1265

FK 506 Modulates D-Galactosamine–Induced Hepatitis in Rats

H. Farghali, A. Gasbarrini, A.B. Borle, M.A. Nalesnik, A. Francavilla, S. Fagiuoli, T.E. Starzl, and D.H. Van Thiel

THE immunosuppressive agent FK 506 has been investigated for its adverse effects on the liver, kidney, and the pancreas.[1] FK 506, however, has multiple biological effects which may be beneficial for the liver. For instance, pretreatment of rats with FK 506 has been shown to both protect the liver from ischemic injury[2] and to ameliorate the hepatic injury associated with ischemia and reperfusion in rats.[3] Other immunomodulators having different chemical structures have been shown to exhibit similar hepatoprotective effects with different types of hepatic injury.[4–6] The aim of the present study was to assess the effect of FK 506 pretreatment on D-galactosamine–induced hepatitis.

METHODS

Male, adult Sprague-Dawley rats weighing 220 to 270 g were divided into four groups of six to twelve each. Groups 1 and 3 were used as controls and were gavaged with water, while animals in groups 2 and 4 were given 0.3 mg/kg of FK 506 suspended in water orally. Twenty-four hours later, acute hepatic failure was induced by a single intraperitoneal injection of D-galactosamine hydrochloride dissolved in 0.9% NaCl at dose of 2 g/kg for groups 1 and 2 and 2.5 g/kg for groups 3 and 4. All rats were observed for 10 days for survival. Blood was collected from the tail vein, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were estimated at 24, 48, and, when possible, 72 hours after GA treatment. Seventy-two hours after the GA insult, 3 rats from groups 3 and 4 were anaesthesized, and the right lobe of the liver was removed and freeze-clamped with liquid nitrogen for ATP enzymatic analysis. The residual left hepatic lobe was utilized for histopathologic examination after the specimens were fixed in 10% formalin, dehydrated, embedded in paraffin, cut at 5 μm, and stained for histological examination with hematoxylin and eosin. The extent of the liver necrosis was estimated semiquantitatively. The number of frankly necrotic hepatocytes per 10 random high-power microscopic fields (Olympus BH-2 microscope, 400×) was counted. A portion of the frozen liver was ground in liquid nitrogen and 4% (wt/vol) precooled perchloric acid (1 × 5 wt/vol) and then homogenized. The homogenized samples were centrifuged at 4°C at 3000 rpm for 10 minutes. The supernatant was brought to pH 7 using 6 mol/L K_2CO_3 and respun at 4°C at 3000 rpm for 5 minutes. The resultant samples were analyzed for ATP by the Sigma kit (Sigma Chemical Co, St. Louis, Mo). All data are reported as mean ± SEM. The

