Toxicity of FK-506 in Human Fetal Pancreas

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The toxic effects of FK-506 (FK), a newly discovered immunosuppressive agent isolated from *Streptomyces tsukubaensis*, were studied in the human fetal pancreas (HFP) system. Human fetal pancreas explants (14–21 wk gestation) were cultured up to 72 h with either 10^{-10} to 10^{-7} M FK or 10^{-8} to 10^{-5} M cyclosporin A (CsA), then examined histologically and functionally with a stimulated insulin-release assay. Additionally, FK and CsA were tested for the ability to suppress cell-mediated immunity with a human mixed-lymphocyte reaction (MLR). Previous studies have demonstrated that near-complete immunosuppression is obtained with FK at 10^{-8} M and CsA at 10^{-6} M. When cultured up to 72 h throughout the concentration range, neither FK nor CsA altered HFP cellular architecture, and indirect immunoperoxidase staining for insulin demonstrated no change in quantity or distribution of insulin-producing β-cells compared with control tissue. Drug-cultured HFP also retained the ability to release insulin in response to a glucose/theophylline challenge. Finally, 50% inhibition of a human MLR was achieved with FK at 2×10^{-9} M and CsA at 2×10^{-7} M, demonstrating the 100-fold greater potency of FK. Our results suggest that further work with FK will show that this immunosuppressive agent can be used with HFP transplantation into diabetic patients without toxicity to HFP. *Diabetes* 38 (Suppl. 1):172–75, 1989

Formerly designated FR 900506, FK-506 (FK) is a newly described immunosuppressive drug isolated in 1984 from the fermentation of *Streptomyces tsukubaensis* strain 9993. It is a member of the macrolide family, has a molecular weight of 822, and is structurally different from cyclosporin A (CsA; 1–3). Early in vitro studies demonstrated potent inhibition of murine and human T-lymphocyte function, including suppression of concanavalin A mitogen stimulation, mixed-lymphocyte reaction (MLR), cytotoxic T-lymphocyte generation, and T-lymphocyte production of interleukin 2 (IL-2) and IL-2 receptor (4). The mechanism of action of FK appears to be similar to that of CsA, but the FK mechanism has ~100-fold greater potency. Reports indicate that near-complete immunosuppression of T-lymphocyte function in vitro is obtained with FK at a concentration of 10^{-8} M and CsA at 10^{-6} M (5,6). Follow-up in vivo studies have demonstrated a similar potent immunosuppressive effect of FK in prolongation of allograft survival for heart transplantations in the rat (7,8) and kidney and liver transplantations in the dog (9–11).

Transplantation of insulin-producing pancreatic islet cells has been investigated over the years in an attempt to reverse the physiological effects of diabetes mellitus. The two major tissue sources receiving most study are isolated adult pancreatic β-cells and human fetal pancreas (HFP) tissue (12). HFP has several advantages as a source of insulin-secreting tissue and offers great potential for practical application. Fetal tissue has a greater proportion of islet tissue than adult tissue, and when transplanted as a free graft, it undergoes selective atrophy of the exocrine component (13). HFP also has the ability to replicate and differentiate with the formation of mature islets. HFP transplantation in the mouse can reverse streptozocin-induced diabetes and provide excellent glucose autoregulatory control (14–17). In addition, a technique has recently been described whereby HFP can undergo indefinite cryopreservation without loss of histologic integrity or endocrine function (K. Bethke, A.S.L., D.K.L., D.A.H., H.W.S., unpublished observations). This technique will allow for HFP tissue banking, with the potential for HLA typing as well as the storage of adequate volumes of tissue needed for transplantation.

