Small Doses of Subcutaneous Insulin as a Strategy for Preventing Slowly Progressive β-Cell Failure in Islet Cell Antibody–Positive Patients With Clinical Features of NIDDM

Tetsuro Kobayashi, Koji Nakanishi, Toshio Murase, and Kinori Kosaka

We report a pilot study to determine the preventive effect of small doses of insulin injected subcutaneously on slowly progressive β-cell damage in islet cell antibody (ICA)-positive patients with apparent NIDDM. Ten NIDDM patients who were ICA$^+$ were divided into two groups of five. In the insulin group (age: 51 ± 8 years [mean ± SD], sex: 3 men and 2 women), intermediate-type insulin (3–16 U/day) was given once or twice daily as a subcutaneous injection. The sulfonylurea (SU) group (age: 48 ± 11 years, sex: 3 men and 2 women) was initially treated with a SU agent. Changes in β-cell function, as indicated by serum C-peptide responses and blood glucose values during a 100-g oral glucose tolerance test, as well as ICA and GAD antibody status, were evaluated for up to 30 months in both groups. ICA status became negative in four of five patients in the insulin group. ICA status did not become negative in any of the patients in the SU group (P = 0.047 vs. insulin group). ICA status was persistently positive in two patients whose β-cell function eventually progressed to an insulin-dependent state and fluctuated in the remaining three patients. In the insulin group, GAD antibody status became negative in one of four initially GAD antibody–positive NIDDM patients. In the SU group, GAD antibody status was persistently positive in three NIDDM patients (NS vs. insulin group). The serum C-peptide response improved significantly within 6 and 12 months in the insulin group, whereas it decreased progressively in the SU group. The changes in C-peptide response were significantly different between the two groups at 6, 12, 24, and 30 months. Two-hour blood glucose and HbA_1 values were unchanged in the insulin group, but they increased in the SU group. Subcutaneous small doses of insulin, resulting in a high rate of negative conversion of ICA and an improved serum C-peptide response, may be effective in treating ICA$^+$ NIDDM patients who are at high risk for slowly progressive β-cell failure. *Diabetes* 45:622–626, 1996

From the Department of Endocrinology and Metabolism, Toranomon Hospital, Okinaka Memorial Institute for Medical Research, Tokyo, Japan.

Address correspondence and reprint requests to Dr. Tetsuro Kobayashi, Department of Endocrinology and Metabolism, Toranomon Hospital, Okinaka Memorial Institute for Medical Research, Tokyo 105, Japan.

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CV, coefficient of variation; FBG, fasting blood glucose; ICA, islet cell antibody; JDF, Juvenile Diabetes Foundation; NOD, nonobese diabetic; OGTT, oral glucose tolerance test; SU, sulfonylurea.

Prospective studies demonstrate that β-cell dysfunction in patients with a late onset of NIDDM who are islet cell antibody (ICA) positive tends to progress slowly to insulin dependency (called slowly progressive IDDM) (1,2). A change in ICA status is a useful predictor of a change in β-cell function in initially ICA$^+$ NIDDM patients: if ICA status is persistently positive or fluctuating, β-cell function will deteriorate progressively, leading to insulin dependency over the years (1,2). In contrast, when patients become ICA$^-$ during the clinical course, the secretion of insulin improves (1).

Other risk factors beside pancreatic autoimmunity are assumed to contribute to the slowly progressive β-cell failure, including sulfonylurea (SU) treatment and sex (3–5). We hypothesized that administration of insulin, rather than a SU agent, would aid in the prevention of slowly progressive β-cell failure in such patients because of improved glucose control, an unknown immunological mechanism, or a combination of these mechanisms. We therefore conducted a prospective pilot study on the effects of small doses of insulin in patients with presumed NIDDM who were ICA$^+$ and thus at high risk for progression to insulin dependency.

RESEARCH DESIGN AND METHODS

Subjects. Ten ICA$^+$ patients with NIDDM were selected from among 1,584 patients with NIDDM. NIDDM patients were selected to take part in this study if they fulfilled the following criteria: *1*) disease diagnosed according to the National Diabetes Data Group (6); *2*) ICA with a titer ≥20 Juvenile Diabetes Foundation (JDF) U (positivity and titer of ICA in NIDDM patients ascertained by second sampling within 1 month after initial measurement), and *3*) patients were not related to each other. Patients were excluded if they had a history of ketonuria, diabetic ketoacidosis, or marked hyperglycemia initially requiring insulin. Initially, a total of 27 patients were ICA$^+$. Seventeen ICA$^+$ NIDDM patients were excluded because of low ICA titers: 5 patients with 5 JDF U and 12 patients with 10 JDF U. All 10 patients with an ICA titer ≥20 JDF U agreed to participate in the trial. Equal numbers of patients were randomly assigned to treatment with insulin or SU. Patients were divided into two groups of five each.

