Long-Term Results of Early Cyclosporin Therapy in Juvenile IDDM

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In juvenile IDDM patients, immunosuppression with cyclosporin A allows partial β-cell function recovery and transient remissions of insulin dependency. The effects of this therapeutic approach, however, have not been evaluated in the long-term, since no reported trial exceeded 1 year. Here we analyze 130 diabetic children followed at our institution during the first years of their disease. Cyclosporin was given to 83 of them at an initial dose of 7.2 ± 0.1 mg · kg^{-1} · day^{-1}, which was decreased stepwise then interrupted after 6–62 months, depending on the response to therapy. A total of 47 diabetic children, who served as control subjects in two trials, were pooled for comparison. Over 4 years, the cyclosporin-treated group kept plasma C-peptide approximately twice as high as the control group ($P < 0.02$). It took 5.8 ± 0.6 years for C-peptide secretion stimulated by glucagon to become undetectable in the cyclosporin group versus 3.2 ± 0.6 years in the control group ($P < 0.02$). Average insulin dose remained lower by 0.2–0.4 U · kg^{-1} · day^{-1} and glycated hemoglobin by ~1% in cyclosporin-treated patients ($P < 0.02$), who also had less hypoglycemia than the diabetic control subjects ($P < 0.05$). After 4 years, differences between the groups became nonsignificant. We observed no significant secondary effects of cyclosporin. In conclusion, positive effects of low-dose cyclosporin in recently diagnosed clinical IDDM patients are prolonged beyond interruption of the drug. The magnitude and duration of the benefit, however, do not appear sufficient to justify this immunosuppressive treatment in clinical practice. ***Diabetes*** **45:101–104, 1996**

Several trials in the mid 1980s documented that cyclosporin A, at doses ranging from 7 to 10 mg · kg^{-1} · day^{-1}, increases residual β-cell function in adult and juvenile patients with recently diagnosed IDDM (1–5). Remissions of insulin dependency were frequent in all studies and lasted ~0.5–3 years (5–7). All protocols were in fact designed for 1-year trials, not to prospectively evaluate the long-term effects of early cyclosporin immunosuppression.

Because of its transient effects on β-cell destruction and its potential toxicity, cyclosporin did not qualify for further therapeutic investigation in IDDM and inclusion in trials stopped in 1990. Since then, neither the evolution of residual insulin secretion nor its long-term effects on glycemic control have been reported.

We describe the results of the 6-year follow-up of 83 diabetic children enrolled in cyclosporin trials from 1986 to 1988 (4,7) compared with 47 age-matched diabetic control subjects treated in parallel with insulin and a placebo. Our data allow evaluation of the long-term influence of early immunosuppression on the evolution of juvenile IDDM. Nevertheless, the present study cannot pretend to the same degree of accuracy as a long-term randomized prospective trial.

RESEARCH DESIGN AND METHODS

Patients. Clinical and biological characteristics of the patients at entry in the study are presented in Table 1. A total of 83 cyclosporin-treated children were included in the survey: 63 from our initial open trial (4) and 20 from a placebo-controlled double-blind study started in 1988. They were selected only on the basis of inclusion in cyclosporin A trials performed at St. Vincent de Paul from 1986 to 1988. Sixty-three patients completed a continuous 6-year follow-up at our institution starting at diagnosis of IDDM. Among the 20 patients who did not complete follow-up, the proportion of remissions was not different from that in those who continued. The diabetic children received cyclosporin twice daily for various durations, depending on their responses to immunosuppression. Remission of insulin dependency was defined as previously proposed (1–5): glycated hemoglobin <7.5%, fasting plasma glucose <140 mg/dl, and postprandial glucose <200 mg/dl in the absence of insulin injection. In the patients considered to be in remission of insulin dependency, cyclosporin doses were 6–7.5 mg · kg^{-1} · day^{-1} in the 1st year of trial and then decreased stepwise. We arbitrarily chose a basal plasma C-peptide concentration of >0.1 nmol/l and a glucagon stimulated value of >0.2 nmol/l as indicators of significant residual β-cell function.

