Cyclosporin A Does Not Delay Insulin Dependency in Asymptomatic IDDM Patients

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OBJECTIVE — To measure the effects of cyclosporin A (CyA) with no insulin therapy on glucose tolerance and β-cell function in the preclinical phase of insulin-dependent diabetes mellitus (IDDM).

RESEARCH DESIGN AND METHODS — β-cell responses to the intravenous glucose tolerance test (IVGTT), hyperglycemic clamp, intravenous arginine, and intravenous glucagon were evaluated before and after a 6-month course of CyA in seven patients (mean age 19.6 years) with asymptomatic IDDM.

RESULTS — Initial insulin secretory responses were severely decreased when the patients were compared with eight healthy control subjects: IVGTT (1 + 3 min): 106 ± 16 vs. 884 ± 190 pmol/l ($P < 0.001$); hyperglycemic clamp: 102 ± 16 vs. 310 ± 42 pmol/l ($P < 0.001$); intravenous arginine: 346 ± 72 vs. 1104 ± 168 pmol/l ($P < 0.01$); and intravenous glucagon: 170 ± 37 vs. 247 ± 35 pmol/l (NS). The β-cell responses remained markedly abnormal after 6 months of CyA, although the response to intravenous glucose and oral glucose tolerance tests improved in three subjects. All the patients became insulin-dependent after 5–36 months.

CONCLUSIONS — CyA alone is not a suitable treatment for asymptomatic IDDM. Earlier identification of subjects with substantial β-cell secretory capacity and newer nontoxic intervention strategies are required for the prevention of IDDM.

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AUC, area under the curve; BMI, body mass index; CyA, cyclosporin A; FBG, fasting blood glucose; HLA, human leukocyte antigen; ICA, islet cell antibodies; IDDM, insulin-dependent diabetes mellitus; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

Cyclosporin A (CyA) treatment has induced transient remissions from insulin dependency in patients with overt insulin-dependent diabetes mellitus (IDDM) (1,2). The best remission rates were observed in patients with IDDM of recent clinical onset, free of ketoacidosis and substantial weight loss, and with significant residual insulin secretion (2–4). The question was therefore raised whether to start immunosuppressive treatments earlier, before the clinical onset of diabetes, to test the hypothesis that the CyA-induced remissions might be more frequent and more prolonged when the insulin secretory capacity is less deteriorated. We report the results of a pilot study initiated in 1991 on seven subjects with established diagnosis of IDDM, but no clinical insulin dependency, who were treated with CyA. Because the subjects did not require insulin therapy, the effects of the treatment were assessed on the β-cell secretory responses to various stimuli before and after the 6-month treatment period.

RESEARCH DESIGN AND METHODS

Subjects

Seven Caucasian subjects were recruited on the basis of family history of IDDM, the presence of persistent circulating islet cell antibodies (ICA), and human leukocyte antigen (HLA) class II risk alleles. The patients presented with no symptoms of overt IDDM or weight loss. Fasting blood glucose (FBG) values were normal or moderately elevated, but oral glucose tolerance tests (OGTTs) were markedly altered (Table 1). No patients presented hepatic or renal dysfunction or any other contraindication to CyA administration. This study was approved as part of a general protocol on the prediction and prevention of IDDM by the French National Ethics Committee. The patients (or their parents, when required) gave their informed consent.

Table 1—*Clinical, immunogenetic, and metabolic characteristics of the patients at entry into the study*

Patient	Age (years)	Sex	Family history of IDDM	BMI (kg/m^2)	HLA-DR	ICA (JDF U)	Blood glucose during OGTT 0 min (mmol/l)	Blood glucose during OGTT 120 min (mmol/l)	HbA_{1c} (%)
1	13	M	grandfather	16.2	4,6	5	7.2	22.3	7.6
2	14	F	sister	17.7	3,4	20	6.3	17.3	5.3
3	11	F	brother	17.6	3,5	2.5	6.5	12.9	5.5
4	30	M	brother	23.2	3,8	20	5.0	8.6	5.3
5	16	M	brother	23.5	3,4	20	5.2	10.1	5.8
6	21	M	brother	23.2	3,3	10	6.4	10.1	7.2
7	32	M	sister	20.4	3,3	2.5	6.9	17.2	6.3

JDF U, Juvenile Diabetes Foundation units.

They were compared with *1*) eight healthy young adults, six men and two women (age: 25.1 ± 2.1 years; body mass index (BMI): 22.2 ± 1.5), ICA negative and with no family history of diabetes (healthy control group); and 2) seven ICA-positive subjects (mean age 21.3 ± 7.8 years) with high-risk HLA markers and severely altered OGTT who remained untreated (untreated group).

