Effect of FK 506 Chronic Administration on Bromosulphthalein Hepatic Excretion in Rats

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THERE are several reports dealing with the hepatic effect of FK 506,[1-4] some of which are beneficial while others are adverse. Because the liver is a major target organ that is often the site of drug toxicity, studies directed at determining whether FK 506 affects specific hepatic function and/or its histologic appearance are important. Therefore, the present study was undertaken to examine bromosulphthalein (BSP) elimination kinetics in rats as a sensitive index of hepatic excretory function under the influence of FK 506 in the rat.

METHODS

Male Sprague-Dawley rats weighing between 200 to 220 g were used. They were divided into three groups (1, 2, and 3) of nine, eight, and eight rats each, respectively.

Group 1 rats served as the controls while groups 2 and 3 were the recipients of 0.4 mg/kg and 0.8 mg/kg FK 506, respectively. FK 506 was administered as a suspension by gavage daily to each animal in groups 2 and 3 while the animals in group 1 were given 0.4 mL/rat of drinking water by gavage. All animals were treated for 5 to 6 weeks. The day after the last dose, the animals were anesthetized with pentobarbitone sodium (50 mg/kg intraperitoneally). After a median laparatomy, the bile duct was cannulated for bile collection using polyethylene tubing (Clay Adams PE-10). The left and right femoral veins were catheterized with polyethylene tubing for administration of BSP and blood sampling, respectively. BSP solution in normal saline was injected as a 5 mg/kg in the left femoral venous PE line. Bile was collected in preweighed Eppendorf polethylene tubes over four successive sampling periods of 20 minutes each, ie, up to 80 minutes. The bile samples were weighed immediately, and kept at −10°C until being analyzed. Blood samples were collected from the right femoral vein at the midpoint of each bile collection interval with the exception of the first blood sample, which was obtained 5 minutes after the IV administration of the BSP. After each blood with-

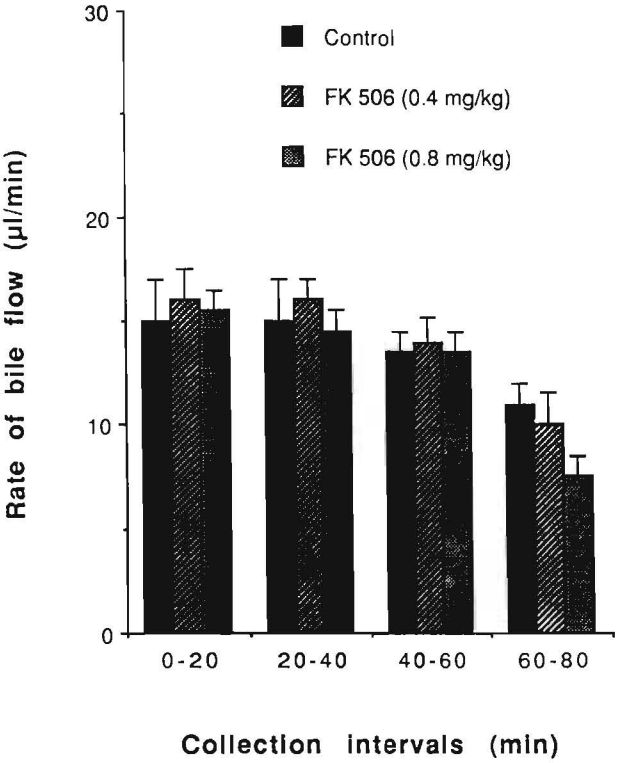

Fig 1. The mean rate of bile flow (μL/min) as a function of collection time intervals in control and FK 506-treated rats at two dose levels for 5 to 6 weeks. Bile was collected for four successive intervals of 20 minutes each (mean of eight to nine ± SEM).

