

1308

# EFFECTS ON PANCREATIC FUNCTION

## Effect of FK 506 on Glucose Metabolism and Insulin Secretion in Normal Rats

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**F**K 506 is a powerful immunosuppressant currently being investigated in clinical transplantation.<sup>1</sup> In vivo administration of this drug resulted in the prolongation of skin, liver, kidney, and heart allografts in large and small animal models.<sup>2,3</sup> The immunosuppressive effect has also been shown recently to be effective in the prevention of development of diabetes in BB rats.<sup>4</sup> In baboons, treatment with FK 506 to prolong kidney allograft survival resulted in hyperglycemia in recipient animals.<sup>5</sup> In vitro, it has been demonstrated that FK 506 does not affect insulin secretion from rat and human islets.<sup>6,7</sup> Such observations suggest that the effect of FK 506 on pancreatic islet cells needs closer examination. The present study was undertaken to assess the in vivo effects of FK 506 treatment on glucose metabolism and insulin secretion by the  $\beta$  cells in normal rats.

### MATERIALS AND METHODS

Inbred male ACI rats (Harlan Sprague Dawley Inc, Indianapolis, Ind) weighing 200 to 300 g were used for all experiments. The animals were provided with rat chow and water ad libitum. Injectable form of FK 506 (Fujisawa Pharmaceutical Co, Tokyo, Japan) was prepared daily in saline and given intramuscularly (IM) at 1 mg/kg per day for 14 consecutive days in ACI rats. The control animals received saline IM.

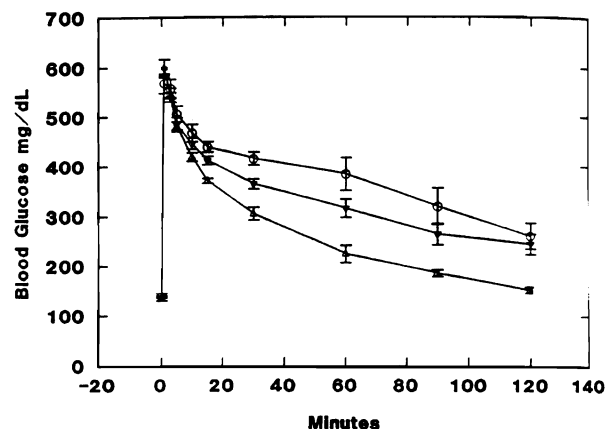
A standard intravenous (IV) glucose tolerance test (IVGTT 1 g/kg body weight of 25% dextrose) was performed in overnight fasted animals prior to, at 1 week during, and immediately after the 2-week course of FK 506 treatment and following discontinuation of FK 506 injections at 2, 4, and 8 weeks. Blood was taken at 0; 1, 3, 5, 10, 15, 30, 45, 60, 90, and 120 minutes for blood glucose (Beckman Glucose Analyzer, Beckman Instruments) and insulin (Pharmacia RIA Kit, Montreal) determination. In addition, an oral glucose tolerance test (OGTT, 2 g/kg body weight of 20% dextrose) was also performed 1 day after the IVGTT in some animals. Blood was taken at 0, 15, 30, 60, 90, and 120 minutes for glucose determination. Rat insulin standard was a generous gift from E. Lilly Co, Indianapolis, Ind.

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

### RESULTS

Fig 1 shows that the glucose tolerance curve in normal ACI rats became abnormal after 1 week of FK 506 injections, and worsened after the second week of injections.

Fig 2 shows that there was improvement in glucose



**Fig 1.** Effect of the duration of FK 506 treatment (1 mg/kg per day IM) on glucose tolerance curves following IVGTT (1 g/kg body weight) in overnight-fasted ACI rats ( $\Delta$ — $\Delta$  before treatment,  $n = 14$ , mean  $\pm$  SEM;  $\nabla$ — $\nabla$  1 week,  $n = 8$ ; and  $\circ$ — $\circ$  2 weeks,  $n = 14$  of FK 506).

tolerance by 2 weeks after the stoppage of FK 506 injections and a near normal curve was obtained after 1 month. Four groups of rats were tested showing insignificant variation in results among the groups. The overall result would indicate that it required approximately 1 month for normalization of the glucose tolerance curve following discontinuation of the medication.

