Cyclosporin-Induced Inhibition of Insulin Secretion in Isolated Rat Islets and HIT Cells

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SUMMARY

The effects of cyclosporin, an immunosuppressive agent used in diabetic and nondiabetic patients, on in vitro glucose-induced insulin secretion were evaluated in isolated islets and in a glucose-responsive clonal β-cell line (HIT cells). Cyclosporin inhibited glucose-induced insulin secretion in a dose-response manner at concentrations commonly found in human blood. With isolated islets, four time periods (0–5, 5–15, 15–30, and 30–60 min) were examined after stimulation with 300 mg/dl glucose. Inhibition of insulin secretion by cyclosporin was evident by 5–15 min as well as during later times with progressively smaller drug concentrations. With HIT cells, longer-term effects were examined after 16 h of incubation with various drug concentrations. Inhibition of insulin secretion was again observed, and these inhibitory effects were not reversed by drug washout. A cyclosporin concentration of 0.1 μg/ml, which is a therapeutic blood level in humans, was sufficient to inhibit insulin secretion in both in vitro models. Patients using this drug should be carefully monitored for signs of deficient insulin secretion. DIABETES 1986; 35:1016–19.

Cyclosporin is an 11–amino acid cyclic polypeptide commonly used as an immunosuppressive agent in patients receiving transplanted tissues and organs. Diabetics receive cyclosporin when transplanted with segments of pancreas or isolated pancreatic islets. Cyclosporin is also used in experimental therapy of recently diagnosed type I diabetes in humans to suppress their autoimmune response.[1,2]

Despite its emerging therapeutic role in diabetes mellitus, there is surprisingly little information available about the effects of cyclosporin on insulin secretion. In the published literature there is disagreement about whether cyclosporin has adverse effects on pancreatic islet function and glucose homeostasis.[3–9] Consequently, we examined the short-term effects of cyclosporin on glucose-induced insulin secretion in islets isolated from Sprague-Dawley rats. We also examined longer-term effects of cyclosporin and assessed whether these effects were reversible in a glucose-responsive clonal β-cell line derived from Syrian hamster pancreatic islets (HIT cells).[10,11]

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MATERIALS AND METHODS

Pancreata were surgically excised from Sprague-Dawley rats. Islets were isolated via digestion with collagenase by a modification of the method of Lacy and Kostianovsky[12] and Ficoll gradient.[13] The portions of the gradient containing 23, 20.5, and 11% Ficoll were collected and diluted with the digestion buffer before centrifugation. Individual islets were picked by pipette from the resuspended pellet and placed in a series of 12 × 75-mm culture tubes. These tubes were modified by removing their bottoms and fusing a nylon mesh to the bottom of the tube to allow drainage of buffer from the islets without allowing passage of the islets through the mesh. Twenty-five islets were placed on top of the mesh, and this tube was then placed inside a 16 × 100-mm glass tube containing Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4). Incubations with various concentrations of glucose and cyclosporin in the buffer were performed at 37°C in a water bath. Experiments involved a 2-h preincubation period with 30 mg/dl glucose with and without cyclosporin (0.01–100 μg/ml). After the preincubation period, the islets were washed with buffer and then exposed to buffer containing 300 mg/dl glucose with and without 0.01–100 μg/ml cyclosporin. Cyclosporin solution was obtained from Sandoz (East Hanover, NJ), diluted in saline, and added to the culture media. Thereafter, four time periods were examined: 0–5, 5–15, 15–30, and 30–60 min. At the end of each period, the smaller meshed tube containing the islets was moved out of the larger tube containing the buffer, quickly rinsed, and then placed into a second incubation tube containing the same glucose and cyclosporin concentrations as the pre-

TABLE 1
Inhibition of glucose-induced insulin secretion by cyclosporin in isolated islets

