ELSEVIER

European Journal of Pharmacology 419 (2001) 99–105

ejp

www.elsevier.nl/locate/ejphar

# Induction of choleresis by immunosuppressant FK506 through stimulation of insulin-like growth factor-I production in the liver of rats

Ikuo Kawamura*, Shigeru Takeshita, Mariko Fushimi, Miyuki Mabuchi, Jiro Seki, Toshio Goto

*Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*

Received 1 February 2001; received in revised form 30 March 2001; accepted 3 April 2001

**Abstract**

FK506 (Tacrolimus) is an effective immunosuppressant currently used worldwide in organ transplantation. Based on our recent findings that insulin-like growth factor-I (IGF-I) is important for the stimulation of choleresis in vivo, in this study we investigated the effect of FK506 on bile flow and the plasma and hepatic levels of IGF-I in rats. Intravenous treatment of rats with FK506 resulted in a significant increase in bile flow, whereas cyclosporin A induced a significant decrease. A significant increase in plasma levels of IGF-I was observed in rats 30 min after a single intravenous administration of FK506. Oral treatment of rats with FK506 for 1 week also resulted in an increase in both plasma and hepatic levels of IGF-I. Overall, this study showed that FK506 treatment increased bile flow and also induced an increase in the plasma and hepatic levels of IGF-I in rats, suggesting that a stimulation of hepatic IGF-I production by FK506 may contribute to its choleretic profile. 

*Keywords:* FK506 (Tacrolimus); IGF-I (Insulin-like growth factor-I); Bile flow; Choleresis; Cyclosporin A; Cholestasis

## 1. Introduction

FK506 (Tacrolimus) is an immunosuppressant isolated from a Streptomyces (Kino et al., 1987a,b). Although FK506 is structurally different from cyclosporin A, FK506, like cyclosporin A, inhibits mixed-lymphocyte reactions, the formation of interleukin-2 by T-lymphocytes and the formation of other mediators such as interleukin-3 and interferon-γ (Kino et al., 1987c; Goto et al., 1991; Sigal et al., 1991). Recently, calcineurin, a $Ca^{2+}$- and calmodulin-dependent protein phosphatase, has been identified as the target molecule for both FK506 and cyclosporin A. Inhibition of calcineurin leads to an alteration in the phosphorylation state of essential signaling molecules during T lymphocyte activation (Friedman and Weissman, 1991; Liu et al., 1991; O'Keefe et al., 1992; Clipstone and Crabtree, 1992). The in vitro immunosuppressive activity of FK506 is approximately 100 times more potent than that of cyclosporin A. In animals, FK506 prevents graft rejection and prolongs graft survival in liver, kidney and heart transplants (Ochiai et al., 1987a,b; Isai et al., 1990; Jiang et al., 1991).

FK506 was first used clinically in 1989 as a rescue immunosuppressant for liver transplantation (Todo et al., 1990) and was approved in 1994 for the prophylaxis of rejection after primary liver transplantation in the United States and in Europe (European FK506 Multicentre Liver Study Group, 1994; The US Multicenter FK506 Liver Study Group, 1994). Currently, FK506, as well as cyclosporin A, is used worldwide as an effective immunosuppressant for organ rejection in patients with allogeneic liver, kidney or lung transplants (Griffith et al., 1994; McDiarmid et al., 1995; Pirsch et al., 1997). The discovery of these immunosuppressants has lowered the incidence of rejection episodes, which are the most important cause of graft failure and morbidity in transplant recipients, and has improved the success rate of organ transplantation (European FK506 Multicentre Liver Study Group, 1994; The US Multicenter FK506 Liver Study Group, 1994; Griffith et al., 1994; McDiarmid et al., 1995; Pirsch et al., 1997; Wiesner, 1998).

Recent clinical findings in liver transplant patients have indicated that FK506, but not cyclosporin A, induces an

* Corresponding author. Tel.: +81-6-6390-5250; fax: +81-6-6304-5367.

*E-mail address:* ikuo_kawamura@po.fujisawa.co.jp (I. Kawamura).

0014-2999/01/$ - see front matter 
PII: S0014-2999(01)00961-X

increase in bile flow and bile acid output, suggesting a different choleretic profile for FK506 and cyclosporin A (Ericzon et al., 1997). Numerous studies have shown that cyclosporin A treatment induces cholestasis in humans (Klintmalm et al., 1981; Laupacis et al., 1981; Theilmann et al., 1991) and rats (Stone et al., 1987; Roman et al., 1990; Chan and Shaffer, 1997). However, it has not been completely elucidated whether FK506 induces cholestasis or not, since contradictory results have been reported with respect to the effect of FK506 on bile flow or bile acid secretion (McCashland et al., 1994; Sanchez-Campos et al., 1998; Mizuta et al., 1999). When we infused insulin-like growth factor-I (IGF-I) into rats with ligated bile ducts, we found that a large amount of bile accumulated in the ligated portion of the bile duct. As this observation led to the hypothesis that IGF-I might stimulate choleresis and induce an accumulation of bile in the bile duct, we investigated the relationship between IGF-I and bile flow or bile acid secretion in rats. As a result, exogenous treatment with IGF-I resulted in an increase in bile flow and bile acid secretion, demonstrating an important role for IGF-I in stimulating choleresis (Kawamura et al., 2000). Therefore, in the present study we investigated the effects of FK506 on bile flow and the plasma and hepatic levels of IGF-I in rats, in order to clarify the relationship between IGF-I production by FK506 and its choleretic action.

