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In Vivo and In Vitro Effect of FK 506 on Rat Leydig Cell Function

J. Tai, W.J. Tze, N. Murase, A. Tzakis, and T.E. Starzl

FK 506 is a macrolide antibiotic with powerful immunosuppressive activities. It is known to prevent allograft rejection in rats, dogs, monkeys, baboons, and humans.[1,2] In vitro studies indicate that, like cyclosporine (CyA), FK 506 acts specifically by inhibiting the transcription of a limited set of early T-cell activation genes, including those encoding interleukin-2 (IL-2), IL-3, IL-4, and interferon-gamma (IFN-γ).[3] There are reports indicating that CyA impairs testicular steroidogenesis and spermatogenesis in rats.[4,5] The immunosuppressive effect of FK 506 is many times more potent than that of CyA and to date there are no published studies concerning toxicity of FK 506 on testicular functions. The present study was undertaken to assess the in vivo and in vitro effects of FK 506 on rat Leydig cell function.

MATERIALS AND METHODS

Animals and FK 506 Treatment Protocol

Adult male ACI rats (Harlan Sprague Dawley Inc, Indianapolis, Ind) weighing 225 to 300 g were used for all experiments. The animals were provided with rat chow and water ad libitum. An injectable form of FK 506 (Fujisawa Pharmaceutical Co) was prepared daily in saline and given IM at 1 mg/kg/d or 2 mg/kg/d for 14 consecutive days to ACI rats. The control animals received saline IM.

Assessment of Testicular Function In Vivo

FK 506-treated and control rats were given a subcutaneous injection of human chorionic gonadotropin (hCG) (Sigma Chemical Co, St Louis, Mo; cat. CG10) at 300 IU/250 g body weight. Blood samples collected at 0, 4, 8, and 24 hours were measured for total testosterone levels by radioimmunoassay (TKTT2, Diagnostic Products, Calif).[6]

Preparation of Dispersed Leydig Cells

Dispersed cells from testes were prepared with a modification of the protocol of Simpson et al with 0.2 mg/mL purified collagenase (Type XI, Sigma).[7] The purity of Leydig cells in the preparation was regularly over 85%, as determined by specific staining for 3β-HSD activity.[8] Cellular viability was >90% by trypan blue dye exclusion test. Cellular viability in each preparation was also determined during and at the end of in vitro incubation period in the presence of varying concentrations of FK 506.

In Vitro Culture of Leydig Cells With FK 506

Stock solution of pure FK 506 (1 mg/mL) (Lot 011050L, Fujisawa) was prepared in absolute ethanol and then diluted in DME nutrient mixture F-12 Ham medium supplemented with 20% newborn calf serum. 0.5 million/mL Leydig cells were incubated in 10 × 75-mm sterile polystyrene culture tubes in concentrations of FK 506 ranging from 10 to 10,000 ng/mL. Leydig cells were incubated for between 15 to 17 hours in a 5% CO_2 incubator at 34°C. At the end of the incubation period, the tubes were shaken to mix the medium and then centrifuged at 600*g* for 7 minutes at 4°C. The supernatant was saved at −20°C for testosterone assay. The cells were then washed two times with culture medium followed by an additional 3 hours of incubation at 34°C. The culture media were saved for testosterone assay.

Data were analyzed statistically by Student's *t* test with the level of significance set at $P < .05$.

RESULTS

Figure 1 shows the serum testosterone levels in response to hCG stimulation in normal ACI rats before, immediately after, and 4 weeks after a course of 14 daily IM injections of FK 506 at 1 mg/kg/d. Though the basal testosterone levels in ACI rats treated with FK 506 were lower than that before the FK 506 treatment, the values were not significantly different. The rats that received a course of FK 506

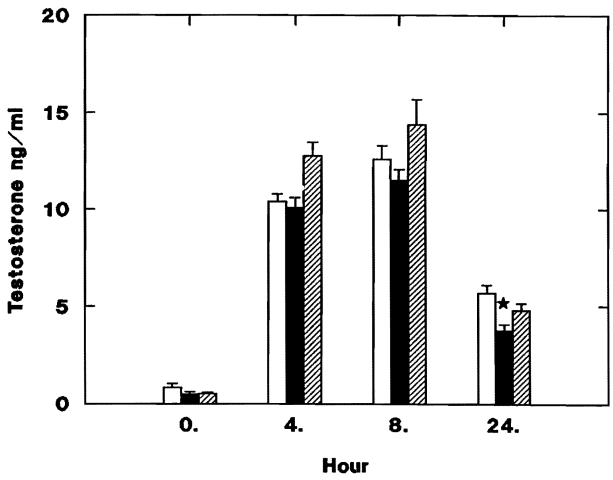

Fig 1. Effect of FK 506 on serum testosterone levels (mean ± SEM) in response to hCG stimulation (300 IU/250 g body weight subcutaneously) in ACI rats before (□), immediately after (■), and 4 weeks after (▨) a course of 14 IM injections of FK 506 at 1 mg/kg/d. Data represent combined results from two separate experiments (n = 12 to 18). *$P < .05$ compared with the control group.

From the Departments of Pathology and Pediatrics, University of British Columbia, Vancouver, BC, Department of Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania.

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Address reprint requests to J. Tai, PhD, c/o Research Center, Room 218B, 950 West 28th Avenue, Vancouver, BC Canada V5Z 4H4.

