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IN VIVO EFFECT OF FK506 ON HUMAN PANCREATIC ISLETS¹

CAMILLO RICORDI,² YIJUN ZENG, RODOLFO ALEJANDRO, ANDREAS TZAKIS,
RAMAN VENKATARAMANAN, JOHN FUNG, DEBORA BEREITER, DANIEL H. MINTZ, AND
THOMAS E. STARZL

The Department of Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania 15213; The Department of Surgery, Institute H. San Raffaele, University of Milan, Milan, Italy 20132; and The Diabetes Research Institute, University of Miami, Miami, Florida 33101

The purpose of this study was to evaluate the in vivo effect of FK506 on human pancreatic islets. Twenty-five nude mice were made diabetic by one intravenous injection of streptozotocin. Approximately 600 islets were administered in the renal subcapsular space 3-5 days following streptozotocin administration. One week after transplantation, the mice were divided in four groups. In group 1, the animals received 1 injection of 0.5 ml of diluent i.p. daily for one week. In groups 2, 3, and 4 the treatments were daily i.p. injection of 0.3, 1, and 3 mg/kg FK506, respectively. After treatment, the functional integrity of the transplanted human islets was tested by measuring the plasma glucose and human C-peptide response to intraperitoneal glucose injection in groups 1 and 4. IPGTT alone was assessed in groups 2 and 3. The results indicate that i.p. administration of

FK506 for one week at a dose 0.3 mg/kg/day did not result in any significant alteration of glucose disappearance and C-peptide response to IPGTT. Higher doses of FK506 produced a significant delay in glucose disappearance in groups 3 and 4, and a significant inhibition of glucose-mediated C-peptide response in group 4.

FK506 has recently joined the growing list of new pharmaceuticals with potent immunosuppressive properties (1). Post-transplant hyperglycemia is a well recognized side effect of most standard immunosuppressive drugs, including prednisone (2) and cyclosporine A (3-5), which are known to be diabetogenic in men. Similar effects have been ascribed to FK506 in both experimental (6, 7) and clinical studies (8). As clinical experience is successfully extended, there will be new applications for the drug besides whole organ transplantation. FK506 has been recently used as a main immunosuppressive agent in clinical trials of islet transplantation (9). In addition, intervention trials of FK506 in the treatment of new-onset type I diabetes mellitus are planned. The aim of this study was to test the in vivo effect of FK506 on human pancreatic islets.

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² Address correspondence to: Dr. Camillo Ricordi, Department of Surgery, University of Pittsburgh, 3610 Fifth Ave., Pittsburgh, PA 15213.

MATERIALS AND METHODS

Human islet isolation. Human islets were prepared from cadaver donor pancreases obtained from multiorgan donors (10, 11). After in situ perfusion of the abdominal aorta with 1500–2000 ml of University of Wisconsin solution (UW) additional 500–1000 ml of UW was infused directly into the liver via the portal vein. Venous hypertension of the pancreas was avoided by venting the portal and/or splenic vein. The pancreas was immersed in UW and packed in ice until the islet isolation began. The islets were isolated as previously described (9, 12). Briefly, after intraductal injection of 350 ml of Hanks' solution containing 2 mg/ml collagenase (Boehringer-Mannheim, type P) the pancreas was loaded into a stainless steel digestion chamber and the islets were separated during a continuous digestion process that lasted 40–50 min (12). The islets were purified by centrifugation on Eurocollins-Ficoll gradients (density = 1.108, 1.096, 1.037) using a COBE 2991 cell separator (COBE Laboratories, Lakewood, CO) (9).

Animals. Male BALB/c nude mice (16–20 g body weight) were used as islet recipients. The animals were made diabetic by one intravenous injection of streptozotocin (165 mg/kg).

Human islet transplantation. Only mice with nonfasting plasma glucose >400 mg/dl were used as recipients of human islets. Each animal received an aliquot of approximately 600 human islets of 150- μ diameter (13) 3–5 days after streptozotocin administration. The islets were transplanted beneath the left renal capsule as previously described (14).

Treatment. FK506 (10 mg) was dissolved in a mixture of cremaphor (625 mg) and ethanol (328 mg). This solution was diluted with normal saline to give a concentration of 100 mg/ml. The saline solution of FK506 was prepared fresh daily for injection.

One week after human islet transplantation the animals were divided in four groups. In group 1 (n=10), the animals received one injection of 0.5 ml of cremaphor-ethanol-saline (without FK506) i.p. daily for one week. In group 2 (n=5) the animals were treated by daily i.p. injection of 0.3 mg/kg FK506, while in groups 3 (n=5) and 4 (n=5) the daily injections were 1 and 3 mg/kg FK506, respectively.