Study design

CyA (Sandimmun, Sandoz) was given orally at a maximal dose of 7.5 $mg \cdot kg^{-1} \cdot day^{-1}$ that was adapted to CyA whole blood trough levels, monitored every 2nd week, and adapted to serum creatinine levels that were maintained below 130% of initial values (5). After 6 months, CyA was continued in four patients at a daily dose not exceeding 5 mg/kg. Patients received no oral hypoglycemic drugs and were fed a normocaloric weight-maintaining diet.

The following metabolic studies were performed in the healthy control subjects and in the patients immediately before and at the end of the 6-month CyA course on 3 separate days in the postabsorptive state: *1*) an OGTT test (OGTT, 1.75 g/kg glucose); *2*) a combined test, performed over 2.5 h on the same morning, comprising an intravenous glucose tolerance test (IVGTT) (0.5 g/kg glucose in 2 min), a hyperglycemic clamp (10 mmol/l for 120 min), and an intravenous arginine bolus (5 g L-arginine hydrochloride); and *3*) a glucagon test (1 mg i.v.). Insulin and C-peptide secretory responses are expressed as the sum of (1 + 3) min values for the IVGTT; the mean of the last two values (100 and 120 min) for the hyperglycemic clamp; the mean of values at 2, 3, 4, and 5 min for the arginine test; and the peak value over 15 min after intravenous glucagon.

Plasma glucose was measured by a glucose oxidase method, HbA_{1c} by high-performance liquid chromatography (normal values ± SD = 4.9 ± 0.7%), and insulin and C-peptide by radioimmunoassay (SB-INSI 5 kit and C-PEP kit, CIS, Gif sur Yvette, France). Whole blood CyA concentrations were measured by fluorescence polarization immunoassay (TDx, Baxter, IL) using an anti-CyA monoclonal antibody. HLA-DR was typed using sequence-specific oligonucleotides. ICAs were detected by indirect immunofluorescence on frozen sections of a human pancreas. The threshold of detection was <2.5 Juvenile Diabetes Foundation units; the specificity was 98.75% and the sensitivity was 69%.

Parametric results are presented as mean values ± SD unless otherwise stated. Data for patients and control subjects were compared using a two-tailed Student's *t* test; those before and after 6 months of CyA treatment using a paired Student's *t* test. Parameters of glucose tolerance and insulin secretion were correlated using linear regression analysis.

RESULTS

Initial metabolic status

Basal glucose levels and HbA_{1c} in the patients were higher than control values: 6.2 ± 0.3 vs. 4.9 ± 0.7 mmol/l and 6.1 ± 0.3 vs. 4.9 ± 0.1% ($P < 0.001$). Basal plasma insulin levels were similar: 41 ± 8 vs. 39 ± 5 pmol/l. The patients' insulin secretory responses were dramatically reduced: 106 ± 16 vs. 884 ± 190 pmol/l after intravenous glucose ($P < 0.001$), 102 ± 16 vs. 310 ± 42 pmol/l during the hyperglycemic clamp ($P < 0.01$), and 346 ± 72 vs. 1104 ± 168 pmol/l after intravenous arginine ($P < 0.01$). The β-cell responses to intravenous glucagon (170 ± 37 vs. 247 ± 35 pmol/l) did not differ significantly from control values.

Metabolic status at 6 months

Two patients developed sustained hyperglycemia and clinical symptoms of diabetes from the 5th month of CyA and were put on insulin. The other five did not require insulin for 10–36 months. Their BMI remained stable; mean FBG values at 6 months were 5.7 ± 0.3 (range 4.8–6.4) mmol/l, and mean HbA_{1c} was 5.7 ± 0.2% (range 5.2–6.6%).

The β-cell responses to stimuli remained markedly abnormal and did not differ from the initial results (Table 2). Three patients improved their acute insulin response to intravenous glucose and the area under the glucose curve (AUC glucose) during the OGTT (Fig. 1). The changes in the acute insulin response from the 1st to the 2nd IVGTT were negatively correlated with the changes affecting the AUC glucose from 1st to 2nd OGTT ($r = -0.98$, $P < 0.01$).

Among the five patients who were insulin-free at 6 months, one (#5) decided to stop using CyA and became insulin-dependent on month 24. Two (#3

Table 2—*Individual plasma insulin values (pmol/l) in the basal state and after various stimuli in asymptomatic IDDM patients at entry and after 6 months of cyclosporin A treatment*

	Basal value		IVGTT (1 + 3)		Hyperglycemic clamp		Intravenous arginine		Glucagon test	
Patient	Before CyA	After CyA	Before CyA	After CyA	Before CyA	After CyA	Before CyA	After CyA	Before CyA	After CyA
1	58	40	87	—	105	—	354	—	246	—
2	36	66	124	—	93	—	246	—	126	288
3	30	30	100	54	81	84	396	486	348	198
4	30	42	147	52	114	72	162	228	114	72
5	78	66	162	306	162	—	522	—	144	138
6	36	24	73	282	126	150	648	648	156	594
7	18	66	46	87	36	60	90	108	60	96

Patients 1 and 2 developed IDDM after 5 months of CyA treatment, before the tests could be repeated.

and 7) of the four patients who continued on CyA required insulin at month 10; the last two patients (#4 and 6) remained non-insulin-dependent until months 21 and 36, respectively. During the insulin-free period, fasting and postprandial blood glucose values remained below 6.8 mmol/l and 10.0 mmol/l, respectively. The mean insulin-free period (16 ± 11 months) did not differ significantly from the untreated group (12 ± 11 months, range 2–34 months).