One group received small doses of insulin (insulin group), and the other received a SU drug (glibenclamide) (SU group). Intermediate-acting human insulin (Novorin N or Monotard insulin, Novo, Copenhagen, Denmark) was injected subcutaneously once or twice daily in the insulin group throughout the study. Glibenclamide (Euglucon, Hoechst, Somerville, NJ) was given orally once or twice a day in the SU group (Table 1). All patients gave their informed consent for participation. All patients studied were instructed on nutrition therapy by a dietitian before study entry using the food exchange table of the Japan Diabetes

TABLE 1
Characteristics of patients with NIDDM who were ICA$^+$ at the beginning of study treated with insulin versus SU

Group	Age (years)	Sex (M/F)	Duration of NIDDM (years)	BMI (kg/m^2)	ICA titer (JDF U)	Insulin (U/day)	SU (mg/day)
Insulin							
1	52	M	2.9	20.7	20	8	—
2	48	F	0.1	18.9	20	10	—
3	61	F	0.2	22.2	80	3	—
4	38	M	0.4	20.5	20	16	—
5	57	M	0.1	23.4	20	4	—
Mean ± SD	51 ± 8	3/2	0.7 ± 1.1	21.1 ± 1.5	20*	8 ± 5	—
SU							
6	55	M	5.5	22.7	20	—	2.5
7	44	F	0	18.4	20	—	10.0
8	60	F	1.3	27.1	20	—	1.25
9	29	M	0.1	21.4	160	—	5.0
10	54	M	0.4	22.5	20	—	7.5
Mean ± SD	48 ± 11	3/2	1.5 ± 2.1	22.4 ± 2.8	20*	—	5.3 ± 3.2

The SU drug used was glibenclamide. *Median value.

Society (Bunkodo, Tokyo, Japan). They were principally assigned to consume a diet containing 30 kcal · kg ideal body wt^{-1} · day^{-1} with 55–60% carbohydrate. The patients were encouraged to document the dose of insulin or oral agent and to bring the data to ensure their compliance. The target of glycemic control was as follows: fasting blood glucose (FBG) <6.7 mmol/l and/or HbA_1 <8.0%. The dose of SU was adjusted based on FBG and HbA_1 values by increasing or decreasing the dose of glibenclamide by 1.25 mg. The criteria for determining failure of oral hypoglycemic agents included FBG becoming >12.2 mmol/l and/or an HbA_1 value >10% with the maximal daily dose of glibenclamide (15 mg). The frequency of the initial insulin dose was basically once a day in the morning. If the FBG level was >6.7 mmol/l, additional insulin was given in the evening.

Follow-up observations. All patients received a 100-g oral glucose tolerance test (OGTT) at the beginning of the study and were instructed to repeat this test at least every 6 months for the measurement of blood glucose, HbA_1, and serum C-peptide. A 100-g OGTT was done in the morning after a 12-h overnight fast. Patients were instructed to consume a diet containing >250 g carbohydrate/day for 3 days before the OGTT. The patients were encouraged to undertake regular exercise. SU or insulin treatment was stopped on the day of the OGTT. A 100-g glucose dose was adopted, based on our previous studies on the natural history of C-peptide response in ICA$^+$ NIDDM patients (1,5). The amount of glucose load can elicit a C-peptide response even in severely insulin-deficient diabetic patients (7), making assessment of β-cell function easy in this study. Insulin was stopped on the morning of the OGTT and given at the completion of the OGTT. The response of serum C-peptide to the OGTT was quantified as the integrated value of the serum C-peptide levels in six samples obtained during the OGTT at 0, 30, 60, 90, 120, and 180 min (Σ C-peptide: normal range 7.9–13.6 nmol/l). Longitudinal changes in C-peptide response, blood glucose, HbA_1, ICAs, and GAD antibodies were studied for up to 30 months. Serum samples were stored at −80°C until assay.