During the trial, we discontinued cyclosporin within 6 months in each patient whose plasma C-peptide values had fallen below these values. Cyclosporin was also discontinued in cases where remission either was not obtained after 4 months or failed later. Trough levels of cyclosporin were monitored weekly for 1 month, then monthly as reported (4). The evolution of cyclosporin doses for the whole group is presented in Fig. 1.

Results were compared with those of 47 diabetic children who had received no immunosuppression and had been treated with insulin from 1987 to 1988 as controls in either cyclosporin trials or a placebo versus hexapentanoic acid study.

In both groups, insulin therapy was given through two or three injections per day at doses adjusted to blood glucose monitoring and aimed at optimizing glycemic control (8).

Methods. Plasma C-peptide concentration was measured 0 and 6 min after the intravenous injection of 1 mg of glucagon, given after an overnight fast after 2–4 days during which blood glucose levels were near normal. Plasma C-peptide was measured using M 1221 antiserum (Novo Nordisk, Bagsvaerd, Denmark) after proinsulin had been removed by polyethylene glycol precipitation (4). The lower limit of detection was 0.02 nmol/l. The average relative precision was 14% at 0.05 nmol/l and 3% at 0.3 nmol/l. The limit of detection was 0.02 nmol/l. In nondiabetic healthy age-matched children, we determined that basal C-peptide averaged 0.42 ± 0.20 nmol/l, and glucagon stimulated C-peptide averaged 1.78 ± 0.69 nmol/l (7,9). HbA_{1c} was measured every 6

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TABLE 1
Characteristics of patients at diagnosis

	Cyclosporin-treated diabetic patients	Control diabetic patients
n	83	47
Age (years)	10.0 ± 3.2	11.1 ± 2.6
Duration of symptoms (days)	43 ± 9	47 ± 7
Weight loss (% body weight)	8.3 ± 2.1	8.0 ± 2.0
Ketoacidosis (*n*)	7	5
Basal C-peptide (nmol/l)	0.14 ± 0.02	0.13 ± 0.02
Postglucagon C-peptide (nmol/l)	0.26 ± 0.03	0.25 ± 0.04
Islet cell antibodies		
Positive (%)	92	91
Mean titer (JDF U)	63 ± 11	62 ± 21
Insulin autoantibodies (% insulin binding)	2.9 ± 0.4	2.6 ± 0.7
HLA DR3,4 (*n*)	39	24

Data are means ± SE unless otherwise indicated. JDF U, Juvenile Diabetes Foundation units.

months with automated high-pressure liquid chromatography (Diamat, BioRad, Richmond, CA) (normal value 5.3 ± 0.7%, mean ± SD).

Intergroup comparisons were performed on an intention-to-treat basis using unpaired Student's *t* test. Values are expressed as means ± SE.

RESULTS

The mean duration of cyclosporin administration was 18 ± 4 months, with an interindividual range of 6–62 months. The evolution of mean cyclosporin dose is depicted in Fig. 1. The numbers of patients receiving cyclosporin were 80 (1 year), 55 (2 years), 38 (3 years), 14 (4 years), 4 (5 years), and 0 (6 years). During the 6-year follow-up period, 20 patients dropped out from the cyclosporin group and 9 from the control group. The characteristics of these patients were comparable, both at diagnosis and at time of dropping out, with those of the patients who remained in the study. The main alleged reason for dropping out was the inconvenience of yearly in-hospital evaluation for patients living far from our center. In the cyclosporin group, the mean time for dropping out was 29 ± 8 months of follow-up after 22 ± 9 months of cyclosporin administration, whereas the 9 control subjects dropped out after 33 ± 7 months.

Islet cell and insulin antibody titers remained lower in the serum of cyclosporin patients during the first 3 years. After cessation of cyclosporin therapy, however, they were comparable to titers in control patients (Table 2).

