARDIAL METABOLISM IN RATS

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ABSTRACT —The effect of a 14-day administration of FK-506 at 1 or 3 mg/kg on plasma lipids and myocardial energy and glucose metabolism in rats was investigated. FK-506 increased the level of blood glucose in rats when given daily at an oral dosage of 3 mg/kg for 14 days. The plasma level of total cholesterol was significantly increased by FK-506 at either 1 or 3 mg/kg, whereas that of triglyceride was not changed. There was no significant difference in energy charge potential and the lactate/pyruvate ratio of the myocardial tissue between FK-506- and solvent-treated groups. Because FK-506 administration for 14 consecutive days did not disturb myocardial energy and glucose metabolism, its cardiotoxic side effects were not recognized in the present experimental toxicological evaluation of rats.

KEY WORDS: FK-506, Cardiotoxicity, Myocardial energy metabolism, Triglyceride, Cholesterol, Rats

INTRODUCTION

FK-506 suppressed immune responses *in vitro* and *in vivo* in mice. It is well known that FK-506 and cyclosporin are effective immunosuppressants with low myelotoxicity and that they inhibit the production of T cell-derived soluble mediators such as interleukin 2, interleukin 3 and gamma-interferon induced by antigens and lectins (Schreiber and Crabtree, 1992; Wolfe *et al.*, 1997). Immunosuppressive effects of FK-506 are more highly potent than those of cyclosporin (Isai, 1990; Atkison *et al.*, 1995). Such powerful immunosuppressive agents which would attack specific cellular target sites are expected to be useful drugs for immunotherapy. However, in toxicological studies, Ohara *et al.* (1990) reported that FK-506 caused renal and pancreatic damage in rats and baboons. Clinically the spectrum of FK-506 side-effects generally resembles that of cyclosporin, including headache, nausea, vomiting, hypertension, hyperglycemia and hyperuricemia (Atkison, 1995). Nomoto *et al.* (1994) have demonstrated that cardiac symptoms such as chest pain and chest discomfort have been reported as adverse

side effects of FK-506 in clinical trials. In addition, potentially toxic effects of the agent on the heart have been reported in rabbits (Noto *et al.*, 1994). However, cardiotoxic side effects are not fully established in the experimental toxicological evaluation of FK-506 in rats.

The purpose of the present study was to investigate whether long-term oral administration of FK-506 in rats affects myocardial metabolic functions, such as energy and glucose metabolism, and shows cardiotoxicity. Blood samples were also collected and the levels of total cholesterol and triglyceride in plasma and those of glucose in whole blood were determined.

MATERIALS AND METHODS

Chemicals

The FK-506 was purchased from Fujisawa Pharmaceutical Co., Ltd. (Ibaraki, Japan). The cytochrome c and bovine serum albumin were from Sigma Chemical Company (St Louis, MO). The polyoxyethylene was from Nikko Chemical KK. (Tokyo, Japan). All other chemicals used were of reagent grades available commercially.

Experimental animals and diets

Sprague Dawley strain male rats (6 weeks old) were purchased from Clea Japan, Inc., Tokyo. The animal room was maintained at $23 \pm 1^\circ\text{C}$, with $50 \pm 5\%$ relative humidity and on a 12 hr light-dark cycle (lights on 6:00 to 18:00). The rats were freely given water and commercial laboratory chow (MF; Oriental Yeast Co., Japan) for at least one week before use. This investigation conforms to the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Treatment and preparation of animals

