

preservation, and the rapidly expanding recipient pool. Larger series will be required to critically evaluate the use of kidneys from the young donor less than two years old.

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EFFECT OF THE IMMUNOSUPPRESSANT FK506 ON GLUCOSE-INDUCED INSULIN SECRETION FROM ADULT RAT ISLETS OF LANGERHANS

The clinical use of cyclosporine has been a key advance in organ transplantation in the past decade (1). This agent is currently used in patients who receive all types of allografts. Also, CsA has been useful in preventing the onset of insulin-dependent diabetes in animal models (2) and inducing remissions in new-onset type I diabetes in humans (3). Despite the impressive achievements with cyclosporine, nephrotoxicity and graft rejection are still significant problems. Additionally, CsA decreases insulin synthesis and secretion in several species (4-7) and decreases glucose induced insulin secretion from human islets in vitro (8). It has been reported to decrease intravenous glucose tolerance when compared with azathioprine used in the same patient (9). The long-term consequences of these findings continue to be of concern.

FK506 is a new immunosuppressive agent that is more potent on a weight basis than CsA (10). These drugs share many similar effects although the binding proteins for these two agents appear to be different. The binding sites share a novel isomerase enzyme system: peptidyl proline isomerase, which may represent an important common site of action (11). FK506 has recently been used successfully in trials in humans undergoing organ transplantation (12).

In baboons, others have raised the issue of its potential

adverse effect on glucose tolerance (13). Preliminary studies in human recipients of liver allografts showed that 6/12 patients had oral glucose tolerance tests that deviated from normal at at least one time point; 2 patients had impaired glucose tolerance, but only one patient had overt diabetes (14). These findings do not appear to differ from historical controls undergoing liver transplantation who did not receive FK506—however, the question of the effect of this agent on glucose metabolism remains an important unanswered question.

FK506 apparently does not alter morphology or interfere with insulin secretion from fetal islets of Langerhans (15). Glucose-induced insulin secretion in neonatal and fetal islets is not comparable to the adult response (16, 17). Since adult tissues are often used in transplant studies, we looked at the effect of FK506 on glucose-induced insulin secretion from adult rat islets of Langerhans.

We used freshly isolated islets obtained by collagenase digestion of the pancreas, from adult male Wistar rats (18). Insulin was measured by standard radioimmunoassay using a charcoal and dextran separation method (19). After isolation, islets were washed 5 times in basal (2.8 or 5.6 mM) glucose containing buffer.

Acute drug exposure studies. We measured insulin released

EFFECT OF FK 506 ON GLUCOSE INDUCED INSULIN SECRETION

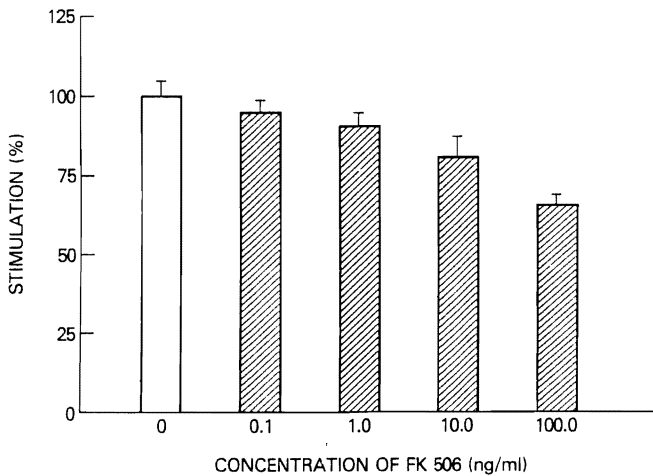


FIGURE 1. The acute effect of FK506 on 16.7 mM glucose-induced insulin release. In this and subsequent figures insulin release was measured at the end of a 90-min incubation in all conditions. Values are expressed as the mean \pm SEM from 36 batches of islets. In this and subsequent figures, 100% secretion corresponds to insulin measured in 16.7 mM glucose after subtraction of basal release. Basal (2.8 mM glucose induced) insulin release was 650 ± 5.8 pg/islet/90 min and stimulated (16.7 mM glucose induced) release was 2208 ± 20.9 pg/islet/90 min ($P < 0.01$). A concentration of 100 ng/ml FK506 produced an average inhibition of 35% ($P < 0.05$). This concentration was inhibitory in every experiment. In one experiment, a concentration of 10 ng/ml was also significantly inhibitory.