## 2. Materials and methods

### 2.1. Drugs

FK506 was synthesized in Fujisawa Pharmaceutical., FK506 oral formulation was suspended in water. FK506 soluble form for intravenous perfusion was diluted in saline. Cyclosporin A soluble form for intravenous perfusion was purchased from Sandoz (Bazel, Switzerland) and was diluted in saline. In the case of intravenous injection, saline was used as a vehicle for both drugs. In the case of oral treatment, the placebo was the vehicle for FK506. The drugs were given intravenously or orally to rats at a volume of 5 ml/kg of body weight.

### 2.2. Animals

Male Sprague–Dawley rats were purchased from Charles River Japan (Kanagawa, Japan). Male hypophysectomized Sprague–Dawley rats were from Japan SLC (Shizuoka, Japan). Hypophysectomy was performed at 4 weeks of age and was verified by the absence of weight gain in rats. The rats were kept under conditions of constant temperature and humidity, and fed on a standard diet and water ad libitum. All the animal experiments in this study were approved by our institutional animal care committee.

### 2.3. Effect on bile flow in rats after single treatment with FK506 or cyclosporin A

Under anesthesia with urethane (1.2 g/5 ml/kg of body weight, i.p.), the abdomen of each rat (6 or 7 weeks old) was opened via midline incision, and the common bile duct was exposed. A PE-10 polyethylene tube (Clay Adams, Parsippany, NJ, USA) was then inserted into the bile duct. After collection of bile for 30 min (baseline), FK506 (1 and 10 mg/kg) or cyclosporin A (10 and 100 mg/kg) was intravenously administered, and bile collection was continued at 30-min intervals for an additional 3 h. During experiments, the rats were kept on heating plates set at 37°C. After the experiments, the rats were killed by cardiac puncture under urethane anesthesia. Bile flow was measured gravimetrically and expressed as the value per body weight of rats.

### 2.4. Effect on plasma IGF-I levels in rats after single treatment with FK506

FK506 at a dose of 10 mg/kg was intravenously administered to 6-week-old normal rats anesthetized with urethane (1.2 g/5 ml/kg of body weight, i.p.), and blood samples were obtained from the subclavian vein. Plasma was then separated by centrifugation and all samples were stored at −80°C until analyzed. The plasma levels of IGF-I were measured using a rat IGF-I radioimmunoassay (RIA) kit (Diagnostic Systems Laboratories, Webster, TX, USA). Plasma samples were extracted with acidic ethanol solution, followed by centrifugation to remove IGF-I binding proteins before the RIA was performed.

### 2.5. Effect on plasma and hepatic IGF-I levels, and blood biochemistry in rats after 1-week oral treatment with FK506

FK506 at doses of 1 and 10 mg/kg was given orally to 6-week-old normal rats once daily for 1 week. Thirty minutes after the last treatment, the rats were anesthetized with sodium pentobarbital (50 mg/5 ml/kg of body weight, i.p.), and a blood sample was taken from the abdominal aorta and the liver was removed for determination of the IGF-I content.

Plasma was then separated by centrifugation and its IGF-I level was measured using a rat IGF-I RIA kit after acidic ethanol extraction. The plasma levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bile acids and bilirubin were measured using Chemical Analyzer Model TBA-80FR (Toshiba, Tokyo, Japan).

The liver extraction procedure for IGF-I measurement was performed according to previously described methods (Thissen et al., 1991). Briefly, pooled liver tissue was powdered in a mortar and pestle under liquid nitrogen. One milliliter of ice-cold acetic acid (1 mol/l) was added to

100 mg of powdered sample and the mixture was vortexed vigorously and allowed to stand on ice for 2 h. After centrifugation at 20,000 × *g* for 30 min at 4°C, the supernatant was transferred to a polypropylene tube and the pellet was re-extracted. Both supernatants were combined and evaporated off in a Centrifugal Concentrator CC-181 (Tomy, Tokyo, Japan) and, after being stored at −20°C, were reconstituted in assay buffer 24 h before RIA. The hemoglobin concentration of liver tissue was measured in order to estimate the volume of blood contaminating the tissue powder. The amount of IGF-I in the extract that could have been derived from blood contamination was subtracted from the total extractable IGF-I.

### 2.6. Effect on bile flow in hypophysectomized rats after single treatment with FK506

After cannulation of the bile duct of hypophysectomized rat (7 weeks old) under anesthesia with urethane, FK506 at a dose of 10 mg/kg was intravenously administered, and bile flow was then monitored for 3 h.