The rats were randomly divided into five treatment groups. The first group served as a control and received distilled water (0.5 ml/100g b.wt./day). The second and third groups received, respectively, FK-506 at a dose of 1 mg/kg and its solvent alone (0.4% castor oil + 1.6% ethanol), twice daily for 14 consecutive days. The fourth and fifth groups received, respectively, FK-506 at a dose of 3 mg/kg and its solvent (1.2% castor oil + 4.8% ethanol), twice daily for 14 consecutive days. All drugs were administered orally. In order to test the induction of the hepatic drug-metabolizing enzymes in animals by FK-506 at 3 mg/kg or its solvent (1.2% castor oil + 4.8% ethanol), rats were sacrificed by decapitation 24 hr after 14 consecutive administrations of the drug or solvent, and the hepatic microsomal fraction was prepared as reported previously (Hoshi *et al.*, 1992). In order to assay the tissue metabolites of the heart, animals were sacrificed 2 weeks after the oral administration of FK-506 under pentobarbital-anesthesia (30 mg/kg, i.p.) with artificial ventilation with room air. After opening the thoracic cavity, the heart was removed and frozen immediately with clamps previously chilled in liquid nitrogen. The frozen myocardial samples were pulverized in a mortar and pestle precooled in liquid nitrogen and were extracted with 3 vol 6% perchloric acid (Nakai *et al.*, 1996).

Assays for drug metabolizing enzymes

The cytochrome P-450 (P-450) content and NADPH-cytochrome P-450 reductase (F_{PT}) activity in the hepatic microsomal fraction were measured by the methods of Omura and Sato (1964) and Phillips and Langdon (1962), respectively. The protein concentrations of the microsomal pellet of the liver were measured according to the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Assay for blood glucose, triglyceride, and cholesterol levels

The blood collection was carried out at 9:00 on days 0 and 14. Tail-vein blood samples were obtained from the 24-hr fasted rats and used for measurements of the glucose levels in whole blood, and triglyceride and total cholesterol levels in plasma. The level of glucose in whole blood was determined by the glucose oxidase method of Dahlqvist (1961). The levels of triglyceride and total cholesterol in plasma were measured enzymatically using the Triglyceride-E-Test Wako kit (Wako Pure Chemical Industries, Ltd., Osaka) and Cholesterol E-Test Wako kit (Wako Pure Chemical Industries, Ltd., Osaka), respectively.

Assays for intermediates in myocardial energy and glucose metabolism

The levels of adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), creatine phosphate (CrP), lactate, pyruvate, glucose 6 phosphate (G6P), fructose 6 phosphate (F6P) and fructose 1,6 bisphosphate (FDP) were determined in the neutralized perchloric acid extract according to standard enzymatic procedures (Gruber *et al.*, 1974).

Statistical analysis

The one-way analysis of variance (ANOVA) technique with the Tukey-Kramer's procedure (Tukey, 1949; Kramer, 1956) was used. Calculated values of $P < 0.05$ were considered as statistically significant. Data are expressed as a percentage of change from control values. Mean values $\pm$ S.E.M. are reported.

RESULTS

Changes in body weight

Changes in the body weight of rats treated with FK-506 for 14 days are shown in Fig. 1. The body weight increased as the rats grew up. Repeated administration of either concentration of solvent or FK-506 at 1 mg/kg did not affect the body-weight gain as compared with the distilled-water-treated group. However, the body-weight gain in the FK-506 at 3 mg/kg-treated group was significantly suppressed from day 5 to day 14 as compared to those in the concentration-matched solvent-treated group (1.2% castor oil + 4.8% ethanol). A transient decrease in body weight was observed on day 0 in all groups, because rats were fasted for 24 hr before blood collection.

Effects of FK-506 and its solvent on hepatic drug metabolism

The effects of FK-506 and its solvents on F_{PT}

Effects of FK-506 on myocardial metabolism in rats.

Fig. 1. Time course of the effects on body weight of repeated administration of FK-506 at two doses. Rats were treated with FK-506 at doses of 1 and 3 mg/kg or the solvent for 14 days. They were killed 24 hr after the last dose. Each value represents the mean $\pm$ S.E.M. of 9-14 animals. * P < 0.05, ** P < 0.01, significantly different from the solvent (1.2% castor oil + 4.8% ethanol)-treated group.