Incubation (min)	Control insulin (μU/ml)	Cyclosporin A (μg/ml)				
		0.01	0.1	1.0	10.0	100
0–5	104 ± 13 (18)	99 ± 8 (9)	101 ± 18 (13)	79 ± 6* (14)	106 ± 23 (5)	101 ± 20 (7)
5–15	230 ± 16 (17)	118 ± 9 (9)	98 ± 9 (13)	92 ± 11 (13)	83 ± 9* (5)	85 ± 3* (7)
15–30	641 ± 45 (16)	97 ± 17 (9)	82 ± 7 (13)	76 ± 15† (15)	63 ± 7‡ (5)	64 ± 11† (7)
30–60	1245 ± 114 (16)	89 ± 7 (9)	71 ± 11* (12)	61 ± 4‡ (15)	58 ± 4† (6)	44 ± 2† (5)

Tubes containing 25 islets were incubated during 4 sequential time periods (see MATERIALS AND METHODS). Control tubes contained glucose, 300 mg/dl, without cyclosporin. Data for cyclosporin-containing tubes are expressed as % control data for appropriate time period. Data expressed as mean ± SE. Numbers in parentheses = number of incubated tubes. *P <.05, †<.01, ‡<.001 compared with control values.

vious tube. Samples were taken from each of the four tubes for determination of insulin secretion during each of the four time periods.

A clonal cell line of glucose-responsive HIT cells were grown in 5% CO_2 and air at 37°C and maintained in RPMI-1640 medium containing 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin.[8,9] Before each experiment was conducted, cells were subcultured in the absence of cyclosporin for 3 days by plating 500,000 cells in 60 × 15-mm culture dishes. On the 4th day the cultures were replenished with fresh incubation medium (containing 200 mg/dl glucose) with and without cyclosporin (0.01–100 μg/ml). Samples were taken from the culture plates 16 h later for determination of insulin concentration. Some control plates and plates containing cyclosporin were washed several times and incubated an additional 16 h to assess insulin secretion after drug washout.

Insulin concentrations were determined by a conventional double-antibody radioimmunoassay modification of the method of Morgan and Lazarow.[14] Statistics were determined with Student's paired t test, comparing cyclosporin-containing tubes and plates to control tubes and plates (not containing cyclosporin).

RESULTS

Isolated islets. The effect of cyclosporin on insulin secretion in the presence of 300 mg/dl glucose was determined in four sequential periods for 1 h (Table 1). Over a cyclosporin concentration range of 0.01–100 μg/ml, no consistent effects on insulin secretion were observed during the 0- to 5-min period. However, during the 5- to 15-min period, significant inhibition of insulin secretion was observed at cyclosporin concentrations of 10 and 100 μg/ml. During the 15- to 30-min period, significant inhibition by cyclosporin was evident with concentrations of 1.0 μg/ml and higher. During the 30- to 60-min period, significant inhibition of insulin secretion was observed at cyclosporin concentrations of ≥0.1 μg/ml (Fig. 1).

HIT cells. Glucose responsiveness of these transformed cells under our experimental conditions was demonstrated by incubating two passages of HIT cells on the 4th day of subculture with various glucose concentrations (0–300 mg/dl) in a Krebs-Ringer phosphate buffer (pH 7.4) containing NaCl (119 mM), KCl (5.94 mM), $CaCl_2$ (2.54 mM), $MgSO_4$ (1.19 mM), KH_2PO_4 (1.19 mM), and Na_2HPO_4 (0.01 M). These incubations were carried out for 1 h and demonstrated stimulation of insulin secretion in a glucose dose-responsive manner (Table 2). When the HIT cells were cultured in media containing 200 mg/dl glucose in the presence and absence of cyclosporin for 16 h, inhibition of glucose-induced insulin secretion was observed in a cyclosporin dose-related manner (Fig. 2). To assess reversibility of this cyclosporin effect within the usual clinical time span of repeated dosage, some of the control and drug-containing plates were washed and then reincubated for 16 h in cyclosporin-free media. Insulin concentrations in the culture plates were determined after this additional incubation. Inhibition of glucose-induced insulin secretion was again observed in a cyclosporin dose-