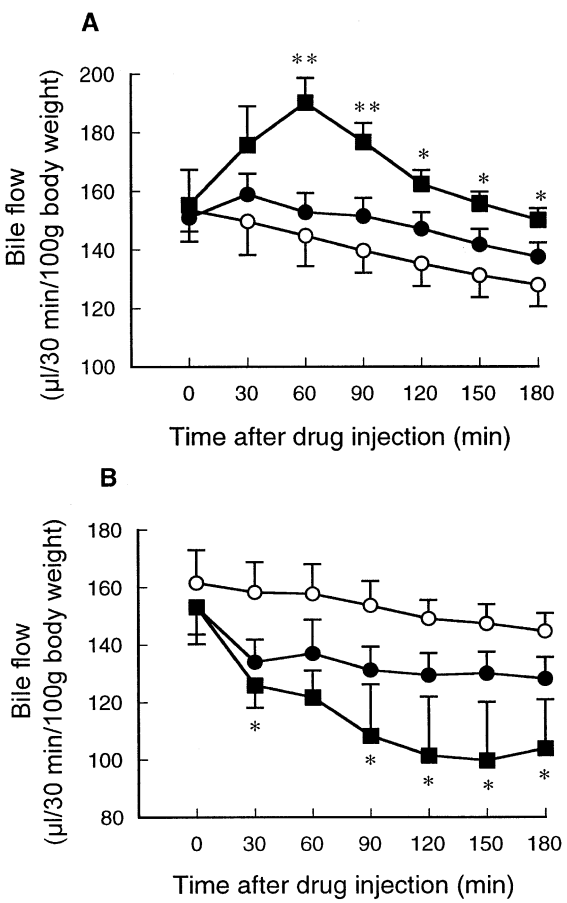


Fig. 1. Effects of FK506 (A) and cyclosporin A (B) on bile flow in normal rats after single administration. After cannulation of the bile duct under anesthesia, rats were treated with saline (○), 1 (●), 10 (■) mg/kg of FK506 (A) or saline (○), 10 (●), 100 (■) mg/kg of cyclosporin A (B) intravenously and bile flow was measured gravimetrically. Each point represents the mean ± S.E.M. for seven rats. * $P < 0.05$, ** $P < 0.01$, compared with the saline-treated rats (Dunnett's test).

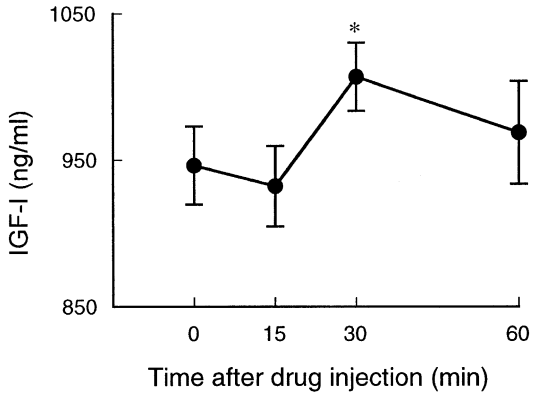


Fig. 2. Effect of FK506 on plasma level of IGF-I after intravenous administration. Rats were treated with 10 mg/kg of FK506 and blood was periodically taken from the subclavian vein. Plasma IGF-I level was measured using a rat IGF-I RIA kit. Each point represents the mean ± S.E.M. for nine rats. * $P < 0.05$, compared with pre-dosing (Student's *t*-test).

### 2.7. Statistical analysis

All values are expressed as the means ± S.E.M. Analysis of variance was performed and the Student's *t*-test or Dunnett's test was used to determine the significance of differences. A *P* value of 0.05 or less was considered significant.

## 3. Results

### 3.1. Effects of FK506 and cyclosporin A on bile flow in normal rats after single intravenous administration

FK506 (1 and 10 mg/kg) or cyclosporin A (10 and 100 mg/kg) was intravenously administered to anesthetized normal rats after cannulation of the bile duct, and bile flow was monitored. As shown in Fig. 1, a gradual and slight decrease in bile flow was observed in the saline-treated rats with increasing time, until 3 h after saline administration. This decrease in bile flow was considered to be due to damage as a result of anesthesia or interception of the enterohepatic circulation of bile acids (Kawamura et al.,

Table 1
Effect of FK506 on body and liver weights of normal rats after a 1-week oral treatment
The method is described in the legend of Fig. 3. Values are shown as the means ± S.E.M. for eight rats.

| Treatment (mg/kg) | Body weight (g) | | Liver weight (g) |
|---|---|---|---|
| | Before treatment | After treatment | |
| Placebo | 185 ± 3 | 236 ± 5 | 11.0 ± 0.4 |
| FK506: 1 | 185 ± 2 | 236 ± 4 | 11.4 ± 0.3 |
| FK506: 10 | 184 ± 2 | 207 ± 5[a] | 9.9 ± 0.6 |

[a] $P < 0.01$ vs. placebo-treated rats (Dunnett's test).

