1086

# Studies of the Hepatotrophic Qualities of FK 506 and CyA

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COMPENSATORY reaction of liver tissue after damage is a typical hepatic response on which most of modern hepatic surgical therapy relies.[1] Characteristic changes in the rate of cellular proliferation (hyperplasia) and hepatocyte volume (hypertrophy) are observed every time a part of the liver is removed or whenever an impairment occurs in the blood supply to the organ (ischemic damage).

Substances which are able to support the liver in those reparative processes or which can prevent hepatocellular damage from different causes are referred to as *hepatotrophic*. Using this definition, hepatotrophic qualities have been associated with several endogenous substances, including insulin[2,3] and cytosolic extract from regenerating livers.[4,5] More recently, a striking hepatotrophic effect has been demonstrated for CyA.[6]

In this study, the hepatotrophic effects of the new immunosuppressive agent, FK 506, were tested using an experimental model (Eck-fistula in dogs) that allows hepatocyte renewal and hypertrophy to be separated.[7] The results were then compared with those obtained with CyA under the same experimental conditions.[6]

## MATERIALS AND METHODS

A functional end-to-side portacaval shunt (Eck-fistula) was created in 21 adult female beagle dogs weighing 10.5 ± 2.3 kg with the surgical technique previously described[6,7] (Fig 1). Different doses of FK 506 were then continuously infused for 4 days into the left branch of the portal vein. Four group of animals were studied: group 1 (n = 4) received an infusion of the vehicle used to solubilize FK 506 (HCO-60, polyoxyethilated hydrogenated castrol oil and D-mannitol) at the rate equivalent to the FK 506 infusion rate of 0.01 mg/kg/d (two animals) and 1 mg/kg/d (two animals); group 2 (n = 6) received FK 506, 0.01 mg/kg/d for 4 days; group 3 (n = 6) received FK 506, 0.1 mg/kg/d for 4 days; and group 4 (n = 5) received FK 506, 1 mg/kg/d for 4 days.

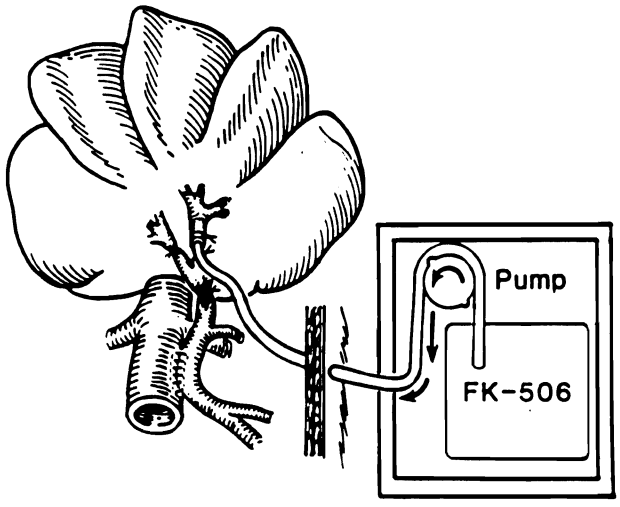


Fig 1. Surgical model of Eck-Fistula (portacaval shunt) with external continuous infusion of CyA and FK 506.

At the end of the infusion period, intravenous [$^3$H]-thymidine was injected 2 hours before biopsy specimens were obtained from left and right lobes of the liver.

As an index of hepatocyte regeneration, the number of mitosis was determined by counting the number of [$^3$H]-thymidine-labeled nuclei per 1,000 hepatocytes. The volume of individual hepatocytes (index of hypertrophy) was determined by determination of hepatocyte cell size units.[2,3]

All data are reported as mean values ± standard deviation (SD). For analysis of variance (ANOVA), the two main populations were control dogs (receiving only the vehicle) and FK 506-treated dogs. An F test value of >3.0 ($P < 0.01$) was considered significant. The comparison between individual experimental groups (differences right/left and CyA/FK 506) was carried out using the Student's $t$ test. A probability of <0.05 was considered to be significant.

## RESULTS

The positive effect of FK 506 on cell renewal and on restoration of the hepatocyte normal size is summarized in Tables 1 and 2.

### Control Animals

The FK 506 vehicle had no significant effect and the hepatocytes suffered the typical changes observed after Eck-fistula.[7] There were slightly more than 4 mitoses/1,000 hepatocytes. The cells were irregular in shape, depleted of glycogen, and shrunken in size. The cell size of the hepatocytes of this group ranged between 0.104 and 0.110. The normal value in dogs is 0.160 ± 0.01 U (Table 2).

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**Table 1. Hepatocyte Mitoses by Autoradiography After Intraportal Continuous Infusion of FK 506 Into the Left Portal Vein of Eck-Fistula Dogs**

| Group No. | No. of Experiments | FK 506 Infusion Rate* (mg/kg/d) | No. of Labeled Hepatocytes Per 1,000 Hepatocytes† (mean ± SD) | | |
|---|---|---|---|---|---|
| | | | Right Lobes | Left Lobes | *P* Value |
| 1 | 4 | 0 (controls)‡ | 4.25 ± 0.33 | 4.47 ± 0.20 | NS |
| 2 | 6 | 0.01 | 4.71 ± 0.63 | 5.46 ± 0.36 | NS |
| 3 | 6 | 0.1 | 5.16 ± 0.44 | 5.98 ± 0.33 | 0.05 |
| 4 | 5 | 1 | 5.48 ± 0.78 | 8.94 ± 0.83 | 0.003 |

*ANOVA (all FK 506 dogs versus controls): F = 12.0325, *P* = 0.0004.
†In normal dogs, the mitoses in left and right lobes are 1.6 ± 0.4.[2,3,6] The increase in mitoses caused by Eck-fistula is known to be significant.
‡Control dogs received only the vehicle of FK 506 (see text).