CyA tolerance

The mean individual (± SD) CyA blood trough levels over the 6-month study period ranged from 122 ± 64 ng/ml to 268 ± 67 ng/ml. There was a nonsignificant increase in serum creatinine levels, from 70.3 ± 7.4 (range 47–94) mmol/l initially to 83.4 ± 7.3 (range 62–112) mmol/l at 6 months. All values returned to baseline when the drug was discontinued and remained in the normal range after a further 3-year follow-up (mean 76.3 μmol/l, range 58–92). No other side effects were observed.

DISCUSSION — Seven subjects with unambiguous IDDM, but still free of symptoms, were given CyA with no other treatment. The goal of this pilot study was to assess whether CyA, given in the preclinical phase of the disease, could improve insulin secretion. Despite a small increase in insulin response to intravenous glucose and a concomitant improvement of oral glucose tolerance in three subjects, the overall β-cell function was not significantly changed after 6 months of treatment. All the patients eventually became insulin-dependent—two before the 6th month of treatment and five after 10–36 months. No long-term toxicity of the CyA course was noted.

Thus, at this asymptomatic stage of the disease, CyA has no effect for the prevention of insulin dependency. Unlike previous trials, the patients in the present

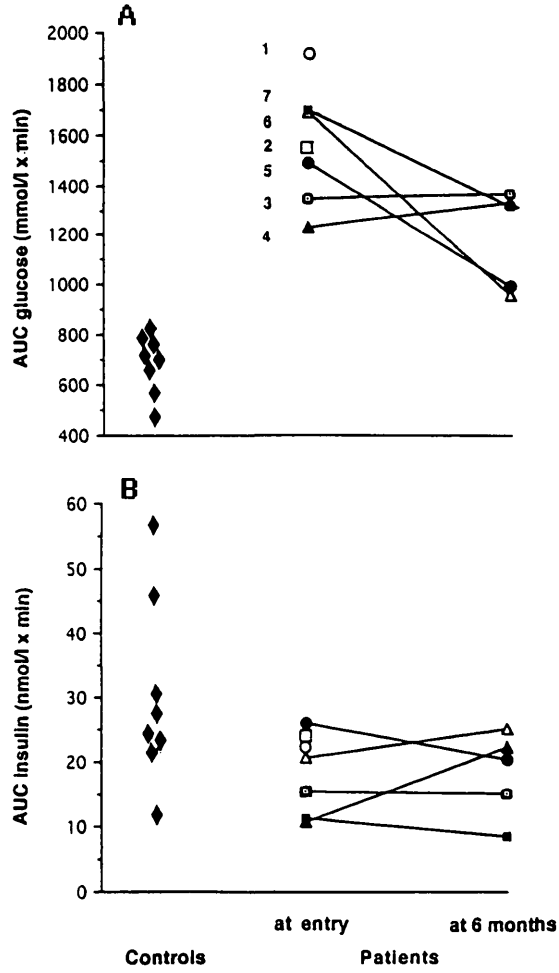

Figure 1—*AUC glucose (A) and AUC insulin (B) during OGTTs in control subjects and in patients with preclinical IDDM at entry and after 6 months of treatment with cyclosporin A. Numbers correspond to patient number. The tests were not repeated in patients 1 and 2, who developed IDDM 5 months after entering the study.*

study did not receive insulin. Insulin may by itself favor recovery of β-cell function and delay the onset of IDDM (6). Hence, it might be important for the efficacy of immune intervention. It is also possible that the treatment still came too late. These patients, who were not prediabetic but IDDM subjects at a non-insulin-requiring stage, had β-cell responses to stimuli already dramatically deteriorated on entry into the study. Their initial metabolic characteristics (insulin response to IVGTT and basal blood glucose values) have been shown to predict the onset of IDDM in the short term (7,8). In subjects with a better-preserved insulin secretory capacity, CyA treatment may yield better results (9).

Immunointervention strategies based on newer concepts (10) are currently focused on individuals at an earlier stage of the β-cell destructive process. This implies a strong effort to identify genuine prediabetic subjects, i.e., subjects with high-risk immunogenetic markers, but a still unaffected or minimally altered β-cell function.

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