In this study, the effects of FK in the HFP model were examined. HFP explants were exposed to FK at varying concentrations and culture time and were subsequently analyzed histologically and functionally via a stimulated insulin-secretion assay. Parallel experiments were performed with CsA. Our results clearly show that FK, at a concentration

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previously shown to be extremely immunosuppressive, does not alter HFP histology or endocrine function when cultured up to 72 h. In addition, we demonstrated that FK has a 100-fold greater immunosuppressive potency in human-derived in vitro MLR compared with CsA. With further clinical development, FK may be a useful immunosuppressive agent for HFP transplantation into diabetic patients.

MATERIALS AND METHODS

HFP. HFP tissue was obtained via suction curettage in the late 2nd trimester (14–21 wk gestation). The tissue was then diced into 1- to 2-mm explants and stored overnight at 4°C in supplemented RPMI medium as described previously (18).

Drugs. FK (lot 011050L) was dissolved in methanol at 1 mM, and CsA (lot 86196.01) was dissolved in 95% ethanol at 1 mM. Both drugs were then diluted in appropriate experimental medium to final concentrations. FK and CsA were generous gifts from Fujisawa (Osaka, Japan) and Sandoz (East Hanover, NJ), respectively.

HFP culture. HFP explants were cultured for varying times in the presence or absence of FK or CsA (FK 10^{-10} to 10^{-7} M; CsA 10^{-8} to 10^{-5} M) at the air-fluid interface, i.e., at 37°C in a 95% air/5% CO_2 humidified atmosphere. Culture medium consisted of Ham's F12 (Gibco, Grand Island, NY) supplemented with 1% fetal calf serum, nonessential amino acids (0.1 mM), dextrose (2.7 mg/ml), transferrin (5 μg/ml), hydrocortisone (1 ng/ml), glutamine (2 mM), insulin 10 μg/ml), penicillin (100 U/ml), and streptomycin (100 μg/ml). Appropriate vehicle controls were included in all groups.

Substrate challenge, insulin-release assay, and radioimmunoassay. After completion of culture, the HFP explants were transferred to fresh petri dishes and exposed to a glucose/theophylline substrate challenge with subsequent measurement of insulin release as previously described (19). Briefly, explants were exposed sequentially to low-glucose buffer (LG; 3 mM), high-glucose buffer (HG; 16.7 mM), and HG supplemented with theophylline (HGT; 10 mM) for 1 h. Insulin release was determined by a standard insulin radioimmunoassay and reported as nanograms insulin released per milligram wet-weight tissue.

Histology. Fresh and cultured HFP tissues were fixed in 10% buffered formalin, dehydrated, embedded in paraffin, and serially sectioned. Samples were stained with hematoxylin and eosin for standard histology and by indirect immunoperoxidase staining (Dako, Santa Barbara, CA; 18) to localize insulin.

Suppression of human MLR. Human peripheral-blood lymphocytes were obtained from heparinized blood of donors with known DR antigen difference with Ficoll-Hypaque (Lymphoprep, Accurate, New York) density centrifugation. Cell culture medium consisted of RPMI-1640 (Gibco) supplemented with 10% heat-inactivated human serum, HEPES (10 mM), glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Responder lymphocytes (10^5) were cultured with an equal number of irradiated (2000 R) stimulator lymphocytes in 0.2 ml culture medium, with or without appropriate drug. FK and CsA were dissolved to 1 mM with methanol and 95% ethanol, respectively, and then were further diluted to appropriate concentrations with culture medium. The cells were incubated at 37°C in a humidified atmosphere of 95% air/5% CO_2 for 7 days. After a 20-h pulsation with [^{3}H]thymidine sp act (2.0 Ci/mM; New England Nuclear, Boston, MA), cultures were harvested on filter paper with a microharvester (Titertek, Skatron, Sterling, VA) and counted with a liquid-scintillation counter. Appropriate vehicle controls were included.