Intraperitoneal glucose tolerance test. At 15 days after islet transplantation, the animals underwent IPGTT (15). The animals were fasted overnight (18 hr) and then injected intraperitoneally with 25% glucose and 0.9% sodium chloride solution (2 g glucose/kg body weight). Blood samples were obtained before (0 min) and 15, 30, and 60 min after glucose injection and analyzed for plasma glucose and human C-peptide levels.

Plasma glucose and human C-peptide determination. Whole-blood (100 μ l) was collected into microhematocrit capillary tubes and placed on ice. Plasma was prepared by centrifugation at 10,000 \times g for 3 min in a microcentrifuge, and plasma glucose was measured with Beckman Glucose Analyzer II (Fullerton, CA). Blood samples for human C-peptide determination were collected as previously described for serum insulin (16). C-peptide was analyzed only in groups 1 and 4. A 20- μ l sample of blood was diluted in 200 μ l of saline, and the serum separated and stored at -20°C until radioimmunoassay. Human C-peptide was assayed by a double-antibody ¹²⁵I radioimmunoassay (17). The human C-peptide standard solutions and the rabbit antihuman C-peptide (serum K6) were obtained from NOVO Biolabs (Danbury, CT). The human mono ¹²⁵I Tyr-C-peptide was a gift from Eli Lilly and Company, Indianapolis, IN.

Determination of FK506 blood levels. Immediately after IPGTT the animals of group 2, 3, and 4 were sacrificed and the blood collected for determination of FK506 levels. The FK506 assay was performed by the modified enzyme immunoassay (18) using a mouse monoclonal anti-FK506 antibody (Fujisawa Pharmaceuticals Co. Ltd, Osaka, Japan).

Histologic studies. The kidneys bearing the human islet transplants were excised for determination of morphologic integrity of the human islets grafts. In group 1 (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 and eosin stain and by immunoperoxidase (insulin) stain.

Statistical analysis. All results are expressed as means \pm SD. ANOVA was used to compare differences between groups, and *P* values are given as measures of statistical significance.

RESULTS

The results are summarized in Tables 1 and 2 and in Figures 1 and 2. Intraperitoneal administration of FK506 for one week at a dose of 0.3 mg/kg/day did not produce any significant alteration of glucose disappearance after intraperitoneal administration of glucose. Higher doses of FK506 produced a significant delay in plasma glucose disappearance. In group 3 and 4, 2 of 10 animals demonstrated hyperglycemia before IPGTT (fasting plasma glucose >200 mg/dl). In these animals, the presence of fasting hyperglycemia despite normal levels of human C-peptide indicated that peripheral insulin resistance could be responsible for the hyperglycemic effect of FK506. Nevertheless, with high-dose treatment insulin secretion appears to be impaired as well. Furthermore, 5 of 10 animals remained hyperglycemic 1 hour after intraperitoneal glucose injection. Human C-peptide levels following IPGTT indicated that abnormal glucose disappearance in group 4 was associated

TABLE 1. Intraperitoneal glucose tolerance test—plasma glucose (mg/dl)*

Group	0'	15'	30'	60'
1	62.5 \pm 12.9	289.8 \pm 77.5	156.6 \pm 110.4	66.7 \pm 16.5
2	65.6 \pm 49.8	296.0 \pm 54.1	114.2 \pm 45.8	70.8 \pm 14.6
3	96.6 \pm 51.2	344.6 \pm 70.2	217.0 \pm 133.8	168.2 \pm 140.1
4	92.8 \pm 90.1	447.0 \pm 64.0	310.8 \pm 198.9	239.6 \pm 204.1

* Plasma glucose levels 0, 15, 30, and 60 min following an intraperitoneal glucose tolerance test performed 15 days after human islet transplantation beneath the renal capsule of diabetic nude mice. Intraperitoneal administration of FK506 for 1 week before the test produced a dose-dependent effect on glucose disappearance.

^b *P*<0.05: 1 vs. 4; 2 vs. 4; 1 vs. 3.

^c *P*<0.05: 1 vs. 4.

TABLE 2. Intraperitoneal glucose tolerance test—human C-peptide (pmol/ml)*

	0	15'	30'	60'	
Group 1 (control)					
1.		0.66	0.99	1.75	2.31
2.		1.54	3.3	4.73	2.09
3.		1.43	2.31	3.52	2.53
4.		1.32	1.98	4.95	2.53
5.		0.66	1.76	1.87	1.98
Mean		1.12	2.07 ^b	3.36	2.24
SD		0.38	0.75	1.36	0.192
Group 4 (1 week of FK506, [3 mg/kg/day])					
1.		1.43	0.88	2.53	2.64
2.		1.21	1.1	1.1	1.98
3.		0.99	0.88	2.53	1.21
4.		1.21	0.77	1.1	1.1
5.		1.65	1.43	2.53	0.66
Mean		1.30	1.01 ^b	1.96	1.52
SD		0.22	0.23	0.70	0.70

* Human C-peptide levels 0, 15, 30, and 60 min following an intraperitoneal glucose tolerance test. At 15 minutes after glucose injection a decrease in human C-peptide levels was observed in animals treated with high doses of FK506 (group 4) that was significantly lower compared with group 1 (control).