TABLE 2
Titers of islet cell and insulin autoantibodies in the studied patients

	Islet cell antibodies (JDF U)		Insulin autoantibodies (% insulin binding)	
Year	Cyclosporin-treated patients	Control patients	Cyclosporin-treated patients	Control patients
0	66 ± 10	58 ± 26	2.7 ± 0.3	2.5 ± 0.7
1	20 ± 4	27 ± 14	8.5 ± 0.5*	26.5 ± 4.1
2	12 ± 3	18 ± 13	12.3 ± 1.2*	26.2 ± 5.2
3	13 ± 7	21 ± 10	19.6 ± 4.2	22.5 ± 7.0
4	13 ± 6	10 ± 7	18.7 ± 5.8	23.6 ± 5.7
5	8 ± 4	4 ± 3	21.2 ± 5.1	25.1 ± 6.2
6	7 ± 4	4 ± 2	25.3 ± 6.4	26.8 ± 7.0

Data are means ± SE. *$P < 0.025$. JDF U, Juvenile Diabetes Foundation units.

The indicators of β-cell function and glycemic control in cyclosporin-treated and control diabetic children are presented in Figs. 2–5. Cyclosporin-treated patients maintained approximately twice greater plasma C-peptide concentrations over 4 years, both in the basal state and after glucagon stimulation. The difference from control diabetic children decreased progressively with duration of follow-up and became nonsignificant 4–5 years after the clinical onset of diabetes (Fig. 2). It took 2.1 ± 0.8 years for individual plasma C-peptide to become <0.1 nmol/l (fasting) and 0.2 nmol/l (postglucagon) in control subjects versus 3.6 ± 0.5 years in cyclosporin-treated patients ($P < 0.02$). Glucagon-stimulated plasma C-peptide became undetectable (< 0.02 nmol/l) at 3.2 ± 0.6 years in the control group versus 5.8 ± 0.6 years in the cyclosporin group ($P < 0.02$). A larger proportion of patients in the cyclosporin group kept a β-cell function considered to be of clinical relevance (10) and maintained for a longer duration (Table 3). We did not find a correlation between plasma C-peptide at diagnosis and values recorded between 1 and 6 years of evolution in the cyclosporin-treated group. However, postglucagon C-peptide concentrations at 2, 3, and 4 years of evolution correlated with those measured at 1 year in cyclosporin patients. The slopes of the respective regression equations were 0.43, 0.33, and 0.16, with correlation coefficients of 0.57, 0.51, and 0.46 ($P < 0.0005$). This was also true for basal C-peptide concentrations. We concluded that the recovery of β-cell function during the 1st year of disease, more than the residual secretion at diagnosis, is predictive of

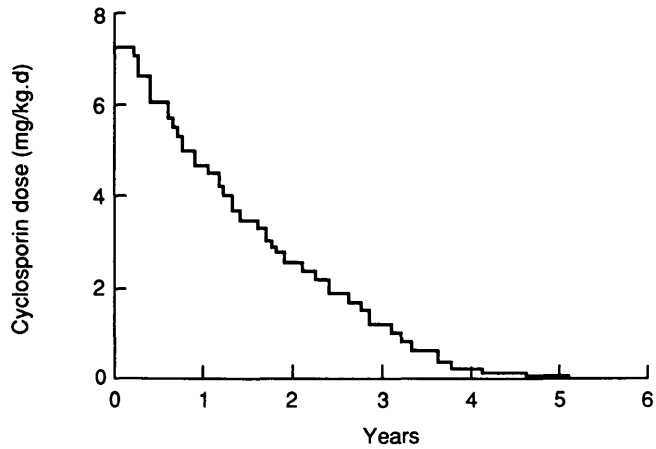

FIG. 1. Mean cyclosporin dose in the studied patients.