ICA and GAD antibody assays. ICAs were measured by an immunofluorescence technique as previously described (8). To keep a consistent assay quality of ICAs, the same pancreas was used for the screening and follow-up studies. Stocked ICA$^+$ control sera with titers of 5 and 10 JDF U and a negative sample were included in the assay samples. All stored samples for each subject in each group were assayed again in the same run at the completion of the study. ICA positivity was judged by two or three investigators in a double-blind manner. Our laboratory participated in the 2nd through the 5th International Workshop on Standardization of the ICA Assay, in which we established a cutoff point of 5 JDF U, sensitivity of 90%, and specificity of 92%. The reproducibility of the ICA assay with 20 JDF U was 100% (62 of 62). GAD antibodies were assayed by a radiobinding assay using porcine native GAD antibodies as previously reported (9). Sensitivity and specificity of our GAD antibodies assay were 80 and 100%, respectively, in the 2nd International GADAb Workshop (10). GAD antibodies were assayed in saved serum samples during the study.

C-peptide and HbA_1 assays. Serum C-peptide levels were measured using a previously reported method (7). Intra-assay and interassay coefficients of variation (CVs) were 3.4 and 8.1%, respectively. HbA_1 levels were measured by high-performance liquid chromatography (Toso, Tokyo, Japan) (normal <7.2%). Intra- and interassay CVs of HbA_1 were 0.9 and 1.2%, respectively.

Statistical analysis. Data are shown as means ± SD, if not otherwise mentioned. The Mann-Whitney *U* test and Wilcoxon's test for paired data were used. Fisher's exact test was used to compare the incidence of ICAs.

RESULTS

Patient characteristics. No significant differences were present among the patients randomly assigned to the two treatment groups (Table 1). The median titer of ICAs was 20 JDF U in both groups at study entry.

Changes in ICA and GAD antibody status during the trial. ICAs disappeared in four of the five (80%) insulin group patients during the observation (Table 2). The duration of negative seroconversion of ICAs after insulin initiation was as short as 6 months in three patients and 12 months in one patient. In one patient (patient 2), ICA status was persistently positive at the same titer. In the SU group, ICAs persisted in all five patients (P = 0.047 vs. insulin group). ICA status was persistently positive in two patients and fluctuated between positive and negative in three patients.

GAD antibodies were detected in four of five patients in the insulin group at study entry. GAD antibodies disappeared in one patient (patient 5) 12 months after insulin, and the other three patients remained GAD antibody–positive (Table 3). In the SU group, GAD antibody status was initially positive in three of five patients. GAD antibody status remained unchanged.

TABLE 2
Longitudinal changes of ICA titer during study

Group	Patient	Follow-up period (month) 0	6	12	18	24	30
Insulin	1	20	<5	<5	<5	<5	<5
	2	20	20	20	20	20	20
	3	80	<5	<5	<5	<5	<5
	4	20	10	<5	<5	<5	<5
	5	20	<5	<5	<5	<5	<5
SU	6	20	10	<5	20	20	20
	7	20	20	40	20	20	20
	8	20	40	20	<5	20	20
	9	160	160	320	320	160	80
	10	20	20	20	<5	20	20

Titers of ICA are expressed in JDF units.

TABLE 3
Changes in GAD antibody status during the study

Group	GAD antibody status	
	Baseline	After study
Insulin		
1	Positive	Positive
2	Positive	Positive
3	Positive	Positive
4	Negative	Negative
5	Positive	Negative (12)
SU		
6	Positive	Positive
7	Positive	Positive
8	Negative	Negative
9	Positive	Positive
10	Negative	Negative

Number in parentheses indicates the time (month) of negative conversion of GAD antibodies.

Changes in C-peptide response, blood glucose response to OGTT, and HbA_1 during the trial. The serum C-peptide response to OGTT (Σ C-peptide) was significantly improved at 6 and 12 months after the initiation of insulin in the insulin group (Fig. 1*A*). In one patient (patient 2) with persistent ICAs, the serum C-peptide level (Σ C-peptide) changed from 3.6 to 3.1 nmol/l over the course of 30 months, with no change in insulin dose.

