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Dose Timing Effect of FK-506 on Liver Regeneration in Partially Hepatectomized Rats

Masahiro OHTAKE[1], Shuntaro KOYAMA[1], Takeo SAKAGUCHI[2], Kazuhiro TSUKADA[1], Keisuke YOSHIDA[1] and Terukazu MUTO[1]

Departments of [1]Surgery I and [2]Physiology I, Niigata University School of Medicine, Asahimachi 1, Niigata 951, Japan

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Summary. **The dose timing effect of FK-506 (FK) on liver regeneration was examined in 66% hepatectomized rats. FK at a dose of 0.1 mg/kg/day was given for three days starting on three different dates: 2 days before hepatectomy (FKI), 1 day before hepatectomy (FKII) and the day of hepatectomy (FKIII). Liver regeneration was evaluated by liver weight as a percentage of the body weight (LRR) and the mitotic index of the hepatocyte (MI) in the 3 days following hepatectomy. No significant change in LRR with an increase in MI was seen when FKI was given. Significant increases in both LRR and MI were obtained following FKII administration, but no change in LRR or MI was noted after FKIII administration. Food and water intake increased only when the rats received FKII. FKI and FKIII administration brought dysfunction of the liver, but no effect on the kidneys was induced by FK. The serum cholesterol concentration remained normal, but fatty tissue parameters were decreased in all animals with FK.**

These observations lead us to conclude that liver regeneration can be stimulated by FK with a reduction in the fatty tissue store, and FK has an optimal dose timing effect on regeneration without causing hepatotoxicity.

INTRODUCTION

It has been shown that liver regeneration after partial hepatectomy is stimulated by an immunosuppressive agent, FK-506 (FK),[1,2] which has newly been developed for organ transplantation.[3] The effect of FK on regeneration has been assumed to be caused by suppression of T lymphocyte proliferation, and the effect of the dose of FK on regeneration has also been evaluated in a dose-response study.[1] These reports suggest that FK is advantageous to both liver regeneration and implantation after transplantation. However, the dose schedule has not yet been examined. On the other hand, FK has been found to affect fat metabolism when used clinically.[4]

This study is designed to determine the dose timing effect of FK on rat liver regeneration in relation to fat metabolism.

MATERIALS AND METHODS

Thirty-six male Wistar rats were used. The animals were housed individually and allowed free access to laboratory chow (MF, Oriental Yeast, Osaka, Japan) and tap water throughout the experiments. The room temperature was controlled at 24±2°C with 12 h : 12 h light: dark cycles (lighting from 08 : 00-20 : 00 h). Estimates of individual body weight, food and water intake and surgery were made between 10 : 00 and 12 : 00 h to eliminate diurnal variation.

When the animals attained a weight of about 180 g, partial hepatectomy was performed under ether anesthesia by the method previously described.[1,5] In brief, the median and left lateral lobes of the liver, constituting two-thirds of the total liver mass, were removed. The abdominal wall was closed in layers. After surgery, the animals were returned to their cages and allowed access to food and water.

Three days after hepatectomy, liver regeneration was evaluated on the basis of the report that FK 0.1 mg/kg evidenely stimulated liver regeneration 3 days after hepatectomy.[1] Under pentobarbital sodim (40 mg/kg, i. p.) anesthesia, the remnant of the liver was removed and the LRR was estimated. Specimens of the caudate lobe of the liver were prepared with hematoxylin and eosin, and the proportion of hepatocytes in mitosis per 1000 counts was expressed as the MI.[1]

Blood samples for chemical analysis, obtained from the tail vein, were cooled immediately with ice water

and centrifuged at 2,200 rpm for 20 min. Then the plasma separated was stored at −20°C until measurement of the following parameters with an autoanalyzer (Hitachi-736, Hitachi, Tokyo, Japan)[5]: total protein (TP, Biuret method), albumin (Alb, Bromcrezol green method), glutamic pyruvic transaminase (GPT, Ultraviolet method), total bilirubin (TB Azobilirubin method), creatinine (Cre, Jaffe method), blood urea nitrogen (BUN, Urease ultraviolet method), and total cholesterol (TC, Cholesterol oxidase colorimetric method).