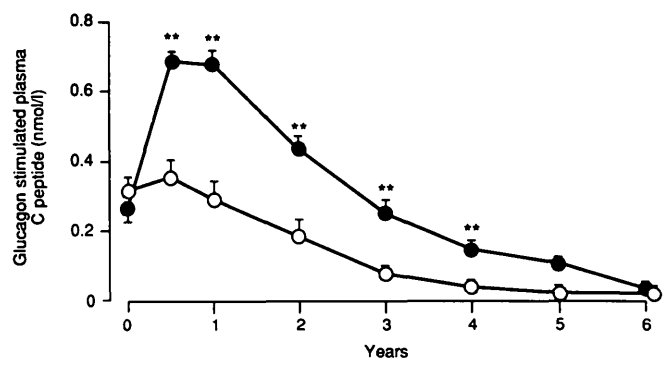

FIG. 2. Evolution of plasma C-peptide concentrations after glucagon stimulation in cyclosporin-treated (●) patients compared with control (○) diabetic patients. **$P < 0.02$.

TABLE 3
Proportion of patients with fasting plasma C-peptide >0.10 nmol/l and glucagon-stimulated C-peptide >0.20 nmol/l

Year	Cyclosporin-treated patients	Diabetic control patients	*P*
0	44	48	NS
1	81	40	<0.0005
2	69	25	<0.0005
3	36	0	<0.0005
4	22	0	<0.005
5	7	0	NS
6	0	0	NS

Data are expressed as percentages.

later evolution of residual β-cell function. After 5 years, C-peptide became very low or undetectable in most patients, precluding any attempt to calculate significant correlations.

Daily insulin doses remained lower by ~0.3–0.4 U/kg during the first 2 years in the cyclosporin-treated diabetic children, then by 0.2 U/kg over the last 4 years (Fig. 3).

During the 1st year of follow-up, remissions of insulin dependency were twice as frequent in the cyclosporin group (61%) as in the control group (32%) ($P < 0.05$) (Fig. 4). During the interruption of insulin therapy, HbA_{1c} averaged 6.1 ± 0.2%, fasting plasma glucose 117 ± 8 mg/dl, and postprandial glucose 143 ± 15 mg/dl, according to previously defined criteria for complete remissions of insulin dependency (1–5). These remissions were more frequent and more prolonged in cyclosporin-treated children who had significant residual insulin secretion at entry (data not shown). The mean duration of remission was 347 ± 23 days (range 31–1,280) in the cyclosporin group and 159 ± 21 days (range 31–407) in the control group ($P < 0.025$). Remissions became rare after 2 years and were only observed in cyclosporin-treated patients.

Levels of HbA_{1c} were lower by ~1–1.5% in cyclosporin-treated children during the first 4 years of follow-up (Fig. 5). From 1 to 3 years of evolution, HbA_{1c} in the cyclosporin-treated patients correlated weakly but significantly with C-peptide concentrations (range of correlation coefficients 0.37–0.47, $P < 0.005$).

During the whole period of observation, the frequency of severe hypoglycemia averaged 0.03 ± 0.03 per patient-year in the cyclosporin group versus 0.23 ± 0.09 per patient-year in the control group ($P < 0.05$).

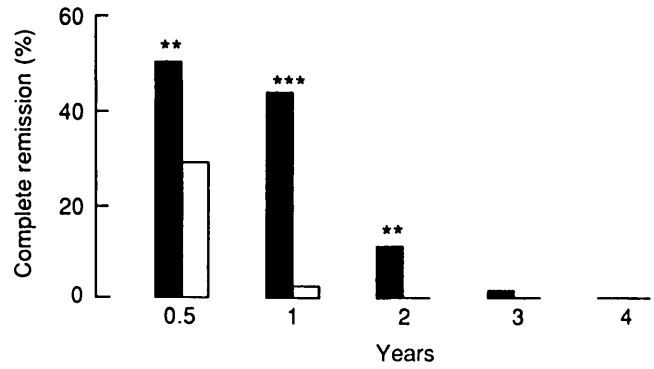

FIG. 4. Frequency of complete remissions of insulin dependency in cyclosporin-treated (■) and control (□) patients.