Fig. 2. Effects on microsomal enzymes responsible for drug metabolism in rats treated with FK-506 at 3 mg/kg or the solvent (1.2% castor oil + 4.8% ethanol) for 14 days. Rats were treated with FK-506 at doses of 1 and 3 mg/kg or the solvent for 14 days. They were killed 24 hr after the last dose. Each value represents the mean $\pm$ S.E.M. of 9-14 animals.

activity and the P-450 content are shown in Fig. 2. Neither a significant induction of the enzyme nor an alteration of the P-450 content was observed in either the FK-506- or the solvent-treated group.

Effects of FK-506 on blood glucose level, and plasma triglyceride and total cholesterol levels

Fig. 3 shows the changes in the percent of the blood glucose level after repeated administration of the solvent or FK-506 at 1 or 3 mg/kg. The increase in blood glucose after 14-day administration is expressed as a percentage of the values obtained before administration in each group. The solvent at either concentration did not alter the blood glucose level. FK-506 at 1 mg/kg did not affect the fasting blood glucose level, while the drug at 3 mg/kg significantly increased the blood glucose level as compared to the concentration matched solvent group (1.2% castor oil + 4.8% ethanol).

Fig. 4 shows the changes in the percent of the plasma triglyceride and total cholesterol levels after administration of the solvent or FK-506 at 1 or 3 mg/kg.

Fig. 3. Effects on the blood glucose level in rats treated with FK-506 at 1 and 3 mg/kg or the solvent for 14 days. Each value represents the mean $\pm$ S.E.M. of 9-14 animals. ** p < 0.01, significantly different from the solvent (1.2% castor oil + 4.8% ethanol)-treated group.

Administration of the solvent appeared to increase the level of triglyceride, and the increase became statistically significant when the higher concentration of the solvent (1.2% castor oil + 4.8% ethanol) was administered. The solvent did not affect the level of total cholesterol. Although FK-506 at either dosage did not change the plasma level of triglyceride, it significantly increased the total cholesterol level, when compared to each concentration-matched solvent.

Effects of FK-506 on myocardial energy and glucose metabolism

The levels of intermediates of energy and glucose metabolism in the myocardium are summarized in Tables 1 and 2, respectively. The level of CrP was significantly decreased by treatment with the higher concentration of solvent (1.2% castor oil + 4.8% ethanol), but the pyruvate level was decreased by treatment with the lower concentration of solvent (0.4% castor oil + 1.6% ethanol) as compared with the corresponding values in the distilled water-treated group. There were no significant differences in the other metabolic parameters between groups. FK-506 at 1 or 3 mg/kg twice dai-

ly administered to the rat for 14 days did not show any deleterious influences on myocardial energy metabolism or glucose metabolism.

DISCUSSION

The body weight progressively increased with physiological growth during the experimental period in the present study. FK-506 at 3 mg/kg resulted in a significant suppression of the body-weight gain from day 5 to day 14 (Fig. 1). It is obvious that 3 mg/kg was toxic as the dose of FK-506 in this study. FK-506 is metabolized in the liver by the cytochrome P-450 III A (Gonschior *et al.*, 1996). We have first tested the effects of the solvent and FK-506 containing ethanol on the hepatic drug metabolizing system, because changes in the system could modify the results obtained in the present experiments. Administration of the solvent (1.2% castor oil + 4.8% ethanol) or FK-506 at 3 mg/kg for 14 days did not affect the activity of F_{PT} and the P-450 content in the liver microsomes (Fig. 2). This implies that the hepatic drug metabolizing system does not have to be taken into account.