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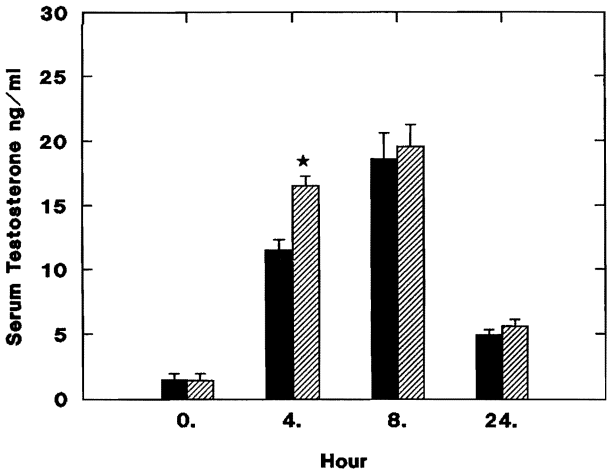

Fig 2. Effect of FK 506 on serum testosterone levels (mean ± SEM) in response to hCG stimulation (300 IU/250 g body weight subcutaneously) in normal ACI rats (■; n = 4) and ACI rats treated with FK 506 at 2 mg/kg/d for 2 weeks (▨; n = 8). *$P < .05$ compared with the control group.

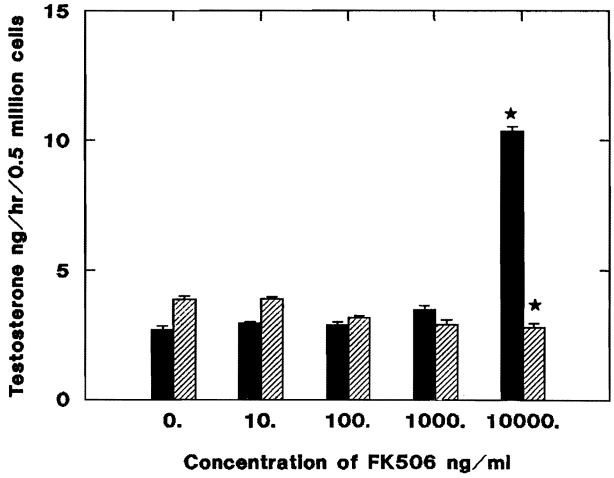

Fig 3. Effect of short-term in vitro treatment of FK 506 on testosterone secretion from ACI rat Leydig cells. The solid bars show the hourly rate of testosterone secretion (mean ± SEM; n = 3) by Leydig cells during the 15 hours of culture in the presence of FK 506 at concentrations varying from 10 to 10,000 ng/mL. The hatched bars show the subsequent rate of testosterone release for an additional 3 hours after the removal of FK 506. *$P < .05$ compared with the control group.

injections showed good response to the hCG stimulation. However, the testosterone levels were significantly lower than the normal controls at 24 hours. The subnormal response was temporary as the testosterone response to hCG stimulation normalized when the animals were retested 4 weeks after the discontinuation of the FK 506 treatment.

Figure 2 shows that 2 weeks of IM treatment with a high dose FK 506 at 2 mg/kg/d did not significantly affect the basal testosterone levels in normal ACI rats. In addition, the testosterone response to hCG stimulation at 8 and 24 hours was not affected. However, the level at 4 hours was significantly higher than in controls.

FK 506 was then tested in vitro on ACI Leydig cells a minimum of three times and since the results were comparable in these experiments only one of them is presented. Figure 3 shows the effect of short-term in vitro treatment of FK 506 in concentrations ranging from 10 to 10,000 ng/mL on testosterone secretion from purified ACI rat Leydig cells. Overnight culture of Leydig cells in the presence of FK 506 up to 1,000 ng/mL did not inhibit the rate of their testosterone secretion. At 10,000 ng/mL, FK 506 showed stimulatory effect on the secretion of testosterone from these cells. The testosterone secretion rates after FK 506 removal were similar in the FK 506-treated cells and the controls except at 10,000 ng/mL samples of which showed significantly lower secretion rate.

DISCUSSION

FK 506 is a novel, potent immunosuppressive agent whose application in experimental and clinical organ transplantations has resulted in markedly improved graft survival.[1,2] Its biologic action is considered to be similar to CyA. CyA has been shown to have a deleterious effect on the male reproductive function of rats[4,5,9] but the effects of FK 506 on steroidogenesis of Leydig cells is not known. The result of this study demonstrated that a regimen of IM injections of FK 506 for 14 days at 1 or 2 mg/kg/d did not significantly alter the basal testosterone level and the testosterone response to hCG stimulation in young adult rats. These two FK 506 treatment regimens were chosen because they have been shown to be effective in the prolongation of allografts across major histocompatibility barrier.[10]

In vitro culture studies were performed to examine the effect of FK 506 on Leydig cell function without involving extragonadal influences that occur in the intact animal. The results indicate that short-term in vitro FK 506 treatment at a dosage range equal or less than 1,000 ng/mL does not appear to have any direct inhibitory effect on testosterone biosynthesis in ACI rat Leydig cells. The Leydig cells cultured in the presence of FK 506 also appeared to be normal morphologically. The dosage of FK 506 used exceeded the plasma concentrations of FK 506 of ≈7 and 100 ng/mL, respectively, following daily IM administration of 1 or 10 mg/kg/d to rats.[11] Whether longer in vitro treatment with FK 506 will affect the steroidogenesis of Leydig cells remains unclear until the problem to maintain consistent testosterone secretion from Leydig cells for more than 3 days in vitro is resolved.[12]

In conclusion, short-term treatment of FK 506 at effective immunosuppressive dosages did not affect Leydig cell steroidogenesis in young adult ACI rats. Short-term in vitro culture of Leydig cells in FK 506 did not depress their testosterone secretion nor reduce their viability. Since most organ transplant recipients remain on immunosup-

pression for life, further investigation using FK 506 treatment for longer duration will be necessary.

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