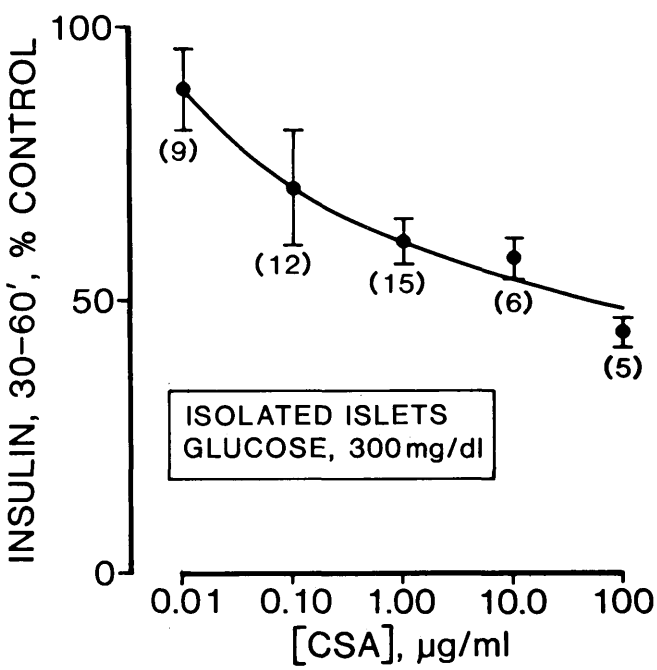

FIG. 1. Inhibitory effect of cyclosporin on glucose-induced insulin secretion by isolated islets. Data correspond to 30- to 60-min period shown in Table 1 and are expressed as % (mean ± SE) of data from control islets not exposed to cyclosporin. Numbers in *parentheses* indicate number of experiments performed with a given drug concentration. Each insulin concentration was determined in duplicate. Insulin concentrations in tubes containing cyclosporin A concentrations of 0.1, 1.0, 10, and 100 μg/ml were significantly less (P values in Table 1) than those in tubes containing no cyclosporin.

TABLE 2
Insulin concentrations (μU/ml) in culture plates after incubating HIT cells with various glucose concentrations for 60 min

	Glucose (mg/dl)					
	0	10	20	100	200	300
#71						
A	315	741	1006	1581	1693	1701
B	277	603	918	1494	2088	1652
C	247	705	1055	1679	2267	1926
#72						
A	132	302	348	515	448	509
B	129	265	368	580	762	477
C	132	331	328	556	508	421

Data are from two separate cell passages (#71 and #72) studied in triplicate. Each insulin value was assayed in duplicate.

related manner in the plates that had been treated with cyclosporin before drug washout (Fig. 2).

DISCUSSION

These studies were performed to assess whether cyclosporin affects glucose-induced insulin secretion in vitro. Isolated islets from Sprague-Dawley rats and HIT cells were used. In both the isolated islets and the HIT cells, cyclosporin inhibited glucose-induced insulin secretion in a cyclosporin dose-responsive manner. Inhibition of insulin secretion was observed in islets and HIT cells with drug concentrations of ≥0.1 μg/ml. To determine whether this inhibitory effect of cyclosporin was reversible, HIT cell cultures that had been exposed to cyclosporin for 16 h were washed and reincubated with glucose but without drug for an additional 16 h. Suppression of insulin secretion persisted. There was no indication of cell death by microscopic visualization or trypan blue dye exclusion.

In the only other publication found dealing with effects of cyclosporin on insulin secretion in isolated rat islets, Laube and Hahn[7] reported that cyclosporin concentrations up to 2 μg/ml did not reduce glucose-induced secretion from rat islets incubated for 60 or 180 min. The reason for the discrepancy between our results is not clear. However, it may be significant that we preincubated the isolated islets for 2 h before stimulation with high concentrations of glucose. In the article by Laube and Hahn it appears that cyclosporin and stimulatory concentrations of glucose were added simultaneously. Using isolated mouse islets cultured for 7 days, Andersson et al.[6] observed inhibition of insulin secretion by cyclosporin in the presence of high glucose concentrations. The inhibitory effects were observed at a drug concentration of 1.0 μg/ml after 2 days of incubation. Our data provide evidence that this effect can occur as early as 5–10 min after glucose stimulation of the β-cell and that inhibition can occur at drug concentrations as low as 0.1 μg/ml. More recently, Yale et al.[8] have reported diminished glucose tolerance and decreased 60-min insulin values after glucose ingestion in rats receiving cyclosporin.