It is known that the immunosuppressive effect of FK-506 was more potent than that of cyclosporin (Atkison *et al.*, 1995). There are several reports of an increased incidence of posttransplant diabetes under FK-506-based therapy (Shapiro *et al.*, 1997). FK-506 can suppress insulin production at the transcriptional step in pancreatic cells without any change in glucagon content (Tamura *et al.*, 1995). We also found a significant increase in the blood glucose level in rats treated with 3 mg/kg of FK-506. The increase in the blood glucose level in the FK-506 at 3 mg/kg-treated group may be explained by the imbalanced glucose metabolism caused by a decrease in insulin production in pancreatic cells. However, the myocardial glucose metabolism assessed by measurements of glycolytic intermediates was not influenced by FK-506, in spite of the imbalance of glucose metabolism in the blood. The increase in the blood glucose level due to FK-506 at 3 mg/kg is not sufficient to modify the myocardial tissue glucose metabolism.

Brown *et al.* (1997) have demonstrated that an increased serum level of lipoprotein is strongly implicated in the development of coronary heart disease by case-control and prospective studies (Mbewu and Durrington, 1990; Webb *et al.*, 1992). Cyclosporin increases serum cholesterol levels, mainly by increasing the low density lipoprotein fraction but not the high-density lipoprotein fraction in renal transplant

Fig. 4. Effects on the levels of total cholesterol and triglyceride in plasma in rats treated with FK-506 at 1 and 3 mg/kg or the solvent for 14 days.

Each value represents the mean $\pm$ S.E.M. of 9-14 animals. $\dagger p < 0.05$, significantly different from the solvent (0.4% castor oil + 1.6% ethanol)-treated group. $**p < 0.01$, significantly different from the solvent (1.2% castor oil + 4.8% ethanol)-treated group. $\#p < 0.05$, significantly different from the distilled water-treated group.

Effects of FK-506 on myocardial metabolism in rats.

recipients and non-transplant recipients (Raine *et al.*, 1988; Ballantyne *et al.*, 1989; Webb *et al.*, 1993). In the present study, a significant increase in plasma total cholesterol level was observed in FK-506-treated rats at either dose. The increase in the total cholesterol level due to FK-506 was similar to that due to cyclosporin (Webb *et al.*, 1993). FK-506 treatment may increase the risk for cardiovascular disease in experimental ani-

mals and humans. In fact, FK-506 causes some side effects, e.g. angina-pectoris-like thoracic pain associated with an abnormal electrocardiogram (Shibata, 1993).

Cardiac symptoms such as chest pain and chest discomfort have also been reported as adverse side effects of FK-506 (Nomoto, 1994). In human liver allograft recipients, disruption of myocardial muscle fibers

Table 1. Effects on the levels of intermediates in myocardial energy metabolism in rats treated with FK-506 or the solvent for 14 days.

	ATP	ADP	AMP	TAN	ECP	CrP
	$\mu\text{mole/g tissue}$					$\mu\text{mole/g tissue}$
Distilled water	4.13 $\pm$ 0.06	1.03 $\pm$ 0.06	0.25 $\pm$ 0.02	5.41 $\pm$ 0.10	0.86 $\pm$ 0.01	5.05 $\pm$ 0.16
0.4% Castor oil + 1.6% Ethanol	4.27 $\pm$ 0.04	1.06 $\pm$ 0.02	0.23 $\pm$ 0.01	5.56 $\pm$ 0.05	0.86 $\pm$ 0.01	5.21 $\pm$ 0.14
FK-506 1 mg/kg	4.40 $\pm$ 0.08	1.21 $\pm$ 0.16	0.29 $\pm$ 0.06	5.90 $\pm$ 0.24	0.85 $\pm$ 0.01	4.72 $\pm$ 0.40
1.2% Castor oil + 4.8% Ethanol	4.14 $\pm$ 0.12	1.18 $\pm$ 0.09	0.31 $\pm$ 0.04	5.62 $\pm$ 0.16	0.84 $\pm$ 0.01	4.39 $\pm$ 0.20 [#]
FK-506 3 mg/kg	4.17 $\pm$ 0.07	1.01 $\pm$ 0.05	0.23 $\pm$ 0.02	5.41 $\pm$ 0.06	0.86 $\pm$ 0.01	$\pm$ 4.81 $\pm$ 0.35

The values are mean $\pm$ S.E.M. of 9-14 rats. [#] $P < 0.05$ when compared with distilled water.