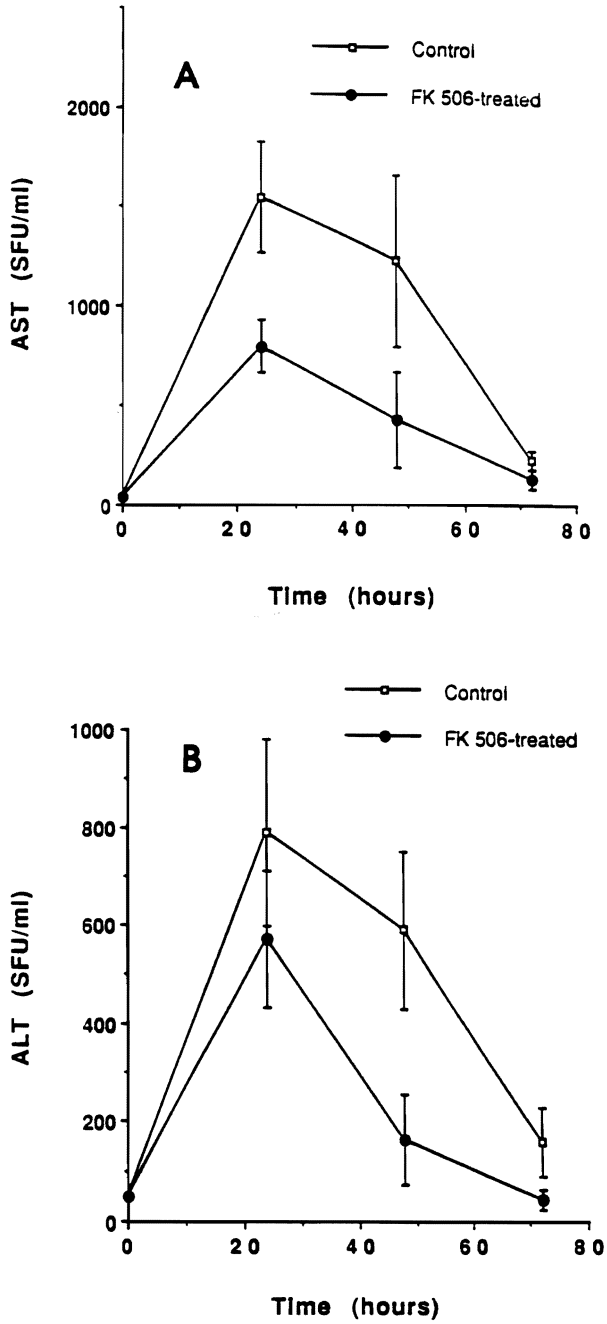
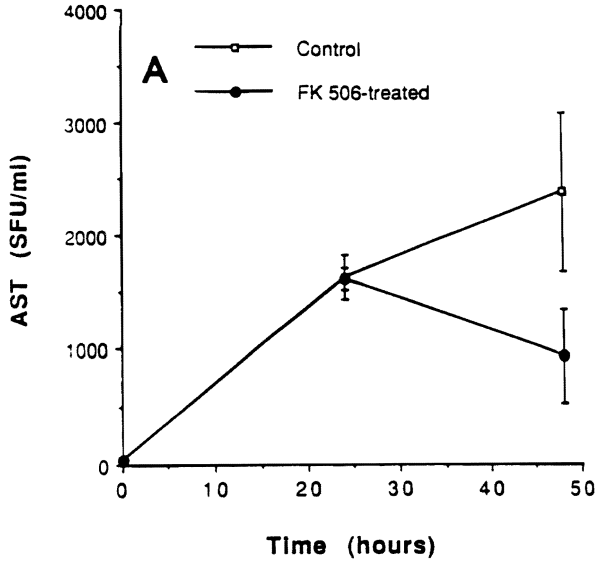


Fig 1. **(A)** Plasma AST (U/L) levels in control rats (GA, 2 g/kg) or FK 506-treated ones (FK 506 + GA) as a function of time after GA; **(B)** plasma ALT (U/L) levels in control rats (GA, 2/kg) or FK 506-treated ones (FK 506 + GA) as a function of time after GA. Points are means ± SEM.

From the Department of Surgery (H.F., A.G., M.A.N., A.F., S.F., T.E.S., D.H.V.T.) and Department of Physiology (A.B.B.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Address reprint requests to D.H. Van Thiel, PhD, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