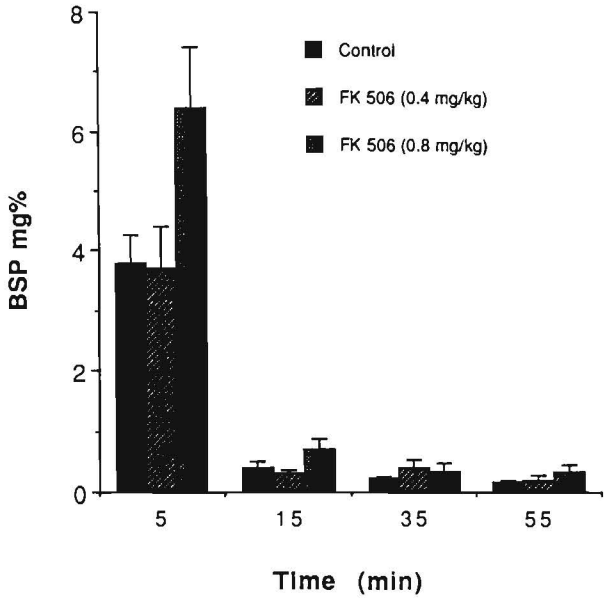

Fig 2. The plasma disappearance profile of BSP after IV administration in control and FK 506-treated rats at two dose levels of 5 to 6 weeks. Values are means of eight to nine plasma samples ± SEM.

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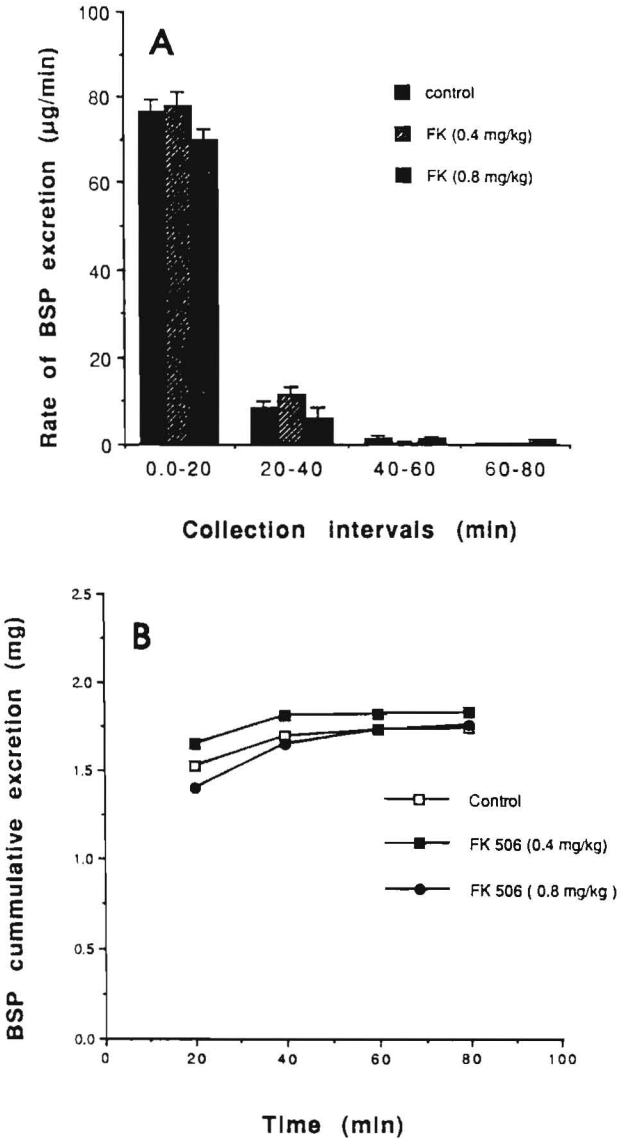

Fig 3. (**A**) The mean rate of BSP excretion (μg/min) as a function of collection intervals in control and FK 506-treated rats at two dose levels for 5 to 6 weeks. Bile was collected for four successive intervals of 20 minutes each. (mean of eight to nine ± SEM). (**B**) The cumulative biliary excretion of BSP as a function of time after IV administration of 5 mg/kg in control and FK 506-treated rats at two dose levels for 5 to 6 weeks.

drawal (0.8 to 1 mL), an equivalent volume of physiologic saline was slowly administered through the left femoral IV line to maintain vascular volume. After the last bile sample was obtained additional blood was withdrawn for enzyme analysis. The concentration of BSP in the plasma and bile was determined spectrophotometrically after an appropriate dilution of the sample with an alkaline buffer containing *p*-toluene sullfonic acid (pH 10.4) at 580 nm with an HP spectrophotometer (nine rats). Plasma aminotransferases levels (alanine transaminase [ALT] and aspartate transaminase [AST]) were determined using standard kits.