Body composition of fatty parameters, Lee-index [body weight $(g)^{0.33}$/nasoanal lenght (mm)×100] and retroperitoneal white adipose tissue weight (RPWT), were estimated.[6,7]

FK (Fujisawa, Osaka, Japan) at a dose of 0.1 mg/kg/day was given once a day for three days, the administration starting either 2 days (FKI) or 1 day (FKII) before, or on the day (FKIII) of hepatectomy. Each injection was given intramuscularly in a volume of 0.1 ml. The control received saline. Preliminarily, it was noted that saline injection according the three different daily schedules produced no difference in the parameters mentioned above.

The statistical significance of the differences among the values was evaluated by ANOVA and Duncan's multiple range test: $p<0.05$ is defined as a significant difference between the values.

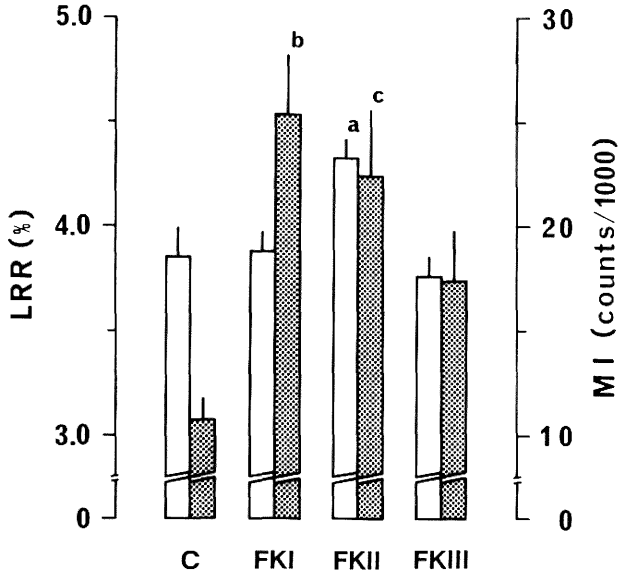

Fig. 1. Liver regeneration caused by differently scheduled administration of FK. FK at a dose of 0.1 mg/kg/day was given for three days starting on three different dates (FKI, FKII and FKIII). The LRR (opened bar) and MI (shaded bar) obtained in the 3 days following hepatectomy are compared. Saline was administered as the control (C). Values are the mean±SEM (n=6). $^{a}p<0.01$ vs C. $^{b}p<0.01$ vs FKIII. $^{c}p<0.01$ vs C.

RESULTS

Figure 1 shows the LRR and MI obtained from the rats treated with FK (0.1 mg/kg) and saline. On comparing the LRR, the LRR obtained from the rats with FKII was found to be higher than that in the rats with FKI or FKIII ($p<0.01$). On the other hand, the MI in the rats with FKI and FKII increased significantly compared to that in the animals with FKIII. No increase in MI was seen following FKIII administration. ANOVA revealed differences of $F_{3,23}=4.395$, $p<0.05$ in the LRR and $F_{3,23}=5.956$, $p<0.05$ in the MI, respectively.

Food and water intake during the 5 days after FK injection are shown in Table 1. FK increased food intake compared to the control, but the amounts of food intake in the rats given FKI, FKII and FKIII did not significantly differ from each other. The rats with FKII had increased water intake, but no significant change in water intake was seen when FKI and FKIII were given. ANOVA revealed a difference of $F_{3,23}=5.388$, $p<0.05$ in food intake and $F_{3,23}=12.644$, $p<0.01$ in water intake.

The results of blood chemistry are shown in Table 2. TP and Alb were not significantly different from each other in the FK treated and control rats. ANOVA showed a difference of $F_{3,23}=0.889$, $p>0.05$ in TP and $F_{3,23}=0.250$, $p>0.05$ in Alb, respectively. GPT increased significantly in the rats with FKI and FKIII compared to the control, but TB did not. ANOVA revealed a difference of $F_{3,23}=57.704$, $p<0.01$ in GPT and $F_{3,23}=0.667$, $p>0.05$ in TB, respectively. Cre and BUN were unaffected by FK administration. ANOVA showed a difference of $F_{3,23}=0.000$, $p>0.05$ in Cre, and $F_{3,23}=2.573$, $p>0.05$ in BUN, respectively. FK had no effect on TC, and the difference in TC was $F_{3,23}=0.444$, $p>0.05$

The Lee-index and RPWT decreased in all groups of rats with FK compared to the control (Table 3). The reduction in the Lee-index and RPWT were unrelated to dose timing. ANOVA brought about a difference of $F_{3,23}=5.867$, $p<0.05$ in the Lee-index, and $F_{3,23}=19.175$, $p<0.01$ in RPWT, respectively.