0041-1345/91/$3.00/+0



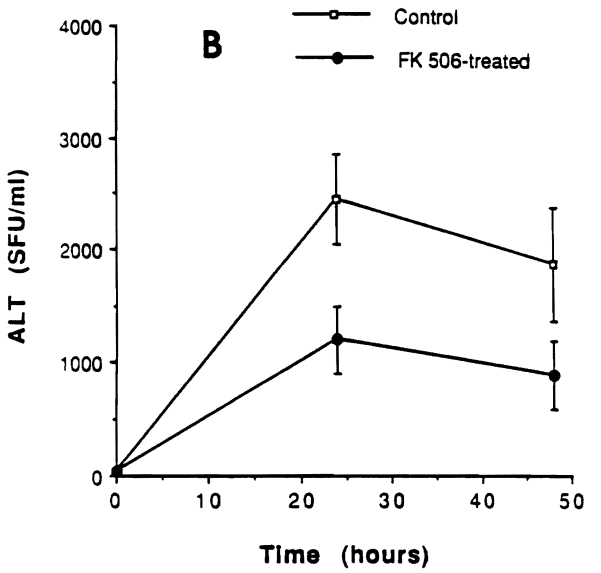


Fig 2. **(A)** Plasma AST (U/L) levels in control rats (GA, 2.5 g/kg) or FK 506-treated ones (FK 506 + GA) as a function of time after GA; **(B)** plasma ALT (U/L) levels in control rats (GA, 2.5 g/kg) or FK 506-treated ones (FK 506 + GA) as a function of time after GA. Points are means ± SEM.

Student's t test was used for statistical analysis. $P < .05$ was considered significant.

RESULTS

FK 506 pretreatment increased the survival of groups 2 and 4, where survival was 90% versus 100% and 50% versus 67% for groups 1, 2, 3, and 4, respectively. The

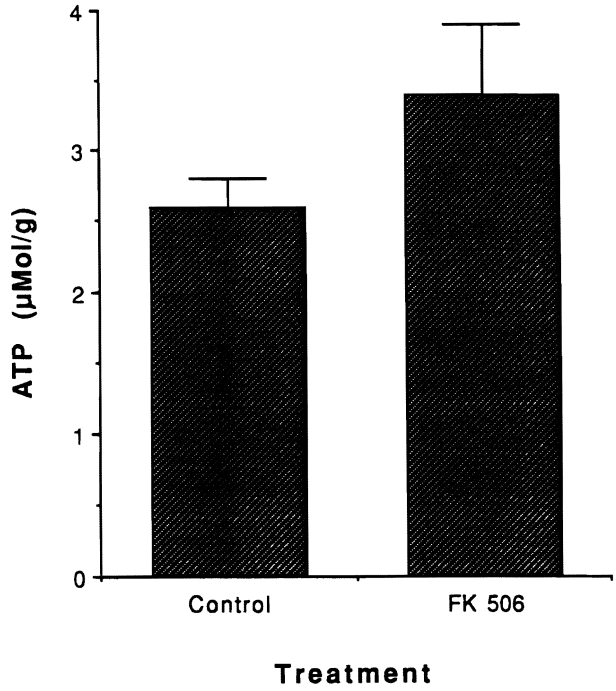


Fig 3. Hepatic ATP contents (μmol/g of liver weight) in control rats (GA-treated) or FK 506-treated ones (FK 506 + GA). Values are means of 3 animals ± SEM.

effect of FK 506 pretreatment on the GA (2 g/kg) induced increases in AST and ALT in groups 1 and 2 is given in Fig 1 and 2, respectively. FK 506 pretreatment was associated with a significant reduction in the plasma levels of AST and ALT at all time intervals tested ($P < .05$). AST and ALT levels for groups 3 and 4 were estimated only after 24 and 48 hours of GA administration, because rats were too ill at 72 hours. Nevertheless, FK 506 pretreatment reduced both the AST and ALT levels at different time intervals as illustrated in Fig 3 and 4, respectively ($P < .05$). Moreover, the ATP levels within the liver of the FK 506-treated rats in group 4 was 3.4 ± 0.5 μmol/L per gram as compared to 2.6 ± 0.2 μmol/L per gram for the controls in group 3 ($P < .01$). The number of frankly necrotic cells in the livers of the FK 506-treated rats of group 4 was significantly reduced ($P < .01$) versus that seen in the control livers of group 3 where they were 27 ± 7.6 versus 61 ± 8.2, respectively.