The serum C-peptide response to the OGTT in the SU group decreased progressively (Fig. 1*A*). Two of five SU group patients required insulin (patient 7, 25 U/day and patient 9, 24 U/day) because of the failure of treatment with secondary oral hypoglycemic agents 8 months in one (patient 7) and 24 months in the other (patient 9) after study entry. The differences in C-peptide response changes between the insulin group and the SU group were apparent from the data, which were expressed as a proportion to baseline value (Fig. 1*B*). Significant differences were noted at 6, 12, 24, and 30 months.

The 2-h blood glucose value during the OGTT tended to decrease from the baseline value in the insulin group, whereas the value increased significantly from the baseline value in the SU group (Table 4). The HbA_1 level remained unchanged in the insulin group and increased in the SU group (Table 4). The HbA_1 value in four NIDDM patients, who were initially ICA^+ and became ICA^- during the study, tended to decrease from 7.8 ± 0.4 (7.2–8.3)% to 6.9 ± 0.2 (6.7–7.1)%.

No significant change in insulin dose was seen in the insulin group. The dose of SU tended to increase in three SU group patients who had fluctuating ICA status (Table 4). No changes in body weight were observed in either group (Table 4). There were no episodes of severe hypoglycemia in either group during the study.

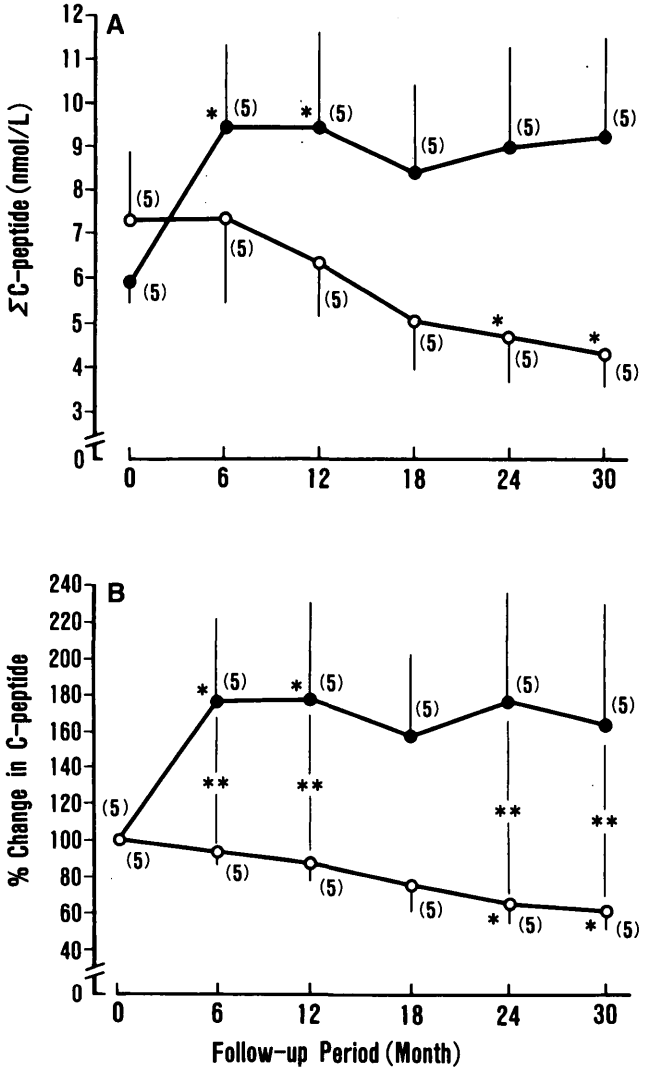

FIG. 1. *A*: changes in serum C-peptide response to an OGTT in patients with NIDDM who were initially ICA^+. They were treated with either a small dose of insulin (insulin group, ●) or SU (SU group, ○), mean ± SE. *$P < 0.05$ vs. baseline value. *B*: changes in the relative values of stimulated C-peptide response by an OGTT (percentage of value at 0 month from each point, mean ± SE) in the insulin and SU groups. *$P < 0.05$ vs. baseline value; **$P < 0.05$, insulin vs. SU groups.

DISCUSSION

The present study demonstrates beneficial effects of small doses of subcutaneous insulin on slowly progressive β-cell failure in initially ICA^+ NIDDM patients. Seroconversion of ICA status from positive to negative occurred in four of five patients shortly after initiation of insulin in the insulin group, suggesting that an immunological marker for ongoing anti–β-cell immunological activation may subside with insulin treatment. The short period before disappearance of ICAs after initiation of insulin treatment in our study was consistent with a previously reported preventive trial of IDDM patients using cyclosporin A (11). Successful results were reported with prophylactic insulin therapy in first-degree relatives of IDDM patients who were at high risk for developing overt IDDM (12), in animal experiments involving nonobese diabetic (NOD) mice and BB rats (13,14), and with intensive treatment of IDDM patients (15,16).