ACUTE EFFECT OF COMBINATION CYCLOSPORINE AND FK 506

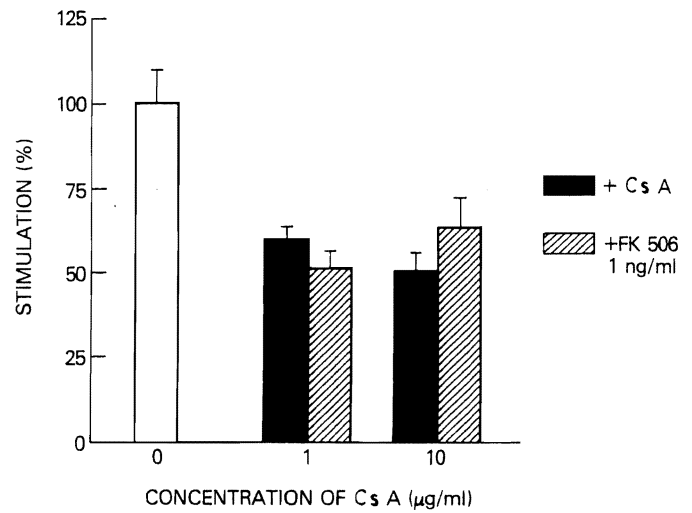


FIGURE 3. The combined effect of CsA and FK506 on glucose-induced insulin release. Results are expressed as the mean \pm SEM from 12 batches of islets. Basal (2.8 mM glucose-induced) insulin release was 268 ± 8.5 pg/islet/90 min, and stimulated (16.7 mM glucose induced) release was 1288 ± 11.4 pg/islet/90 min ($P < 0.01$). CsA produced a 40% and 50% reduction of glucose-induced insulin release at 1 and 10 μ g/ml, respectively ($P < 0.05$). The effects of CsA at either concentration and FK506 (1 ng/ml) were not additive to inhibit insulin release further.

ACUTE EFFECT OF CYCLOSPORINE A ON GLUCOSE INDUCED INSULIN SECRETION

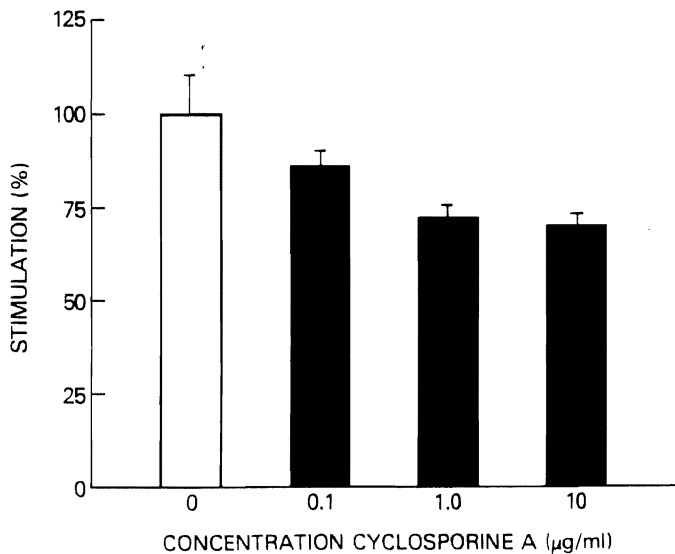


FIGURE 2. The acute effect of CsA on glucose induced insulin release. Values are expressed as the mean \pm SEM from 12 batches of islets. Basal (2.8 mM glucose induced) insulin release was 229 ± 0.93 pg/islet/90 min and stimulated (16.7 mM glucose induced) was 1556 ± 10.2 pg/islet/90 min ($P < 0.01$). CsA at concentrations of 1 and 10 μ g/ml produced about a 30% inhibition of stimulated insulin release ($P < 0.05$).