^b *P*<0.025.

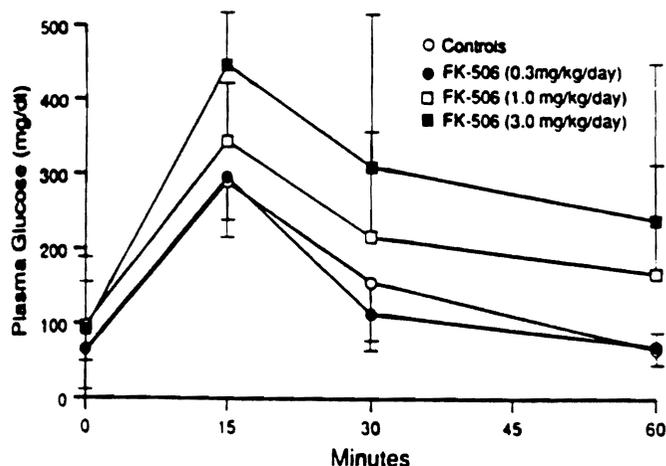


FIGURE 1. Plasma glucose levels during intraperitoneal glucose tolerance test 15 days after human islet transplantation in the renal subcapsular space of diabetic nude mice.

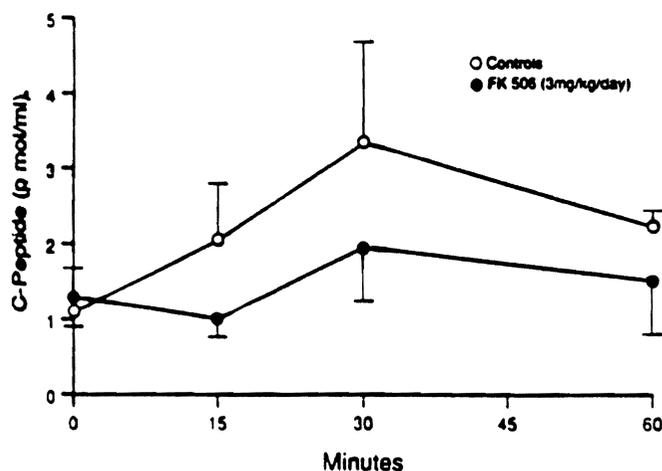


FIGURE 2. Human C-peptide levels during an intraperitoneal glucose tolerance test 15 days after human islet transplantation in the renal subcapsular space of diabetic nude mice.

with an initial impairment of insulin secretion from the engrafted islets. In fact, 15 min after glucose injection a decrease in human C-peptide levels was observed in group 4, in contrast to the control groups, in which a two fold increase in C-peptide was observed. The difference in C-peptide levels between the two groups was statistically significant only at the 15-min level ($P < 0.028$).

The determination of plasma FK levels after IPGTT resulted in an average of 3.8 ± 2.8 ng/ml (group 2), 106.6 ± 6.3 ng/ml (group 3), and 303.5 ± 173.7 ng/ml (group 4).

Histologic studies of the renal subcapsular islets indicated that human islets were present in the renal subcapsular space of all transplanted animals. Nevertheless, in the 2 animals (1 in group 3 and 1 in group 4) who were hyperglycemic, the beta cells appeared degranulated. In the remaining animals the human islets appeared well-preserved, with no significant difference between FK506 treated and control animals. In group 1 (control) a nephrectomy of the kidneys bearing the grafts produced a rapid return to the diabetic state, indicating that the human islets transplanted were responsible for the maintenance of normoglycemia.

DISCUSSION

This study indicates that FK506 did not produce significant alteration of glucose homeostasis in animals treated with a dose of 0.3 mg/kg/day for 7 days. Nevertheless, the effects of the drug on insulin secretion and glucose disappearance after intraperitoneal glucose have been observed at higher doses. These effects appear to be dose-dependent and are observed with serum FK506 levels that are significantly higher than therapeutic levels achieved in man. Although these levels are significantly higher than the therapeutic levels in current use in patients, the potential accumulation during chronic treatment with the agent in the pancreas, like cyclosporine, may induce islet secretory defects even at therapeutic levels. In addition, the pharmacodynamics of FK506 may differ between the two species.

These findings confirm the results of previous reports on the effect FK506 on rat and human islets in vitro (19). Further studies are needed to determine whether the toxic effect of FK506 observed at higher doses is reversible upon discontinuation of treatment.

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