Secondary effects of cyclosporin administration at the present doses were rare and minimal. No children showed abnormal elevation of blood pressure. Slight-to-mild hypertrichosis developed on the arms, back, or face of 39% of patients, then disappeared within 6–18 months of cessation of cyclosporin. In comparison, 11% of patients receiving the placebo reported similar effects. Only one cyclosporin-treated patient had marked hypertrichosis. Gingival hypertrophy was always very mild and affected 19% of patients. Distal paresthesias were seen in 9% and unspecific abdominal pain in 11% of cyclosporin-treated patients.

Plasma creatinine did not change significantly in the cyclosporin-treated group. Cyclosporin-treated patients had creatinine values of 60 ± 1 μmol/l at diagnosis, 64 ± 1 μmol/l at 1 year, 66 ± 2 μmol/l at 2 years, 64 ± 2 μmol/l at 4 years, and 67 μmol/l at 6 years. Corresponding values in control patients were 61 ± 1 μmol/l at diagnosis, 64 ± 1 μmol/l at 1 year, 65 ± 2 μmol/l at 2 years, 66 ± 2 μmol/l at 4 years, and 66 μmol/l at 6 years. Creatinine values were reported normal after 6 years of evolution in all patients who dropped out of follow-up at our institution. Glomerular filtration was measured in 41 patients 2–3 years after cessation of cyclosporin and was found to be within the normal range (not shown). Albuminuria and microproteinuria remained negative in all cyclosporin-treated patients. We obtained 25 kidney biopsies. Of the 19 biopsies performed after 9–12 months of cyclosporin administration, none showed signs of cyclosporin nephrotoxicity, as detailed in a previous report (4). Six

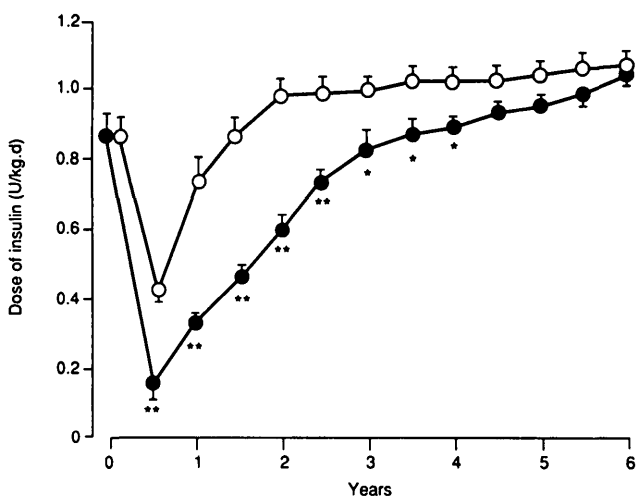

FIG. 3. Changes in daily insulin dose in cyclosporin-treated (●) and control (○) diabetic children. *$P < 0.05$; **$P < 0.02$.

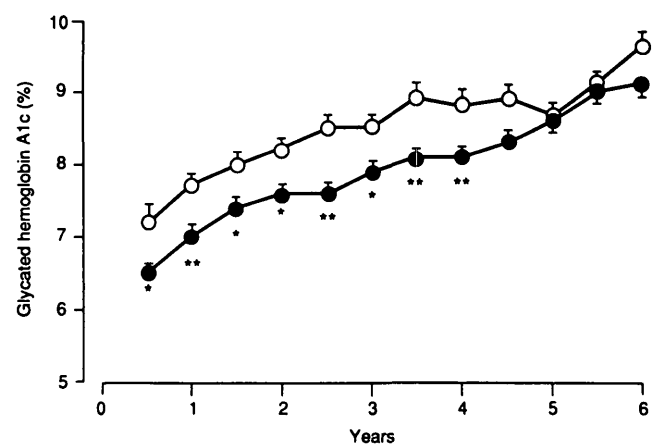

FIG. 5. HbA_{1c} in cyclosporin-treated (●) and control (○) diabetic patients. *$P < 0.05$; **$P < 0.02$.