GLUCOSE INDUCED INSULIN SECRETION AFTER 24 HR. CULTURE WITH FK 506 OR Cs A

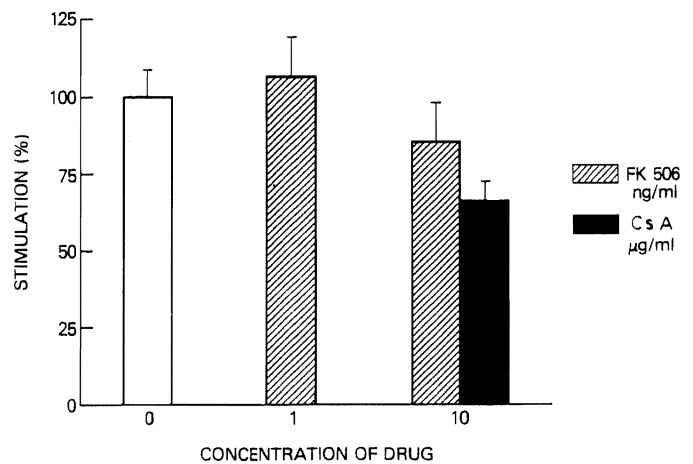


FIGURE 4. Effect of 24-hr incubation with immune suppressant drugs on glucose induced insulin release. After 24-hr culture in the presence of CsA or FK506 in the concentrations shown, the islets were washed $5 \times$ then preincubated in 5.6 mM glucose containing buffer without drug. The medium was removed and insulin release was measured at the end of a 90-min incubation in all conditions. Values are expressed as the mean \pm SEM from 12 batches of islets. Basal (5.6 mM glucose-induced) insulin release was 236 ± 3.4 pg/islet/90 min and stimulated (16.7 mM glucose-induced) release was 1020 ± 6.9 pg/islet/90 min ($P < 0.01$). Previous exposure to CsA produced a 33% inhibition ($P < 0.05$) of glucose-induced insulin release. FK506 did not produce a significant inhibition.

by 2.8 mM or 16.7 mM glucose during a 90-min static incubation of groups of 5–6 freshly isolated islets incubated in the presence or absence of CsA (provided by Sandoz Research Institute East Hanover, NJ), FK506 (provided by Dr. Raman Venkatarmanan, School of Pharmacy University of Pittsburgh), or a combination of these two agents.

Incubation with immunosuppressive agents. For a 24-hour culture period, groups of 5 islets each were transferred to wells containing 1 ml of minimal essential culture medium (Biofluids, Rockville, MD), supplemented with 10% fetal calf serum. The medium was also supplemented with penicillin (100 units/ml) and streptomycin (100 μ g/ml) and were maintained at 37°C in an atmosphere of 5% CO₂. Groups of islets were maintained in the presence or absence of drugs, and after the 24 hr period were washed five times with 5.6 mM drug-free buffer and then preincubated in buffer containing 5.6 mM glucose for 1 hr without drug. After the preincubation, the medium was changed and we measured the insulin released by 5.6 or 16.7 mM glucose. For all experiments, a modified Krebs buffer was used and contained (mM): 120 NaCl, 5 KCl, 25 NaHCO₃, 2.5 CaCl₂, 1.1 MgCl₂, equilibrated with 95% O₂/5% CO₂ and supplemented with 5 mg/ml bovine serum albumin, pH 7.4 at 37°C.

Results are expressed as mean \pm SEM. The statistical significance of the insulin release data were analyzed by Student's *t* test. Data with multiple groups were analyzed using a one way analysis of variance (ANOVA) and the nonparametric Wilcoxon ranked sum test. Conclusions drawn from parametric and nonparametric analyses were the same. We used a stepwise multiple-comparisons procedure to assess dose-response results (20).

In all experiments, glucose (16.7 mM) produced a marked rise in insulin secretion (3–7-fold) compared with basal (2.8 or 5.6 mM) ($P < 0.01$) (data not shown).

Figure 1 summarizes the results of short-term incubations with FK506. Concentrations of 100 ng/ml produced a 35% inhibition of the 16.7 mM glucose-induced insulin release ($P < 0.05$). This concentration produced a significant inhibition in every experiment. In one experiment a 10 ng/ml concentration was also clearly inhibitory ($P < 0.05$).

The results of acute (90-min) incubation with CsA are shown in Figure 2. CsA, at concentrations of 1 and 10 μ g/ml, produced approximately a 30% inhibition of the glucose-induced insulin secretion ($P < 0.05$). These results are similar to those obtained by others in studies using normal rat islets or an insulin-secreting cell line (6).

In animal studies the immune suppressant effects of cyclosporine and FK506 appear to be additive (21). During graft rescue there may be a brief period of exposure to both drugs while being switched to FK506. We therefore looked at the effect of a 90-min exposure to a combination of FK506 and CsA on glucose-induced insulin release. Figure 3 summarizes these results. CsA, 1 and 10 μ g/ml, produced 40 and 50% reductions in glucose induced insulin release, respectively ($P < 0.05$). Addition of 1 ng/ml FK506 did not produce an inhibition in addition to that obtained with cyclosporine alone.