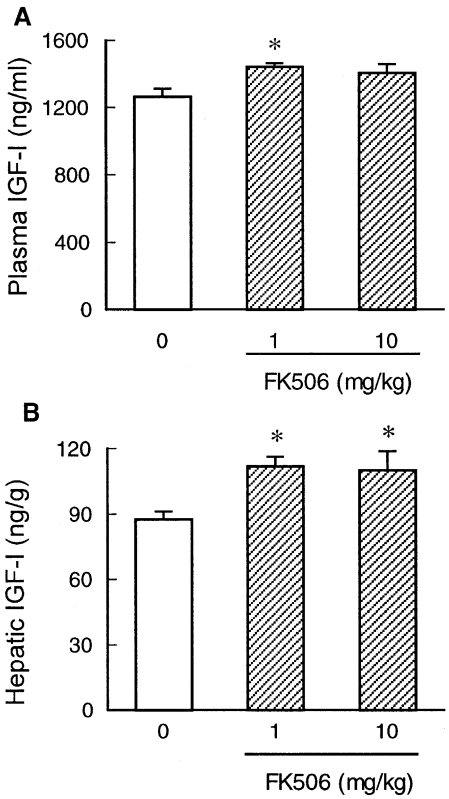


Fig. 3. Effect of FK506 on plasma (A) and hepatic (B) levels of IGF-I in rats. Placebo or FK506 was given orally once daily for 1 week to rats. Thirty minutes after the last treatment, a blood sample was taken from the abdominal aorta and then the liver was removed. Plasma and hepatic levels of IGF-I were measured using a rat IGF-I RIA kit. Rats were used in groups of eight. Each column represents the mean ± S.E.M. * $P < 0.05$, compared with the placebo-treated rats (Dunnett's test).

2000). FK506 treatment resulted in a dose-dependent increase in bile flow. A significant increase in bile flow was observed in the 10 mg/kg FK506-treated rats, and the increase remained throughout the experimental period, after reaching a peak value at 60 min after the injection of FK506 (Fig. 1A). In the case of cyclosporin A-treated rats, a dose-dependent decrease in bile flow was observed throughout the experimental period of 3 h (Fig. 1B). Thus, an opposite effect on bile flow was evident in FK506- and cyclosporin A-treated rats after a single intravenous administration.

### *3.2. Effect of FK506 on plasma and/or hepatic IGF-I levels in normal rats after single intravenous or 1-week oral consecutive treatment*

Recently, we found that exogenous treatment with IGF-I resulted in a significant increase in bile flow and bile acid secretion in rats, suggesting an important role for IGF-I in stimulating choleresis in vivo (Kawamura et al., 2000). Taken together, the findings of this study have led to the hypothesis that the increase in bile flow induced by FK506 might be mediated by a stimulation of IGF-I production in rats. Therefore, to examine the validity of this hypothesis, we next measured the circulating levels of IGF-I in rats after FK506 treatment. As shown in Fig. 2, an elevation of the plasma IGF-I levels was observed 30 min after a single intravenous administration of 10 mg/kg FK506 and after 60 min the IGF-I level declined to the level before treatment.

IGF-I stimulation following 1-week treatment with FK506 was also investigated in rats. FK506 (1 and 10 mg/kg) was orally given once daily for 1 week, and 30 min after the last administration, plasma and hepatic IGF-I levels were measured. After 1 week of oral treatment, 1 mg/kg of FK506 did not have any effect on body weight gain, but 10 mg/kg of FK506 significantly decreased body weight. Liver weight was unchanged by FK506 treatment (Table 1). As shown in Fig. 3, FK506-treated rats showed significantly elevated levels of both plasma and hepatic IGF-I. Plasma IGF-I level was increased by 14% and 11% in 1 and 10 mg/kg FK506-treated rats, respectively. Treatment with 1 and 10 mg/kg of FK506 resulted in an increase in hepatic levels of IGF-I by 28% and 26%, respectively. Thus, our data suggest that hepatic IGF-I production might be stimulated by FK506 treatment.

### *3.3. Effect of FK506 on blood parameters in normal rats after 1-week oral treatment*

In order to examine whether FK506 induced hepatotoxicity, blood parameters were monitored in normal rats after 1 week of oral treatment with FK506. As shown in Table 2, FK506 had almost no effect on the plasma levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bile acids and bilirubin. Blood

Table 2
Effect of FK506 on blood parameters in normal rats after a 1-week oral treatment
The method is described in the legend of Fig. 3. Values are shown as the means ± S.E.M. for eight rats.

| Treatment (mg/kg) | Aspartate aminotransferase (IU/l) | Alanine aminotransferase (IU/l) | Alkaline phosphatase (IU/l) | Total bile acids (μmol/l) | Bilirubin (mg/dl) |
|---|---|---|---|---|---|
| Placebo | 104 ± 8 | 32 ± 2 | 1018 ± 88 | 47 ± 11 | 0.8 ± 0.1 |
| FK506: 1 | 78 ± 5[a] | 26 ± 3 | 806 ± 41 | 45 ± 22 | 0.8 ± 0.0 |
| FK506: 10 | 86 ± 4 | 30 ± 2 | 731 ± 58[a] | 33 ± 7 | 0.8 ± 0.1 |

[a] $P < 0.05$ vs. placebo-treated rats (Dunnett's test).