RESULTS

The chronic administration of FK 506 at either dose level (0.4 mg/kg or 0.8 mg/kg) did not produce a significant change in the rate of bile flow as compared with that of the controls with the exception of the last sample collected from animals receiving the higher dose of FK 506 (0.8 mg/kg) as shown in Fig 1 ($P < .05$). The plasma disappearance profile of BSP after IV administration in the three groups is illustrated in Fig 2. The rats receiving 0.4 mg/kg of FK 506 exhibited the same plasma concentrations as did the controls at different sampling intervals. The rats that were given the higher FK 506 dose had statistically greater plasma BSP concentrations than did the controls at both the first and last time points (6.4 ± 1.06 and 0.3 ± 11 in the treated group as compared with 3.7 ± 0.77 and 0.2 ± 0.07 in controls, respectively). The rate of biliary BSP excretion was plotted as the amount excreted per 20 minutes as a function of time. Both the control rats and the FK 506 lower dose group had similar rates of BSP excretion (Fig 3A). The BSP biliary excretion rate was highest during the first 20 minutes. The BSP biliary excretion rate for the animals receiving the 0.8 mg/kg of FK 506 dose was reduced at the first time point. The cumulative biliary excretion of BSP as a function of time was similar in all the three groups after 80 minutes, however, as shown in Fig 3B. Nearly the entire BSP dose was excreted by 80 minutes (range 99.5% to 110%).

The values for serum ALT and AST in the three groups of rats are shown in Fig 4. Rats that received the lower dose of FK 506 did not differ from the controls. On the

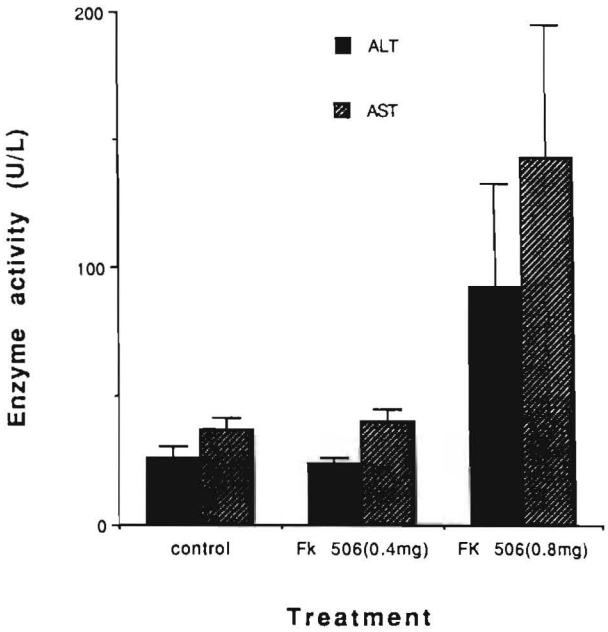

Fig 4. The mean values of plasma ALT and AST in control and FK 506-treated rats at two dose levels for 5 to 6 weeks (mean of eight to nine ± SEM).

other hand, the rats receiving 0.8 mg/kg FK 506 exhibited a threefold and fourfold increase in plasma ALT and AST levels compared with the controls ($P < .05$).

DISCUSSION

There are few available reports that deal with the interaction between FK 506 and the liver,[1-5] especially those concerning effects after multiple dose administration. The present study was designed to shed light on the effect of subacute (5 to 6 weeks) administration of FK 506 at two doses on the biliary excretion of BSP. These experiments demonstrate that, after subacute treatment of rats for 5 to 6 weeks with 0.4 mg/kg, BSP uptake by the liver, bile flow, BSP excretion rate, and total excretion are normal. This is true whether one considers BSP retention at different blood sampling times or BSP rate or cumulative excretion into the bile. The BSP excretion test is a sensitive measurement of liver excretory function.[6] Thus, the present data demonstrate that in rats at the lower dose of FK 506, subacute treatment does not modify hepatic excretory function. Plasma AST and ALT levels, which measure liver cell damage or necrosis,[7] were also not raised with the low dose of FK 506. However, at the higher dose level a significant increase in both enzyme levels occurred. This was accompanied by an initial increase in the plasma value of BSP 5 minutes after IV administration. Moreover, the rate of BSP disappearance from the blood and appearance in the bile was slightly reduced compared with the controls. However, no cholestasis occurred as evidenced by a normal rate of bile flow. In conclusion, within the present experimental limitation, subchronic administration of FK 506 at a dose of 0.4 mg/kg did not produce a deleterious effect on hepatic excretory function. The higher dose produced a slight inhibition of hepatic excretory function and moderately increased the plasma aminotransferases levels.

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