Figure 4 shows the results obtained after a 24-hr incubation in the presence of 10 μ g/ml CsA compared with 1 and 10 ng/ml FK506. After washing the islets with drug-free medium and an additional 1 hr preincubation in without drugs, the cyclosporine-treated islets still showed a 34% decrease in the insulin response to 16.7 mM glucose ($P < 0.05$). The insulin response in the FK506-treated islets was not significantly inhibited.

In summary, the present study shows that FK506 inhibits insulin secretion at the highest concentrations used (10 and 100 ng/ml). The amount of inhibition is similar to that produced by 1 and 10 μ g/ml CsA. Since FK506 is a more potent agent, the serum levels required to suppress the immune response are less than 5 ng/ml. The level of CsA, 1 μ g/ml, that inhibited insulin secretion in our studies is within the range that we see in recipients of kidney, liver, and pancreas transplants who are treated with CsA and steroids. The results of the combination study suggest that the effects of cyclosporine and FK506 on glucose-induced insulin secretion are not additive at lower concentrations of FK506. The results obtained on cultured islets (24 hr) suggest that, at lower concentrations of FK506, there is no inhibition, or that any inhibition of glucose-induced insulin release produced by FK506 is quickly reversible.

When used long-term intramuscularly, FK506 appeared to be diabetogenic in baboons (13). When the immunosuppression was changed at 4 days to an oral regimen, a diabetogenic effect was not observed in baboons undergoing renal transplantation (22) or in cynomolgus monkeys undergoing pancreaticoduodenal allotransplantation (23). FK506 appears to have a considerable steroid-sparing effect (12). In that case, there may be fewer problems with the diabetogenic effect of this agent except when used intravenously or in very high concentrations. In addition, there are important species differences reported with side effects of this agent (24). Since there are potentially important applications of this drug in the field of diabetes, further studies of the long-term effects of FK506 on glucose metabolism in vitro and in vivo in humans are required.

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SUCCESSFUL ORTHOTOPIC LIVER TRANSPLANTATION IN A CHILD WITH LANGERHANS CELL HISTIOCYTOSIS¹

Langerhans cell histiocytosis, previously termed "histiocytosis X," is a rare childhood disorder, characterized histologically by the proliferation of specialized dendritic (Langerhans) cells. Clinical presentation varies from benign localized involvement to fulminant disseminated disease associated with high rates of morbidity and mortality. The latter presentation is seen most often in infants and young children with multisystem involvement and organ dysfunction (hepatic, pulmonary, and hematopoietic) (1).

Children treated successfully for multisystem Langerhans cell histiocytosis (LCH) are at risk for long-term treatment- or disease-related sequelae, including diabetes insipidus, neurologic deficits, pulmonary fibrosis, and biliary cirrhosis (2). The goal of an optimal therapy that would both improve cure rates and decrease the frequency and severity of these late sequelae remains to be realized.

Cirrhosis is a potentially fatal complication of LCH that may arise from histiocytic proliferation in the portal triads, leading to fibrosis, intrahepatic biliary obstruction, and sclerosing cholangitis (3). To our knowledge, there have been no detailed

reports of liver transplantation in patients with irreversible liver damage secondary to LCH (4). We report a case in which an orthotopic transplantation was successful in a child with progressive liver cirrhosis.

A 20-month-old male infant was referred to St. Jude Children's Research Hospital in September 1975 with multisystem disease involving the skin, liver, spleen, lymph nodes, and skeleton. Biopsies of skin and lymph node were diagnostic of Langerhans cell histiocytosis (LCH).^{*} A percutaneous liver biopsy showed portal triaditis with extensive bile duct proliferation and biliary fibrosis but no infiltrates of Langerhans cells (Fig. 1). He was treated initially with prednisone and vinblastine, and later with cyclophosphamide. Because of residual skin disease, methotrexate was administered for 1 year beginning in July 1977. The patient then remained off therapy for 3 years, after which time the disease recurred in the skin and bones.

After methotrexate failed to produce a second complete response, multiple chemotherapeutic agents were tried, including 6-mercaptopurine, cyclophosphamide, vincristine, and chlorambucil. Craniospinal radiation (800 cGy) was given in March

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^{*} Abbreviation: LCH, Langerhans cell histiocytosis.