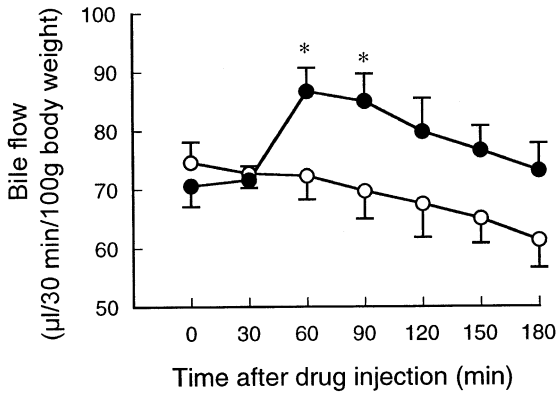


Fig. 4. Effect of FK506 on bile flow in hypophysectomized rats. After cannulation of the bile duct under anesthesia, hypophysectomized rats were treated with saline (○) or 10 mg/kg of FK506 (●) intravenously and bile flow was measured. Each point represents the mean ± S.E.M. for seven rats. * $P < 0.05$, compared with the saline-treated rats (Student's *t*-test).

levels of aspartate aminotransferase and alanine aminotransferase, however, were slightly decreased after FK506 treatment. These results suggest that FK506 does not have a hepatotoxic effect in rats after a 1-week oral treatment.

### *3.4. Effect of FK506 on bile flow in hypophysectomized rats*

To determine whether the choleretic action of FK506 is direct or mediated through the hypothalamic–pituitary growth hormone (GH) axis, the effect of FK506 on bile flow was investigated in 7-week-old hypophysectomized rats, 3 weeks after hypophysectomy. As shown in Fig. 4, the basal bile flow in hypophysectomized rats was 74.6 ± 3.0 μl/30 min/100 g of body weight, and this value was about half of that of normal rats (153.6 ± 13.7 μl/30 min/100 g of body weight in saline-treated rats, in Fig. 1). This decrease in bile flow observed in the hypophysectomized rats was thought to be due to a reduction in the IGF-I level as a result of the GH deficiency, as already discussed in our recent report (Kawamura et al., 2000). Treatment with 10 mg/kg of FK506 resulted in a significant increase in bile flow in hypophysectomized rats. The maximum increase was obtained at 60 min after FK506 injection, and bile flow gradually declined throughout the experiment period of 3 h (Fig. 4). The percent increase in bile flow in the 10 mg/kg FK506-treated hypophysectomized rats was almost equal to that in the 10 mg/kg FK506-treated normal rats.

## 4. Discussion

FK506 is currently used as an effective immunosuppressant to control rejection episodes in patients undergoing organ transplantation. In order to determine whether FK506 is choleretic or cholestatic, we first investigated the effects of FK506 and cyclosporin A on bile flow in normal rats. Single intravenous administration of cyclosporin A resulted in a dose-dependent decrease in bile flow in normal rats (Fig. 1B). This result is consistent with numerous previous reports describing the cholestatic characteristics of cyclosporin A in both humans (Klintmalm et al., 1981; Laupacis et al., 1981; Theilmann et al., 1991) and animals (Stone et al., 1987; Roman et al., 1990; Chan and Shaffer, 1997). In contrast, FK506 caused a significant and dose-dependent increase in bile flow in rats. In 10 mg/kg FK506-treated rats, bile flow started to increase at least 30 min after injection of FK506, reached a peak value at 60 min and remained at this level throughout the experimental period of 3 h (Fig. 1A). These results indicate a choleretic profile for FK506. Previously, contradictory results have been reported with respect to the choleretic or cholestatic characteristics of FK506 (McCashland et al., 1994; Sanchez-Campos et al., 1998; Mizuta et al., 1999). We do not have any explanation for the discrepancy between these results. However, because in our present study an increase in bile flow was evident in FK506-treated rats, we believe that FK506 really has the ability to stimulate choleresis in vivo.

Recently, we have found that exogenous treatment with IGF-I resulted in an increase in bile flow and bile acid secretion in rats, suggesting an important role for IGF-I in the stimulation of choleresis in vivo (Kawamura et al., 2000). The finding in the first experiment of this study led us to hypothesize that FK506 might have the potential to induce an increase in hepatic IGF-I levels, thereby leading to an increase in bile flow, mediated by the choleretic action of IGF-I. To investigate the validity of this hypothesis, we next evaluated the effect of FK506 on the circulating or hepatic levels of IGF-I in rats following single intravenous or 1-week oral treatment with the drug. Interestingly, a significant increase in the plasma levels of IGF-I was observed 30 min after a single intravenous administration of FK506. One-week oral treatment with FK506 also resulted in a significant elevation of both plasma and hepatic IGF-I levels in rats. These results are of particular interest in that FK506 has the ability to stimulate an increase in IGF-I production in vivo. As IGF-I is abundantly present in the blood and its major source is the liver (Rotwein, 1986; Murphy et al., 1987), the findings of this study suggest that hepatic IGF-I production was enhanced by FK506 treatment. This is the first report of a stimulation of IGF-I production in the liver by FK506. Interestingly, it implies a possible causative relationship between the stimulation of hepatic IGF-I production by FK506 and the choleretic action of FK506 in rats.

