Linear Loss of Insulin Secretory Capacity During the Last Six Months Preceding IDDM

No effect of antiedematous therapy with ketotifen

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OBJECTIVE — To investigate the effect of an antiedematous therapy with the histamine antagonist ketotifen on β-cell function in late prediabetes.

RESEARCH DESIGN AND METHODS — In a randomized double-blind placebo-controlled study, ketotifen was administered for 3 months to 9 islet cell antibody positive (ICA^+) prediabetic patients with a first-phase insulin response (FPIR) below the 2.5th percentile to preserve residual β-cell function. Patients were followed by intravenous glucose tolerance tests (IVGTTs) every 4–6 weeks for determination of FPIR, HbA_1, ICAs, and insulin autoantibodies. In 5 patients, the immune activation state was followed by determination of serum levels of tumor necrosis factor-α (TNF-α), $β_2$-microglobulin, and C-reactive protein (CRP).

RESULTS — Seven of nine patients developed diabetes within one year of follow-up. Irrespective of treatment with ketotifen, a slow and linear decline ($P < 0.05$) of 1 + 3-min insulin values was observed in sequential IVGTTs in those 7 patients who developed insulin-dependent diabetes mellitus (IDDM) during follow-up. The 2 other patients showed wide fluctuations of the insulin response with a threefold increase of initial insulin levels. HbA_1 did not correlate with FPIR. Fasting blood glucose increased significantly during the study ($P < 0.05$). Individual levels of serum TNF-α, CRP, and $β_2$-microglobulin did not change during the study.

CONCLUSIONS — The study could not demonstrate preservation of β-cell function by ketotifen in the late stage before manifestation of clinical diabetes. Manifestation is preceded in the last 6 months by a steady loss of the FPIR without rapid deterioration immediately before diagnosis and without signs of increased immune activity.

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IDDM, insulin-dependent diabetes mellitus; ICA, islet cell antibody; FPIR, first-phase insulin response; IVGTT, intravenous glucose tolerance test; IAA, insulin autoantibodies; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; IRI, immunoreactive insulin; JDF U, Juvenile Diabetes Foundation units; HLA, human leukocyte antigen.

In general, the manifestation of insulin-dependent diabetes mellitus (IDDM) appears to be preceded by a long preclinical phase characterized by islet-specific autoimmune phenomena and some deterioration of β-cell function (1,2). This has led to earlier attempts of an immune intervention therapy before the onset of the disease by immunosuppression (3,4), immunomodulation (5), and improving β-cell regeneration (6,7). The therapeutic approach described herein was based on the observation of vascular leakage in inflamed islets of animal models such as the BB rat (8) as well as the low-dose streptozocin diabetes mouse model (9,10). In the latter, an antiallergic therapy with a serotonin/histamine antagonist (pizotifen) administered before the development of insulitis has been shown to suppress macrophage and T-cell infiltration and prevent diabetes manifestation (11). Therefore, we decided to investigate the effect of the well-established (12) histamine antagonist ketotifen on residual β-cell function in pre-IDDM. To treat only patients with a very high risk of developing diabetes, we included only normoglycemic islet cell antibody positive (ICA^+) individuals with an first-phase insulin response (FPIR) (1 + 3-min insulin value) to intravenous glucose below the 2.5th percentile.

RESEARCH DESIGN AND METHODS

— We studied 9 ICA^+ individuals (mean age 12 ± 8.4 years). The individual patient data are given in Table 1. All patients or their parents gave written, informed consent and underwent at least two intravenous glucose tolerance tests (IVGTTs) and determination of ICAs before the study. The inclusion criteria were as follows: absence of overt diabetes according to World Health Organization criteria (13), ICA titers ≥20 Juvenile Diabetes Foundation units (JDF U), FPIR (IVGTT 1 + 3-min values) below the 2.5th percentile on repeated testing, and genetic predisposition (hu-