**Table 2. Hepatocyte Size After Intraportal Continuous Infusion of CyA in Eck-Fistula Dogs**

| Group No. | No. of Experiments | FK 506 Infusion Rate* (mg/kg/d) | Cell Size Units (Mean ± SD)† | |
|---|---|---|---|---|
| | | | Right Lobes | Left Lobes |
| 1 | 4 | 0 (controls) | 0.104 ± 0.018 | 0.110 ± 0.016 |
| 2 | 6 | 0.01 | 0.103 ± 0.074 | 0.111 ± 0.083 |
| 3 | 6 | 0.1 | 0.113 ± 0.081 | 0.137 ± 0.076 |
| 4 | 5 | 1 | 0.134 ± 0.059 | 0.161 ± 0.011 |

*ANOVA (all FK 506 dogs versus controls): F = 20.034, *P* = 0.001.
†In normal dogs, hepatocyte size units are 0.160 ± 0.01.[2,3,6]

### FK 506 Infusion

A dose response relation was evident in the increase in mitotic index (Table 1) and the hepatocyte size (Table 2) in the FK 506-treated dogs. The increased hepatocyte mitoses as well as the restoration of normal hepatocyte size was most striking in the lobes infused with FK 506, but there was also a spillover effect to the right lobes.

The right (non-infused) lobes had changes similar to, although less than, those in the left (infused) lobes (Tables 1 and 2). The results observed with FK 506 were similar to those previously reported[6] with CyA (Table 3), although FK 506 was infused at one fourth to one sixth of the dose used for CyA. Besides the prevention of cellular atrophy after Eck-fistula, high-dose FK 506 (1 mg/kg/d) was also able to induce a significantly stronger cell renewal than that observed with CyA (Table 3).

## DISCUSSION

In effect, the Eck-fistula model used in the present studies splits the liver into two fragments that differ only by what is infused into one of the two main branches of the portal vein. Therefore, hepatotrophic actions of a given substance can be studied comparing, in the same liver, hepatocytes from the infused (treated) lobes versus hepatocytes from non-infused (control) lobes. With this model, the portal route of drug delivery which follows oral ingestion of CyA or FK 506 was simulated in the left lobes but not in the right lobes.

Hepatic regeneration is augmented by CyA.[8-10] In Eck-fistula dogs, CyA prevents hepatocellular atrophy and restores the normal cellular size and morphology and, in addition, it augments the low-grade hyperplasia characteristic of this model.[6] The effects of FK 506 are much the same. With both drugs there appear to be no harmful consequences of delivering the drugs directly to the liver. In fact, the infused (left) lobes exhibited higher mitotic index and healthier hepatocytes when compared with the control (right) lobes.

There were no statistically significant differences between results obtained with FK 506 and CyA (Table 3), although FK 506 was infused at one fourth to one sixth the dosage used for CyA. Therefore, a stronger hepatotrophic

**Table 3. Comparison Between Hepatotrophic Qualities of FK 506 and CyA***

| Group Comparison | | | | |
|---|---|---|---|---|
| FK 506 (mg/kg/d) | | CyA (mg/kg/d) | Mitosis | Cell Size Units |
| Controls | *v* | Controls | NS | NS |
| 0.01 | *v* | 0.06 | NS | NS |
| 0.1 | *v* | 0.6 | NS | NS |
| 1 | *v* | 4 | <0.003 | NS (normal value restored in both treatments) |

Note: FK 506 has a hepatotrophic effect more potent than CyA on a molar basis. At high infusion rate, FK 506 further stimulates the cell renewal component of liver regeneration.
*Complete data on CyA are reported in reference 6.

effect of FK 506 was present with FK 506 versus CyA on a molar basis.

The hepatotrophic (liver-supporting) qualities of FK 506 are in accord with the clinical observations about the value of this new immunosuppressive agent for salvage of normal liver grafts which were severely damaged by ischemic and immunologic reactions.[11,12]

## REFERENCES

1. Weinbren K, Hadjis NS: In Blumgart LH (ed): Surgery of the Liver and Biliary Tract. London, Churchill Livingstone, 1988, p 49

2. Starzl TE, Francavilla A, Halgrimson CG, et al: Surg Gynecol Obstet 137:179, 1973

3. Starzl TE, Watanabe K, Porter KA, et al: Lancet 1:821, 1976

4. Starzl TE, Jones AF, Terblanche J, et al: Lancet 1:127, 1979

5. Terblanche J, Porter KA, Starzl TE, et al: Surg Gynecol Obstet 151:538, 1980

6. Mazzaferro V, Porter KA, Scotti-Foglieni CL, et al: Surgery (in press)

7. Starzl TE, Porter KA, Francavilla A: Curr Probl Surg 20:688, 1983

8. Makowka L, Svanas G, Esquivel CO, et al: Surg Forum 37:352, 1986

9. Kahn D, Lai HS, Romovacek H, et al: Transplant Proc 20:850, 1988 (suppl 3)

10. Kim YI, Salvini P, Auxilia F, et al: Am J Surg 155:245, 1988

11. Starzl TE, Todo S, Fung J, et al: Lancet 2:1000, 1989

12. Fung J, Todo S, Jain A, et al: Transplant Proc (this issue)