It was recently reported that FK506 stimulated GH secretion (Murao et al., 1996). As IGF-I production in the liver is regulated by GH (Rotwein, 1986; Murphy et al., 1987), the induction of choleresis by FK506 was next examined in hypophysectomized rats in order to determine

whether the choleretic action of FK506 is mediated through the hypothalamic–pituitary GH axis. FK506 treatment increased bile flow in hypophysectomized rats as well as in normal rats and its potency to do so was almost the same as that in normal rats (Figs. 1A and 4). This implies that the choleretic action of FK506 is not mediated through the hypothalamic–pituitary GH axis but possibly via a direct action. Like cyclosporin A, FK506 binds to its intracellular receptor and has the ability to associate with calcineurin, finally leading to an inhibition of phosphatase activity (Friedman and Weissman, 1991; Liu et al., 1991; O'Keefe et al., 1992; Clipstone and Crabtree, 1992). However, as FK506 stimulates an increase in either IGF-I production or bile flow, whereas cyclosporin A decreases bile flow in rats, we speculate that the mechanism of stimulation of IGF-I production by FK506 might be unrelated to its phosphatase inhibition. Thus, it is interesting to note that FK506 is, to the best of our knowledge, the first chemical compound to stimulate an increase in hepatic IGF-I production, although GH is known to regulate the expression of IGF-I in the liver endogenously (Rotwein, 1986; Murphy et al., 1987). Unfortunately, the scope of the present study did not allow us to elucidate the mechanism underlying this effect of FK506; however, it will be important to clarify this in the future.

Recent clinical studies showed that bile flow and bile acid output 10 days after the start of drug treatment were significantly higher in liver transplant patients treated with FK506 than in those treated with cyclosporin A, although bile flow and bile acid output were similar in the two groups immediately after transplantation. This suggests that FK506 caused a more rapid recovery of bile secretion after transplantation than did cyclosporin A (Ericzon et al., 1997). Taken together with the findings of our present study, it is likely that hepatic IGF-I production is stimulated in FK506-treated patients, and that this increase in IGF-I may contribute to an increase in bile flow and bile acid output. It has been reported that hepatic dysfunction, especially cholestatic dysfunction, frequently occurs in the transplanted liver in the early period after liver transplantation, owing to ischemic damage and thrombosis of hepatic blood vessels (Herrera et al., 1989; Ericzon et al., 1990; Lang et al., 1997). Therefore, it is possible that the choleretic action of FK506, mediated through the stimulation of hepatic IGF-I production, might benefit patients with liver transplants. However, because IGF-I is known to exert a variety of actions against many types of cells, there is a possibility that stimulation of hepatic IGF-I production by FK506 may contribute to some of the adverse effects associated with the clinical use of this drug. The major adverse effects observed in transplant patients treated with FK506 are hyperglycemia, neurotoxicity, renal toxicity and hypertension (European FK506 multicentre liver study group, 1994; The US Multicenter FK506 Liver Study Group, 1994). Recent evidence has shown that FK506 treatment causes a decrease in insulin production at the transcriptional step in pancreatic β-cells, leading to hyperglycemia (Tamura et al., 1995). Conversely, IGF-I, like insulin, has the ability to lower blood glucose levels and also possesses potent neuroprotective activity (Dore et al., 1997). Moreover, there has been no published evidence of a relationship between IGF-I and hypertension or renal toxicity. Thus, it is possible that the stimulation of IGF-I production by FK506 might not be associated with its adverse effects. Although changes in the blood levels of IGF-I or its binding proteins, so far as we know, have never been examined in transplant patients treated with FK506, it is important to verify whether IGF-I induction by FK506 really benefits transplant patients.

In conclusion, the present study demonstrates that FK506 treatment increased bile flow and also induced an increase in the plasma and hepatic levels of IGF-I in rats. This suggests that a stimulation of hepatic IGF-I production by FK506 may contribute to its choleretic profile.

## References

Chan, F.K.L., Shaffer, E.A., 1997. Cholestatic effects of cyclosporine in the rat. Transplantation 63, 1574–1578.

Clipstone, N.A., Crabtree, G.R., 1992. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 357, 695–697.

Dore, S., Kar, S., Quirion, R., 1997. Rediscovering an old friend, IGF-I: potential use in the treatment of neurodegenerative diseases. Trends Neurosci. 20, 326–331.