The inhibitory effect of cyclosporin on pancreatic β-cell function raises questions regarding possible adverse effects of cyclosporin on insulin secretion in patients receiving this drug.[1–5] If this in vitro effect also occurs in vivo, it is of particular interest to diabetes mellitus research, because patients receiving pancreatic transplants commonly receive cyclosporin therapy. Moreover, some individuals with type I diabetes mellitus are being treated experimentally with both cyclosporin and insulin.[1,2] The cyclosporin trough level, normally sought 12 h after ingestion of the drug, is ~0.200 μg/ml. Our isolated-islet and HIT cell data suggest that this concentration of cyclosporin might paradoxically inhibit glucose-induced insulin secretion while benefiting the patient through its immunosuppressive actions. Peak levels of cyclosporin can be anticipated to be ≥0.200 μg/ml. In this regard, our data and those of others indicate that with progressively greater concentrations of cyclosporin, there is more efficacious inhibition of insulin secretion.[6,8,9] The drug-washout experiments in the HIT cells indicate that washing the cultures does not remove the inhibitory effect of cyclosporin. This raises the possibility that the drug may continually affect β-cells if it is given in the conventional time interval of once every 12 h. This is clinically relevant because it is not uncommon for patients to use cyclosporin therapy for years, allowing the possibility that time-related exposure to cyclosporin may result in a cumulative toxic effect on pancreatic islet function. The mechanism of this inhibitory effect on insulin secretion is not fully identified, but Andersson et al.[6] reported inhibition of DNA synthesis as a likely mechanism, and Helmchen et al.[9] reported severe degranulation of β-cells.[9]

Note that there are no data yet available from well-controlled human studies evaluating effects of cyclosporin on insulin secretion in nondiabetics and that it has not yet been determined whether this drug is deleterious to normal β-cell function after prolonged use. We are currently closely monitoring secretion of insulin and C peptide as well as carbohydrate tolerance in a group of nondiabetic multiple sclerosis patients on a randomized double-blind 2-yr program of cy-

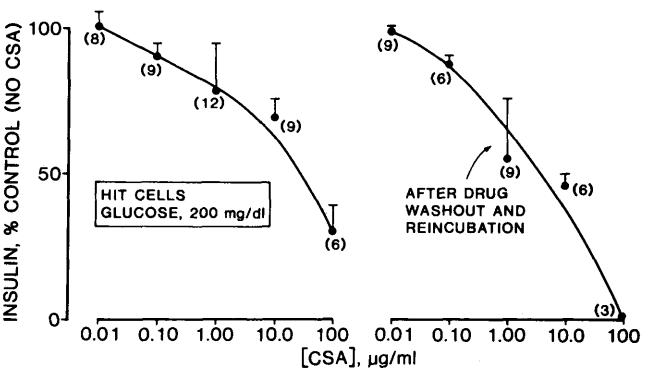

FIG. 2. Inhibitory effect of cyclosporin on glucose-induced insulin secretion by HIT cells. *Left panel* depicts data from experiments involving 16-h incubations in glucose, 200 mg/dl, with and without cyclosporin. *Right panel* depicts data from cultures that underwent subsequent 16-h incubation in glucose but no cyclosporin after drug washout period. Despite washout, inhibition persists. Data expressed as means ± SE % of control data from culture plates containing glucose but no cyclosporin. Numbers in *parentheses* indicate number of experiments performed with a given drug concentration. Each insulin concentration was determined in duplicate. Insulin concentrations in plates containing cyclosporin A concentrations of 10 and 100 μg/ml were significantly less ($P < .01$) than those in plates containing no cyclosporin. After drug washout and reincubation, insulin levels in plates that had contained cyclosporin concentrations of 1, 10, and 100 μg/ml were significantly less ($P < .01$) than those in plates without cyclosporin.

closporin or placebo therapy to ascertain whether the in vitro inhibitory effect of the drug has in vivo relevance to humans.

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