Fig 3 shows the OGTT glucose curves in ACI rats treated with 1 mg/kg per day for 2 weeks. The glucose tolerance curve was abnormal following FK 506 treatment in these animals, and it worsened after 2 weeks of discontinuation of medication. However, a normal glucose curve returned 4 weeks after stoppage of FK 506 treatment.

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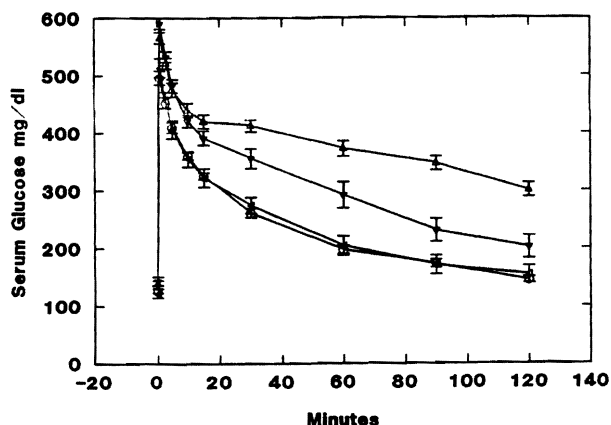


Fig 2. Effect of FK 506 treatment (1 mg/kg per day IM for 2 weeks) on glucose tolerance curves following IVGTT (1 g/kg BW) in overnight-fasted ACI rats (○—○ before treatment,  $n = 35$ , mean  $\pm$  SEM;  $\Delta$ — $\Delta$  2 weeks of FK 506,  $n = 33$ ;  $\nabla$ — $\nabla$  2 weeks,  $n = 14$ ; and  $\triangleleft$ — $\triangleleft$  4 weeks,  $n = 16$ , after discontinuation of FK 506).

Fig 4 summarizes the effect of 2 weeks of FK 506 treatment at 1 mg/kg per day on the insulin profiles in ACI rats following IVGTT. Before FK 506 treatment, two insulin peaks were observed. The initial peak at 1 minute was significantly higher than the second peak at 15 minutes and then the insulin levels gradually declined to above basal level at 2 hours. After the 2-week course of FK 506 treatment, the first insulin peak in response to IVGTT was similar to the controls, while the second peak disappeared. Complete normalization of insulin profile was observed when the animals were retested 8 weeks after discontinuation of FK 506 treatment.

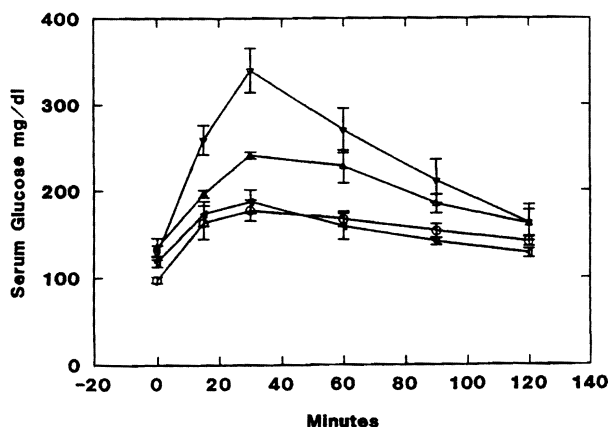


Fig 3. Effect of FK 506 treatment (1 mg/kg per day IM for 2 weeks) on glucose tolerance curves following OGTT (2 g/kg body weight) in overnight-fasted ACI rats (○—○ normal controls,  $n = 24$ , mean  $\pm$  SEM;  $\Delta$ — $\Delta$  2 weeks of FK 506,  $n = 8$ ;  $\nabla$ — $\nabla$  2 weeks; and  $\triangleleft$ — $\triangleleft$  4 weeks after discontinuation of FK 506).