The mechanisms for the apparent beneficial effects of insulin with seroconversion of ICA status in our study remain unclear. One possibility is that insulin triggers a repair mechanism and/or interruption of immunological destruction of β-cells. Our previous prospective study indicated that SU treatment in ICA^+ NIDDM patients poses a risk independent from ICAs for further progression of β-cell dysfunction (3,4). Insulin therapy may reduce a risk of possibly interrupting a self-repair mechanism that sometimes occurs in early-stage IDDM (1,17). Exogenous insulin treatment resulted in β-cell growth and repair in a study of neonatal streptozotocin-induced diabetic rats (18). Exog-

TABLE 4
Changes in HbA_1, fasting blood glucose, 2-h blood glucose, BMI, and insulin dose before and after study in patients with NIDDM who were ICA^+ and treated with a small dose of insulin (insulin group) and a SU drug (SU group)

	Insulin group		SU group	
	Baseline	After study	Baseline	After study
HbA_1 (%)	7.8 ± 0.4	7.3 ± 0.9	8.5 ± 0.6	11.2 ± 1.3*
Fasting blood glucose (mmol/l)	9.6 ± 1.4	7.3 ± 1.7	9.6 ± 1.9	10.1 ± 1.4
2-h blood glucose (mmol/l)	19.1 ± 6.4	15.5 ± 4.4	17.2 ± 3.1	22.6 ± 4.2*
BMI (kg/m^2)	21.1 ± 1.5	21.4 ± 2.3	22.4 ± 2.8	22.7 ± 3.4
Insulin dose (U/day)	8 ± 5	6 ± 3	—	24†, 25‡
SU (glibenclamide, mg/day)	—	—	5.3 ± 3.2	8.8 ± 5.3

Data are means ± SD, and $n = 5$ patients in the insulin and SU groups. *$P < 0.05$ vs. baseline value. †Patient 9; ‡Patient 7.

enously administered insulin can achieve a near-normoglycemic state in NIDDM patients and set β-cells at rest, providing less islet antigen and thus preventing exaggerated islet autoimmunity. β-Cells have been shown to be more resistant to some cytotoxic insults at rest than during active secretion of insulin, as reported in a suppressed β-cell model in diazoxide-treated BB rats (19) and an interleukin 1 model (20). Unknown mechanisms such as insulin may enhance a stage of tolerance. Oral tolerance of insulin was reported in NOD mice (21). Active suppressive action of the B-chain of insulin was also reported (22). Subcutaneously administered insulin may also induce self-tolerance to islet antigen.

Although C-peptide responses were favorably affected by insulin, the 2-h blood glucose responses to an OGTT in insulin-treated patients remained diabetic at the study end. The patients in non–insulin-receiving remission induced by cyclosporin also exhibited diabetic glucose intolerance to an OGTT (23). The HbA_1 value at the study end in insulin-treated patients in our study was ~7.3% (normal <7.2%), whereas the value in the patients in non–insulin-receiving remission by cyclosporin was 6.0–8.0% (23).

Massive screening for ICAs among NIDDM patients and early initiation of small doses of insulin rather than of SU in ICA^+ patients may have widespread clinical importance because the prevalence of patients with ICAs is as high as 10% of the NIDDM population, and NIDDM patients are sometimes treated with SUs (24). The HbA_1 value attained a preferable level after negative conversion of ICAs in four NIDDM patients. However, some may continue to have diabetes in spite of the fact that they are ICA^-. Primary prevention of IDDM at an earlier stage may be needed.

Investigation of larger patient populations over longer periods of time is needed, with randomization of subjects for GAD antibodies, insulin autoantibodies, and HLA, significant predictors of the progression of β-cell dysfunction in the early stage of IDDM (25).

In conclusion, subcutaneous small doses of insulin, resulting in a high rate of negative conversion of ICA and an improved serum C-peptide response, may be effective in preventing progressive β-cell failure in ICA^+ patients with the clinical features of NIDDM (slowly progressive IDDM).

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