Ericzon, B.G., Eusufzai, S., Kubota, K., Einarsson, K., Angelin, B., 1990. Characteristics of biliary lipid metabolism after liver transplantation. Hepatology 12, 1221–1228.

Ericzon, B.G., Eusufzai, S., Söderdahl, G., Duraj, F., Einarsson, K., Angelin, B., 1997. Secretion and composition of bile after human liver transplantation: studies on the effects of cyclosporine and tacrolimus. Transplantation 63, 74–80.

European FK506 Multicentre Liver Study Group, 1994. Randomised trial comparing tacrolimus (FK506) and cyclosporine in prevention of liver allograft rejection. Lancet 344, 423–428.

Friedman, J., Weissman, I., 1991. Two cytoplasmic candidates for immunophilin action are revealed by affinity for a new cyclophilin: one in the presence and one in the absence of CsA. Cell 66, 799–806.

Goto, T., Kino, T., Hatanaka, H., Okuhara, M., Kohsaka, M., Aoki, H., Imanaka, H., 1991. FK506: historical perspectives. Transplant. Proc. 23, 2713–2717.

Griffith, B.P., Bando, K., Hardesty, R.L., Armitage, J.M., Keenan, R.J., Pham, S.M., Paradis, I.L., Yousem, S.A., Komatsu, K., Konishi, H., Fung, J.J., Starzl, T.E., 1994. A prospective randomized trial of FK506 versus cyclosporine after human pulmonary transplantation. Transplantation 57, 848–851.

Herrera, J., Codoceo, R., Mora, N.P., Pereira, F., Jara, P., Pardo, F., Cienfuegos, J.A., Castillo-Olivares, J.L., 1989. Bile acid profile as an early indicator of allograft function during orthotopic liver transplantation. Transplant. Proc. 21, 2313–2314.

Isai, H., Painter, D.M., Sheil, A.G.R., 1990. FK506 and orthotopic liver transplantation in the rat. Transplant. Proc. 22, 2125–2126.

Jiang, H., Takahara, S., Takano, Y., Li, D., Kyo, M., Valdivia, L.A., Kokado, Y., Ishibashi, M., Sonoda, T., 1991. Effect of FK506 on heart allograft survival in highly sensitized recipient rats in comparison with cyclosporine. Transplant. Proc. 23, 540–541.

Kawamura, I., Takeshita, S., Fushimi, M., Mabuchi, M., Seki, J., Tomoi,

M., Goto, T., 2000. Stimulation of choleresis by insulin-like growth factor-I in rats. Endocr. J. 47, 249–255.

Kino, T., Hatanaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H., Imanaka, H., 1987a. FK-506, a novel immunosuppressant isolated from a Streptomyces: I. Fermentation, isolation, and physico-chemical and biological characteristics. J. Antibiot. (Tokyo) 40, 1249–1255.

Kino, T., Hatanaka, H., Miyata, S., Inamura, N., Nishiyama, M., Yajima, T., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H., Ochiai, T., 1987b. FK-506, a novel immunosuppressant isolated from a Streptomyces: II. Immunosuppressive effect of FK-506 in vitro. J. Antibiot. (Tokyo) 40, 1256–1265.

Kino, T., Inamura, N., Sakai, F., Nakahara, K., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H., Ochiai, T., 1987c. Effect of FK-506 on human mixed lymphocyte reaction in vitro. Transplant. Proc. 19 (Suppl. 6), 36–39.

Klintmalm, G.B., Iwatsuki, S., Starzl, T.E., 1981. Cyclosporin A hepatotoxicity in 66 renal allograft recipients. Transplantation 32, 488–489.

Lang, T., Sendl, A.F., Esquivel, C.O., Berquist, W.E., Cox, K.L., 1997. Cholic acid synthesis is reduced in pediatric liver recipients during graft dysfunction due to ischemic injury and allograft rejection. Transplantation 64, 1585–1590.

Laupacis, A., Keown, P.A., Ulan, R.A., Sinclair, N.R., Stiller, C.R., 1981. Hyperbilirubinaemia and cyclosporine A levels. Lancet II 1426–1427.

Liu, J., Farmer Jr., J.D., Lane, W.S., Friedman, J., Weissman, I., Schreiber, S.L., 1991. Calcineurin is a common target of cyclophilin-cyclosporine A and FKBP-FK506 complexes. Cell 66, 807–815.

McCashland, T.M., Donovan, J.P., Amelsberg, A., Rossi, S.S., Hofmann, A.F., Shaw Jr, B.W., Quigley, E.M.M., 1994. Bile acid metabolism and biliary secretion in patients receiving orthotopic liver transplants: differing effects of cyclosporine and FK506. Hepatology 19, 1381–1389.

McDiarmid, S.V., Busuttil, R.W., Ascher, N.L., Burdick, J., D'Alessandro, A.M., Esquivel, C., Kalayoglu, M., Klein, A.S., Marsh, J.W., Miller, C.M., Schwartz, M.E., Shaw, B.W., So, S.K., 1995. FK506 (tacrolimus) compared with cyclosporine for primary immunosuppression after pediatric liver transplantation: Results from the US multicenter trial. Transplantation 59, 530–536.