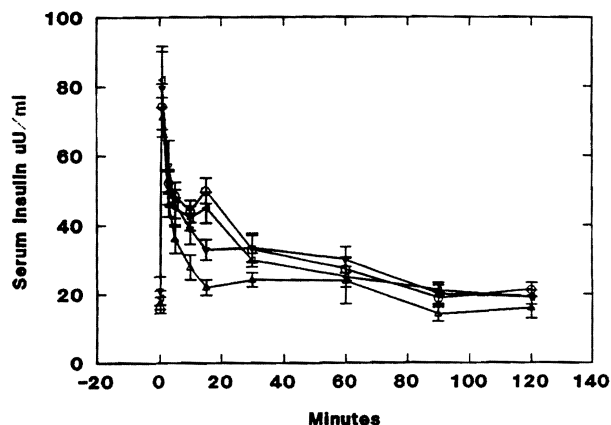


Fig 4. Effect of FK 506 treatment (1 mg/kg per day for 2 weeks) on insulin profiles following IVGTT (1 g/kg body weight) in overnight-fasted ACI rats (○—○ before treatment,  $n = 10$ , mean  $\pm$  SEM;  $\Delta$ — $\Delta$  2 weeks of FK 506,  $n = 9$ ;  $\nabla$ — $\nabla$  4 weeks,  $n = 13$ ; and  $\triangleleft$ — $\triangleleft$  8 weeks,  $n = 8$ , after discontinuation of FK 506).

#### DISCUSSION

FK 506 is a powerful immunosuppressant which has been used in a clinical trial in organ transplantation, and also found to be effective in the prevention of the development of diabetes in BB rats.<sup>1,4</sup> The biological activities of this drug are similar to CyA, and CyA at high doses is known to affect the glucose metabolism of recipients.<sup>8</sup> It was previously reported that rats on oral form of FK 506 between 1 and 4 mg/kg per day for 34 days tend to have high blood glucose levels but no detailed study was conducted.<sup>9</sup> This observed side effect would be more important when FK 506 is used as immunosuppressant in the treatment of newly diagnosed type I diabetics and as an antirejection agent in islet cell transplantation. We presently observed that FK 506 treatment at 1 mg/kg per day for 2 weeks did not significantly affect the basal glucose and insulin levels in the treated ACI rats. However, this treatment with FK 506 altered their glucose tolerance. The derangement of glucose metabolism in the rats treated with FK 506 was not permanent. Glucose profiles following IVGTT were nearly normalized by about 4 weeks after stoppage of the agent, and return to normality was observed when the animals were retested by 8 weeks. The normalization of BG profile preceded that of insulin profile by 2 to 4 weeks. The mechanism underlying the effect of FK 506 on glucose metabolism remains to be determined. The insulin profiles following IVGTT in the treated rats would support the view that the effect was most likely due to decreased insulin release from  $\beta$  cells.

This study confirmed that FK 506, at high doses, can affect glucose metabolism in rats *in vivo*. This would support the *in vitro* observation that FK 506 affected islet insulin synthesis and response to glucose challenge *in vitro* at a high concentration.<sup>6</sup> The FK 506 treatment regimen is currently being used in our laboratory for islet allotrans-

plantation studies. It was found to be effective in the prolongation of allografts across a major histocompatibility barrier.

The present data showed that FK 506 given IM at 1 mg/kg per day for up to 2 weeks can cause temporary deterioration of the glucose metabolism in the recipients. This effect on carbohydrate metabolism is reversible and will not limit the value of FK 506 in transplantation.

#### REFERENCES

1. Starzl TE, Todo S, Fung J, et al: Lancet ii:1000, 1989
2. Thomson AW: Immunol Today 10:6, 1989
3. Murase N, Kim DG, Todo S, et al: Transplant Proc 22(suppl 1):74, 1990
4. Murase N, Lieberman I, Nalesnik MA, et al: Diabetes 39:1584, 1990
5. Thiru S, Collier DSTJ, Calne R: Transplant Proc 19(suppl 6):98, 1987
6. Tze WJ, Tai J, Cheung S: Transplantation 49:1172, 1990
7. Leonard DK, Landry AS, Sollinger HW, et al: Diabetes 38(suppl 1):172, 1989
8. Teraoka S, Kawai T, Yamaguchi Y, et al: Transplant Proc 21:2774, 1989
9. Nalesnik MA, Todo S, Murase N, et al: Transplant Proc 19(suppl 6):89, 1987
10. Murase N, Todo S, Lee P-H, et al: Transplant Proc 19(suppl 6):71, 1987