DISCUSSION

Recently, an interplay between pharmacologic immunomodulation and hepatic function has been shown to exist in several types of hepatic injury, which can be induced experimentally. In recent years, several reports demonstrating a hepatotrophic effect of FK 506 when administered before or immediately after a variety of hepatic injuries have been published.[2,3] The study herein reported was directed to determine the effect of FK 506 pretreat-

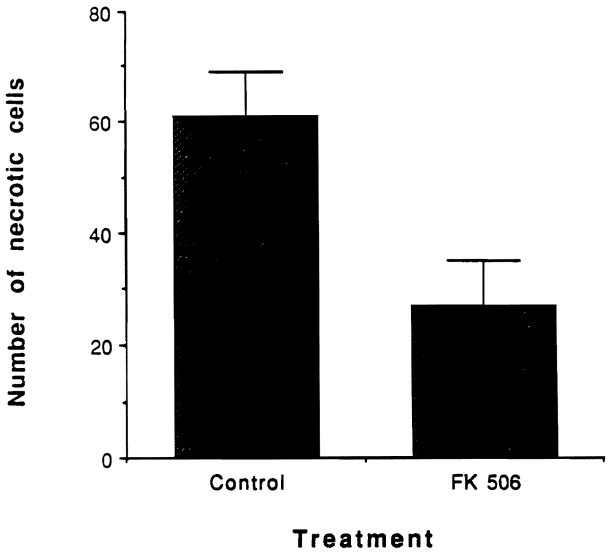


Fig 4. Number of hepatic parenchyma demonstrating necrosis in GA-treated rats (control, 2.5 g/kg) and FK 506-treated ones (FK + GA) 72 hours after GA administration.

ment on yet another type of hepatic injury produced by D-galactosamine (GA). GA is a highly specific hepatotoxin that causes a dose-dependent depletion of uridine metabolites.[7] A hepatoprotective effect of several immunomodulators against chemically induced liver injury has been documented previously.[4–6,8,9] The present data indicate a similar effect of FK 506 pretreatment in modulating the GA-induced hepatotoxicity at both dose levels used. Hepatoprotection was demonstrated by reduced plasma levels of aminotransferases, increased survival, conservation of the energy status of the liver as evidenced by measurement of hepatic ATP levels, and a reduced necrotic index as assessed histologically.

A somewhat similar immunosuppressive agent, cyclosporine, was also a hepatotrophic agent in rats or mice after partial hepatectomy,[10–12] and acts as chemoprotectant against the potent hepatotoxin microcystin, LR.[13] It appears that FK 506 has a similar cytoprotective effect, as it is also protective against various kinds of hepatic insults ranging from surgical to chemical injury, and is shared by other immunomodulators, not all of which are immunosuppressive. The molecular mechanisms responsible for their cytoprotective action are not apparent and deserve further investigation.

REFERENCES

1. Nalesnik MA, Todo S, Murase N, et al: Transplant Proc 19:89, 1987

2. Mazzaferro V, Scotti-Foglieni CL, Porter KA, et al: Transplant Proc 22:93, 1990

3. Sakr MF, Zetti GN, Hassanein TI, et al: Hepatology 13:947, 1991

4. Yoshikawa T, Furukawa Y, Wakamatsu Y, et al: Experientia 38:501, 1982

5. Farghali H, Machkova Z, Kamenikova L, et al: Exp Clin Pharmacol 6:449, 1984

6. Farghali H, Buchar E, Machkova Z, et al: Exp Clin Pharmacol 8:469, 1986

7. Rogers VK, Wu GY: Cancer Chemother Pharmacol 26:93, 1990

8. DeBrabander M, Van Belle H, Aerts F, et al: Int Immunopharmacol 1:93, 1979

9. Farghali H, Buchar E, Jiricika Z, et al: In Kotyk, et al (eds): Highlight of Modern Biochemistry. Zeist, VSP International, 1989, p 1539

10. Kim YI, Caine RY, Nagasue N: Surg Gynecol Obstet 166:317, 1988

11. Kahn D, Lai HS, Romovacek H, et al: Transplant Proc 20:850, 1988

12. Yoshimura S, Kamada N: Transplant Proc 21:911, 1989

13. Hermansky SJ, Casey PJ, Stohs SJ: Toxicol Lett 54:279, 1990