Mizuta, K., Kobayashi, E., Uchida, H., Fujimura, A., Kawarasaki, H., Hashizume, K., 1999. Influence of tacrolimus on bile acid and lipid composition in continuously drained bile using a rat model: Comparative study with cyclosporine. Transpl., Int. 12, 316–322.

Murao, K., Sato, M., Niimi, M., Wada, Y., Matsubara, S., Takahara, J., 1996. Immunosuppressant agent FK506 stimulates growth hormone (GH) secretion and gene expression of hypothalamic GH-releasing hormone in the rat. Endocrinology 137, 1118–1122.

Murphy, L.J., Bell, G.I., Friesen, H.G., 1987. Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. Endocrinology 120, 1279–1282.

Ochiai, T., Nagata, M., Nakajima, K., Suzuki, T., Sakamoto, K., Enomoto, K., Gunji, Y., Uematsu, T., Goto, T., Hori, S., Kenmochi, T., Nakagouri, T., Asano, T., Isono, K., Hamaguchi, K., Tsuchida, H., Nakahara, K., Inamura, N., Goto, T., 1987a. Studies of the effects of FK506 on renal allografting in the beagle dog. Transplantation 44, 729–733.

Ochiai, T., Nakajima, K., Nagata, M., Suzuki, T., Asano, T., Uematsu, T., Goto, T., Hori, S., Kenmochi, T., Nakagoori, T., Isono, K., 1987b. Effect of a new immunosuppressive agent, FK506, on heterotopic cardiac allotransplantation in the rat. Transplant. Proc. 19, 1284–1286.

O'Keefe, S.J., Tamura, J., Kincaid, R.L., Tocci, M.J., O'Neill, E.A., 1992. FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. Nature 357, 692–694.

Pirsch, J.D., Miller, J., Deierhoi, M.H., Vincenti, F., Filo, R.S., 1997. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. Transplantation 63, 977–983.

Roman, I.D., Monte, M.J., Gonzalez-Buitrago, J.M., Esteller, A., Jimenez, R., 1990. Inhibition of hepatocytary vesicular transport by cyclosporine A in the rat: relationship with cholestasis and hyperbilirubinemia. Hepatology 12, 83–91.

Rotwein, P., 1986. Two insulin-like growth factor I messenger RNAs are expressed in human liver. Proc. Natl. Acad. Sci. U. S. A. 83, 77–81.

Sanchez-Campos, S., Lopez-Acebo, R., Gonzalez, P., Culebras, J.M., Tunon, M.J., Gonzalez-Gallego, J., 1998. Cholestasis and alterations of glutathione metabolism induced by tacrolimus (FK506) in the rat. Tranplantation 66, 84–88.

Sigal, N.H., Lin, C.S., Siekierka, J.J., 1991. Inhibition of human T-cell activation by FK506, rapamycin, and cyclosporine A. Transplant. Proc. 23 (Suppl. 2), 1–5.

Stone, B.G., Udani, M., Sanghvi, A., Warty, V., Plocki, K., Bedetti, C.D., Van Thiel, D.H., 1987. Cyclosporine A-induced cholestasis. The mechanism in a rat model. Gastroenterology 93, 344–351.

Tamura, K., Fujimura, T., Tsutsumi, T., Nakamura, K., Ogawa, T., Atumaru, C., Hirano, Y., Ohara, K., Ohtsuka, K., Shimomura, K., Kobayashi, M., 1995. Transcriptional inhibition of insulin by FK506 and possible involvement of FK506 binding protein-12 in pancreatic β-cell. Transplantation 59, 1606–1613.

The US Multicenter FK506 Liver Study Group, 1994. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. N. Eng. J. Med. 331, 1110–1115.

Theilmann, L., Otto, G., Arnold, J., Gmelin, K., Stiehl, A., 1991. Biliary secretion of bile acids, lipids, and bilirubin by the transplanted liver: A quantitative study in patients on cyclosporine. Transplantation 52, 1020–1023.

Thissen, J.P., Triest, S., Moats-staats, B.M., Underwood, L.E., Mauerhoff, T., Maiter, D., Ketelslegers, J.M., 1991. Evidence that pretranslational and translational defects decrease serum insulin-like growth factor-I concentrations during dietary protein restriction. Endocrinology 129, 429–435.

Todo, S., Fung, J.J., Starzl, T.E., Tzakis, A., Demertris, A.J., Kormos, R., Jain, A., Alessiani, M., Takaya, S., Shapiro, R., 1990. Liver, kidney, and thoracic organ transplantation under FK506. Ann. Surg. 212, 295–307.

Wiesner, R.H., 1998. A long-term comparison of tacrolimus (FK506) versus cyclosporine in liver transplantation. A report of the United States FK506 study group. Transplantation 66, 493–499.