DISCUSSION

The finding that FK stimulates liver regeneration after partial hepatectomy (Fig. 1) is consistent with the previous report stating that FK increased liver regeneration.[1]

An optimal dose timing of FK has been presumed to exist when heart graft survival was examined with

Table 1. Food and water intake by partially hepatectomized rats following administration of FKI, FKII or FKIII.

	Saline	FKI	FKII	FKIII
Food intake (g)	70.5±5.2	90.5±2.6[a]	81.0±3.5	86.7±3.2[a]
Water intake (ml)	72.3±2.2	75.0±2.6	116.5±9.8[b]	74.6±3.2

Values are the amounts of food and water intake during the 5 days after FK (0.1 mg/kg) administration. Values are the mean±SEM (n=6). [a]$p<0.01$ compared to the saline. [b]$p<0.01$ compared to the FKI.

Table 2. Serum chemical scores in rats with FK administration.

	Saline	FKI	FKII	FKIII
TP (g/dl)	4.5±0.3	4.7±0.1	4.7±0.1	4.9±0.1
Alb (g/dl)	1.5±0.1	1.6±0.1	1.6±0.1	1.6±0.1
GPT(U)	29±4	86±3[a]	30±2	55±5[b]
TB (mg/dl)	0.3±0.1	0.4±0.1	0.2±0.1	0.3±0.1
Cre (mg/dl)	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1
BUN (mg/dl)	11.3±1.8	16.6±1.0	15.8±1.7	15.1±1.2
TC (mg/dl)	72±4	74±4	74±4	78±3

These values were obtained 3 days after partial hepatectomy. Values are the mean±SEM (n=6). [a]$p<0.01$ compared to the FKIII. [b]$p<0.01$ compared to the saline and FKII.

Table 3. Body composition of fatty tissue following FK administration.

	Saline	FKI	FKII	FKIII
Lee-index	316.7±3.2	306.7±1.3*	305.2±2.0*	307.5±1.6*
RPWT (g)	1604.2±74.5	580.7±48.7*	787.2±129.3*	923.6±128.1*

These values were obtained 3 days after hepatectomy. Values are the mean ±SEM (n=6). *$p<0.01$ compared to the saline.

differently scheduled administrations.[8] In this experiment schedule, the rats which received FKII evidently had an increase in the LRR and MI (Fig. 1). A recent study has suggested that the action of FK on liver regeneration is caused by the suppression of T lymphocyte proliferation, based on the finding that liver regeneration induced by FK can be blocked by interleukin (IL) 1α and 2; the immunosuppression of FK is mediated through the inhibition of IL-2 production, and IL-2 induces T lymphocyte activation.[1] Thus, if the time required to complete these T cell activation processes were constant at approximately 3 days, the observed regenerative difference may be derived from the time constancy.

Food intake in all animals treated with FK increased compared to the control (Table 1). This is in keeping with the report that rats can tolerate FK therapy well.[1,9] On the other hand, the finding that water intake increased only when the rats received FKII suggests that the dose timing of FK affects the mechanism related to drinking behavior. Further work will be needed on this.

The fact that FK had no effect on TP or Alb (Table 2) suggested that the nutrient condition of the animals remained normal. Concerning the serum scores indicating liver function, an inhibitory effect was seen when FKI and FKIII were given (Table 2). This could mean that the appearance of hepatotoxicity with FK was dependent on its does timing. Although a previous study reported renal toxicity caused by FK,[11] in the present study Cre and BUN in the rats with FK were unaffected. It is likely that renal dysfunction was not accompanied by FK.

An immunosuppressant, Cyclosporine A, which stimulates liver regeneration,[12,13] has been shown to bring about hypercholesteremia in the blood when used clinically.[4] In this study the cholesterol concentration was unaffected by FK administration (Table 2), but the fatty metabolic parameters in the body were decreased

(Table 3) in spite of the fact that FK failed to reduce food intake (Table 1). It should be remembered that a non-oral supply of nutrients to animals is demanded to maintain the intrinsic fat store.

Because the dose of FK utilized in this study has been shown to be effective in organ transplantation,[14] we can anticipate advantageous applications of FK for liver regeneration and implantation without causing hepatotoxicity when FK is administered in a timely way.

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