additional patients underwent biopsies after 18–24 months of remission and continuous cyclosporin administration. Four were completely normal, with a nephrotoxicity score of 0 or 1. The other two showed isolated minimal tubular atrophy and interstitial fibrosis, with a corresponding total nephrotoxicity score of four. This score, designed for quantifying specific kidney lesions caused by cyclosporin, is the sum of five items, each being ranked 0–4: glomeruli obsolescence, segmental focal glomerulosclerosis, arteriopathy, interstitial fibrosis, and tubular atrophy (4). The total score ranges from 0 to 20, the values in normal kidneys being 0–4 (4). All 25 biopsies could therefore be classified within normal limits (7).

Cyclosporin-treated children had a slight nonsignificant decrease of hemoglobin (12.7 ± 0.1 g/l) at 6 and 12 months when compared with values at entry (13.1 ± 0.1 g/l). Values at 6 and 12 months in the control group were 13.2 ± 0.1 and 13.1 ± 0.1 g/l. Values were identical in both groups between 2 and 6 years of evolution.

DISCUSSION

Administration of cyclosporin for 18 ± 4 months to children with recent IDDM has positive effects on β-cell function and transiently improves diabetes control. Effects are maximal within 3–4 years of diagnosis, then decrease progressively. In cyclosporin-treated patients, plasma C-peptide values at 1 year are predictive of evolution of residual β-cell function for the next 4–5 years. When administered to patients with significant C-peptide secretion at diagnosis, cyclosporin shows more prolonged effects. However, even in these patients, no benefits of immunosuppression remain detectable after 5 years of evolution.

Because of the relatively low dose and close monitoring of cyclosporin therapy, as well as the young age of our patients (11), no significant toxicity was observed. This confirmed previous reports from our group (4,7,11).

Several comments can be derived from these observations. It is clear that the present regimen of cyclosporin treatment induces a transient amelioration of diabetes control, lasting 4–5 years, with a decrease of HbA_{1c} by ~1% and less frequent hypoglycemia. These results could have been even better had we not decided to discontinue cyclosporin in patients with low residual C-peptide. However, the improvement of C-peptide secretion and diabetic control does not appear sufficient to qualify cyclosporin as an adjunct therapy of insulin to be used routinely in young patients with IDDM. The main reason for this conclusion is that even in young individuals treated at low or medium doses, cyclosporin toxicity cannot be completely excluded and could occur if large cohorts of patients were treated outside of the careful monitoring characterizing clinical trials. It is possible that higher doses and longer administration of cyclosporin could obtain larger and more prolonged effects. This approach, however, was not tested in our juvenile diabetes patients, since we aimed at minimizing drug toxicity. For the future, we do not support more intensive trials (dose, duration) of cyclosporin in overt IDDM, since the main limiting factor of immunosuppression efficacy is late intervention at a stage when most β-cells are already destroyed (4,7). Low-dose cyclosporin may instead find more relevance at a late preclinical stage of the disease.

Evidence was recently obtained in the Diabetes Control and Complications Trial that a diminution of HbA_{1c} levels from 8.9 to 7.1% for 8–9 years is associated with a remarkable decrease of background retinopathy and microproteinuria (12). An improvement of HbA_{1c} by ~1% for 4–5 years, as observed in the present study, may therefore be important for the primary prevention of diabetic microangiopathy. In this respect, immunosuppressive drugs devoid of toxicity could still find useful medical applications in IDDM, even at the stage of clinical manifestations of insulin dependency. This may be of particular importance in adolescents, in whom glycemic control is known to be difficult, even during the first years of disease (8,13–17).

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