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Effect of FK 506 on Function of Human Islets of Langerhans

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FK 506 is a new immunosuppressive agent that has been used successfully in human organ transplantation.¹ This agent has a favorable profile on pancreatic islets studied *in vitro*²; however, in high concentrations, it may suppress insulin release from both rodent^{3,4} and human islets³ studied *in vitro*. More importantly, a diabetogenic effect of this agent has been reported in man.^{5,6} Since this agent may have potential application in the field of pancreas and islet cell transplantation, we examined the effect of FK 506 on glucose metabolism and glucose-induced insulin release using the model of human islets transplanted into male balb/c nude mice.⁷

METHODS

Human Islet Isolation

Human islets were prepared from cadaveric donor pancreata obtained from multiorgan donors.^{8,9} After *in situ* perfusion of the abdominal aorta with University of Wisconsin (UW) solution, the pancreas was transported on ice. The islets were isolated using the automated method as previously described.^{10,11}

Transplantation

Male balb/c mice ($n = 25$) (16 to 20 g) were made diabetic by a single IV injection of streptozotocin (165 mg/kg). Diabetes was confirmed if random plasma glucose following the injection was >400 mg/dL. Three to 5 days later, each animal received an aliquot of approximately 600 150- μ m human islet equivalents that were transplanted beneath the left renal capsule.⁷

FK 506 (10 mg) was dissolved fresh daily in a mixture of Cremaphor (625 mg) and ethanol (328 mg). This solution was diluted with normal saline to give a final FK 506 concentration of 100 mg/mL.

Seven days after human islet transplantation, the animals were randomly assigned to four groups. Group A ($n = 10$) animals received 7 daily IP injections of 0.5 mL of Cremaphor-ethanol-saline vehicle. Group B ($n = 5$) animals were treated by 7 daily IP injections of 0.3 mg/kg FK 506. Groups C ($n = 5$) and D ($n = 5$) animals received 7 daily injections of 1 and 3 mg/kg FK 506, respectively.

Metabolic Testing

Fifteen days after human islet transplantation, the animals underwent a standard IP glucose tolerance test (IPGTT).¹² Following an overnight fast, the animals were injected with 2 g/kg IP of a 25% glucose solution. Samples were obtained for analysis of plasma glucose and C-peptide at 0, 15, 30, and 60 minutes following the IP injection. C-peptide was measured by standard radioimmunoassay using human C-peptide standards.

Immediately following IPGTT, the animals in groups B, C, and D were killed, and blood was collected for determination of FK 506 levels using an enzyme immunoassay (Fujisawa Pharmaceuticals Co, Osaka, Japan).

Histologic Studies

The kidneys bearing human islet transplants were excised for determination of morphologic integrity of the grafts. In group A (control), a nephrectomy of the kidney bearing the graft was performed to demonstrate that the human islet transplants were responsible for the maintenance of normoglycemia. Histologic integrity of the renal subcapsular graft was determined by hematoxylin-eosin stain and by immunoperoxidase (insulin) stain. Results are expressed as mean \pm SEM, and analysis of significance of difference was assessed by Student's *t* test.

RESULTS

Administration of 0.3 mg/kg per day of FK 506 for 1 week did not produce any significant alteration of glucose disappearance. Animals receiving 1 and 3 mg/kg per day FK 506 had a significant delay in plasma glucose disappearance rate. In addition, 2 of 10 animals in these groups had a fasting glucose >200 mg/dL. The abnormal glucose disappearance rate in group D was associated with impairment of insulin secretion from the engrafted islets. Fifteen minutes after glucose injection, a decreased C-peptide level compared to control group A was observed (1.01 ± 0.25 vs 2.07 ± 0.38) ($P < .03$).

Plasma FK 506 levels were 3.8 ± 1.25 ng/mL (group B), 106.6 ± 2.82 ng/mL (group C), and 303.5 ± 77.7 ng/mL (group D) ($P < .05$).

Histologic studies indicated that human islets were present in the renal subcapsular space of all transplanted animals. In the two animals (1 in group C and 1 in group D) that had fasting hyperglycemia, the β cells appeared degranulated. In the remaining animals, human islets appeared well preserved with no significant difference between FK 506-treated and control animals. Nephrectomy produced a rapid return to the diabetic state, indicating that the transplanted human islets were responsible for maintaining normoglycemia.

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The data indicate that FK 506 did not produce a significant alteration of glucose homeostasis in animals treated with a dose of 0.3 mg/kg per day for 7 days. Nevertheless, prolonged glucose disappearance was observed in the groups that received higher FK 506 doses. These effects appear to be dose dependent, and were observed at serum FK levels that are significantly higher than therapeutic levels achieved in man (0.5 to 3 ng/mL). Furthermore, in the group that received the highest dose of FK 506, a small decrease in insulin secretion was observed.

These findings in vivo confirm the results of previous reports that high concentrations of FK 506 inhibit insulin secretion from rat and human islets in vitro.^{3,4} Both metabolic and histologic findings confirm a lack of significant toxic effects of FK 506 on islets.² Further studies are required to assess if FK 506 has an effect to increase insulin resistance that would explain the diabetogenic effect observed in vivo.

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