RESULTS

Histology. After HFP culture with FK and CsA, the tissue was examined histologically with either standard hematoxylin and eosin staining or indirect immunoperoxidase staining for insulin. When cultured up to 72 h, the HFP tissue was not altered histologically with FK or CsA at any concentration tested (data not shown). Cellular architecture remained unchanged compared with control tissue, with no structural damage to cellular or stromal elements. Immunoperoxidase staining for insulin demonstrated similar quantity and distribution of insulin-secreting islet cells between FK- and CsA-cultured and control tissue.

Postculture substrate challenge and insulin release. No significant difference in stimulated insulin release was noted between control tissue and tissue cultured 72 h in FK (Table

TABLE 1
Effect of 72-h FK-506 and cyclosporin A culture on functional release of human fetal pancreas insulin

Tissue culture drug (M)	Insulin released (ng/mg wet wt tissue)			Stimulation index (LG/HGT)*
	LG	HG	HGT*	
Control	7.9 ± 1.3	6.5 ± 1.3	38.8 ± 5.6	5.0 ± 1.4
FK-506 vehicle (n = 3)	3.8 ± 1.3	3.9 ± 0.9	24.7 ± 13.2	7.1 ± 4.0
10^{-7}	3.4 ± 0.6	5.0 ± 1.5	38.1 ± 6.4	11.8 ± 3.9
10^{-8}	4.0 ± 1.9	3.9 ± 1.9	30.4 ± 14.5	7.8 ± 2.3
10^{-9}	5.5 ± 0.6	5.5 ± 1.7	45.2 ± 15.8	8.1 ± 1.9
10^{-10}	4.8 ± 0.6	4.9 ± 1.8	39.5 ± 5.6	8.3 ± 0.2
Cyclosporin A vehicle (n = 2)	6.8 ± 3.6	5.4 ± 0.3	67.9 ± 20.7	10.8 ± 2.7
10^{-5}	3.7 ± 0.8	4.3 ± 0.7	29.0 ± 7.4	8.3 ± 3.8
10^{-6}	6.3 ± 2.6	7.5 ± 1.4	58.0 ± 2.1	10.1 ± 3.9
10^{-7}	5.1 ± 0.9	4.4 ± 1.3	47.8 ± 0.6	9.5 ± 1.4
10^{-8}	6.4 ± 2.0	4.9 ± 0.9	47.9 ± 12.7	8.2 ± 4.5

Values are means ± SE. LG, low-glucose buffer; HG, high-glucose buffer; HGT, HG + theophylline (see MATERIALS AND METHODS).
*$P \geq .2$ with Wilcoxon's t test.