Table 2. Effects on the levels of intermediates in myocardial glucose metabolism in rats treated with FK-506 or the solvent for 14 days.

	G6P	F6P	FDP	([G6P]+[F6P]) / FDP
	$\mu\text{mole/g tissue}$			
Distilled water	0.28 $\pm$ 0.03	0.07 $\pm$ 0.01	0.03 $\pm$ 0.01	13.05 $\pm$ 1.76
0.4% Castor oil + 1.6% Ethanol	0.28 $\pm$ 0.02	0.07 $\pm$ 0.01	0.03 $\pm$ 0.01	13.16 $\pm$ 1.55
FK-506 1 mg/kg	0.29 $\pm$ 0.03	0.06 $\pm$ 0.01	0.03 $\pm$ 0.01	14.67 $\pm$ 2.64
1.2% Castor oil + 4.8% Ethanol	0.27 $\pm$ 0.02	0.06 $\pm$ 0.01	0.04 $\pm$ 0.01	9.49 $\pm$ 1.10
FK-506 3 mg/kg	0.25 $\pm$ 0.02	0.05 $\pm$ 0.01	0.03 $\pm$ 0.01	14.56 $\pm$ 2.91
	Lactate		Pyruvate	Lactate / Pyruvate
	$\mu\text{mole/g tissue}$			
Distilled water	1.10 $\pm$ 0.19		0.10 $\pm$ 0.02	12.24 $\pm$ 2.26
0.4% Castor oil + 1.6% Ethanol	0.77 $\pm$ 0.07		0.07 $\pm$ 0.01 [#]	13.50 $\pm$ 1.91
FK-506 1 mg/kg	0.90 $\pm$ 0.18		0.11 $\pm$ 0.04	10.25 $\pm$ 1.64
1.2% Castor oil + 4.8% Ethanol	0.81 $\pm$ 0.08		0.11 $\pm$ 0.02	10.88 $\pm$ 2.28
FK-506 3 mg/kg	1.14 $\pm$ 0.20		0.08 $\pm$ 0.01	14.08 $\pm$ 0.01

The values are mean $\pm$ S.E.M. of 9-14 rats. [#] $P < 0.05$ when compared with distilled water.

has been noted (Isai, 1990). However, these side effects have been evaluated by the pathological changes in the myocardium in the FK-506-treated animals and humans (Isai, 1990). In the present study, we tried to estimate the influence of FK-506 on the myocardium by means of measuring the myocardial levels of energy and glucose metabolites. Although the level of CrP significantly decreased 14 days after repeated administration of the solvent (1.2% castor oil + 4.8% ethanol), FK-506 at either dose did not alter the myocardial levels of energy metabolites as compared with the concentration-matched solvent (Table 1). The levels of glycolytic intermediates were not modified by the administration of FK-506, although the level of pyruvate was significantly decreased by the lower concentration of the solvent (Table 2). At present, we cannot explain the reason why the CrP and pyruvate levels decreased in the solvent-treated group. It may be due to some artificial changes. These findings suggest that FK-506 does not cause any deleterious changes in the myocardial energy and glucose metabolism at least 14 days after administration. Ohara *et al.* (1990) have reported that the main toxic changes are seen in the kidneys in a 13-week oral administration of FK-506 to the rat, but cardiotoxicity is not observed. The most common side effects such as headache, nausea, vomiting, and flushing have been reported only after intravenous administration of FK-506 (Shapiro *et al.*, 1990).

In conclusion, the high dose of FK-506 (3 mg/kg) caused a significant suppression of the body-weight gain from day 5 to day 14. This suggested that 3 mg/kg was toxic as the dose of FK-506 in this study. However, oral administration of FK-506 at 1 or 3 mg/kg twice daily to the rat for 14 days did not show any deleterious influences on myocardial energy metabolism or glucose metabolism.

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