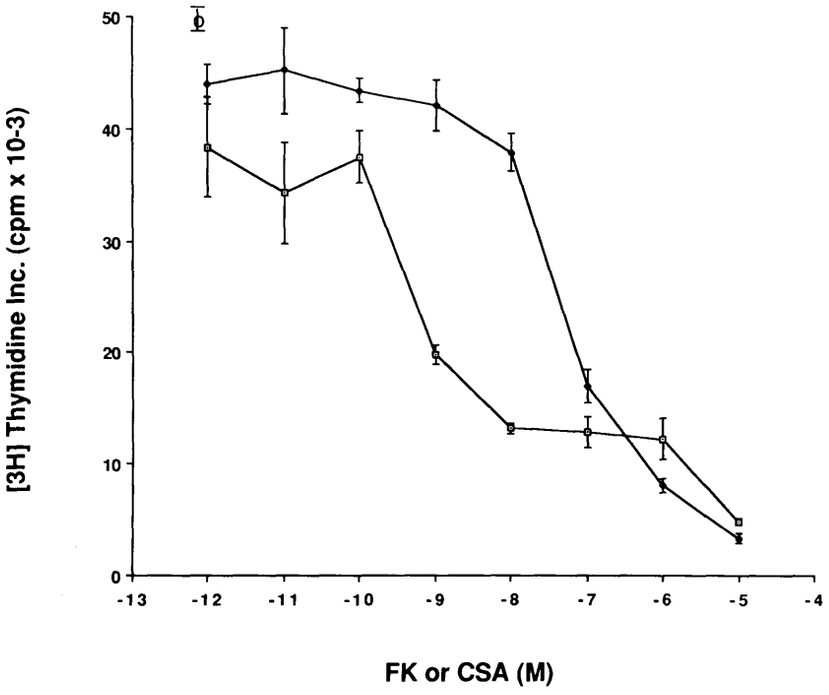

FIG. 1. Dose-dependent mixed-lymphocyte reaction (MLR) inhibition by FK-506 (FK) or cyclosporin A (CsA). Human T-lymphocytes were placed in standard MLR conditions in absence (○) or presence of 10^{-12} to 10^{-5} M FK (□) or CsA (●). [^{3}H]thymidine incorporation was measured on day 7, after a 20-h pulsation.

1). To better illustrate the ability of HFP tissue to functionally respond to theophylline stimulation, a stimulation index (SI) was calculated by dividing the LG response into the HGT response. Throughout the concentration ranges of FK and CsA, 72 h of culture did not significantly decrease the SI below that of control tissue (5.0 ± 1.4). Similar results were observed for 24- and 48-h cultures (data not shown). For both drugs, vehicle alone was without effect.

FK suppression of human MLR. By adding increasing concentrations of FK and CsA to the culture, a dose-dependent inhibition of the MLR was achieved (Fig. 1). Fifty-percent inhibition was detected with 2×10^{-9} M FK and 2×10^{-7} M CsA, demonstrating the 100-fold greater potency of FK over CsA. For both drugs, culture with vehicle alone did not significantly alter proliferation (data not shown).

DISCUSSION

The results presented here represent the first investigation of FK in the HFP model. Our experiments demonstrate that a 72-h HFP culture with FK is not toxic: HFP retains its histological and functional integrity. Both cellular architecture and insulin-producing islet cell distribution are unchanged, and cultured tissue functionally responds to a glucose/theophylline substrate challenge with increased insulin release. These findings support the conclusion that FK at a concentration range of 10^{-10} to 10^{-7} M is not toxic to HFP. Additionally, we were able to reproduce earlier findings that FK is a potent inhibitor of the human in vitro MLR with a 100-fold greater potency than CsA (5).

Early investigation of FK has yielded exciting results. In vitro experimentation has demonstrated FK's potent ability to suppress T-lymphocyte–mediated immune reactions with a mechanism of action directed at the early phase of lymphocyte activation by inhibition of IL-2 and IL-2–receptor activity (4). In vivo transplantation studies have shown marked prolongation of allograft survival in the heart, kidney, and liver organ systems in several species (7–10). These reports, however, have been tempered by studies observing prohibitive toxicity in dogs and baboons (20,21).

CsA has been shown to have deleterious effects on isolated human islet β-cells. At a high concentration (10 μg/ml, 8×10^{-6} M), CsA has been shown to cause severe cytoplasmic vacuolization and degranulation of isolated islet cells and reduction of mRNA synthesis (22). Even at a more clinical concentration (0.1 μg/ml, 10^{-7} M), CsA has been shown to inhibit release of insulin from islet cells despite normal insulin content (23). Our results demonstrate that even at a high CsA concentration (12 μg/ml, 10^{-5} M), HFP is not histologically altered, and insulin release is not decreased. This finding is in marked contrast to the effect of CsA on isolated human islet β-cells.

HFP offers promise to diabetic patients as a source of transplantable insulin-producing tissue. Studies in the nude mouse have demonstrated that after transplantation under the renal capsule, HFP tissue matures into histologically adult islet endocrine structures (19), increases in both absolute weight and total insulin content (16), and can reverse streptozocin-induced diabetes with maintenance of glucose autoregulatory control (17). Both fetal pancreas transplantation and FK require further laboratory and clinical investigation if they are to be utilized in the transplantation arena; however, our results suggest that FK is not toxic to HFP and has potential application in this tissue system.

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