Table 1—*Individual patient data of all patients in the ketotifen study*

Patient	Age (years)	Sex	HLA-DR	Treatment	Reasons for ICA test	ICA titer (JDF U)	Fasting blood glucose at study entry (mM)	Cause of study termination	Days to diabetes
A	3	F	3,4	Placebo	Diabetic siblings	40	4.1	End of study	—
B	18	M	7,w8	Verum	Diabetic sibling	20	3.8	End of study	—
C	31	F	3,4	Verum	Diabetic sibling	320	4.4	Blood glucose > 11.1 mM	139
D	8	M	3,4	Placebo	High normal blood glucose	80	4.8	Blood glucose > 11.1 mM	148
E	8	F	4	Verum	High normal blood glucose	80	5.8	Blood glucose > 11.1 mM	183
F	12	F	4	Placebo	High normal blood glucose	160	4.4	Blood glucose > 11.1 mM	181
G	8	F	3,4	Verum	High normal blood glucose	40	4.0	End of study	209
H	6	F	3,4	Placebo	High normal blood glucose	40	3.6	Blood glucose > 11.1 mM	98
I	14	M	4	Verum	High normal blood glucose	20	5.6	End of study	209

High-normal blood glucose is defined as 6.7 mM ≤ random capillary blood glucose ≤ 8.3 mM.

man leukocyte antigen [HLA]-DR3 and/or HLA-DR4, and/or HLA identity to a sibling with IDDM). After randomization, all patients received 2 mg of slow-release ketotifen (Sandoz AG, Basel, Switzerland) or placebo daily during the first 2 weeks in a double-blinded fashion. Thereafter, the dose was increased to 2 × 2 mg ketotifen or placebo per day in patients 14 years of age or older. Medication was discontinued after 3 months. Patients were followed for an additional 3 months without medication. IVGTTs were performed before medication and after 1, 2, 3, 4.5, and 6 months. Before each IVGTT, blood was drawn for determination of various immunological and metabolic parameters (see below). Sera were aliquoted and stored at −20°C.

All sera were tested for ICAs or insulin autoantibodies (IAAs) in one single assay to avoid interassay variation. ICAs were determined by immunofluorescence, and IAAs were determined with a fluid-phase radioimmunoassay as described previously (14,15). Our laboratory participates in the worldwide ICA proficiency test (16); the specificity was rated as 100%. A complete set of sera was available in 5 patients for the detection of tumor necrosis factor-α (TNF-α), β_2-microglobulin, and C-reactive protein (CRP). TNF-α, β_2-microglobulin, CRP, HLA typing, immunoreactive insulin (IRI), blood glucose, and HbA_1 were determined by standard laboratory methods.

The IVGTT was performed after an overnight fast between 0730 and 1000. Glucose (20%) (0.5 mg/kg body weight; maximum 35 g glucose) was given manually through an indwelling intravenous cannula within 3 min. Blood was drawn for measurement of IRI and C-peptide at 0, 1, 3, 5, 10, and 20 min after the termination of glucose infusion. The percentile of the 1 + 3-min insulin value was determined according to the IVGTTs of 100 healthy control subjects (17).

Statistical analysis

$P < 0.05$ was considered statistically significant. Where applicable, we used Wilcoxon's rank test for between-group comparisons. Data are presented as means ± SD. Statistical analyses were

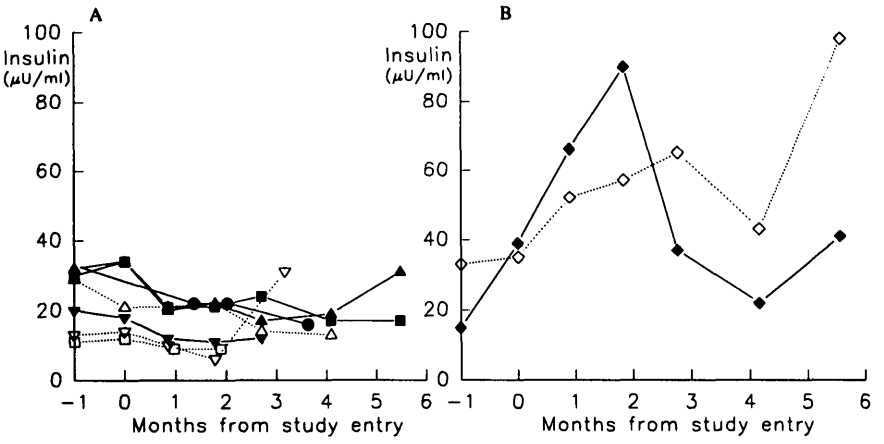

Figure 1—*IVGTT: FPIR (1 + 3-min values) during the ketotifen study for patients who developed diabetes (A) and those who did not develop diabetes (B). Each symbol represents one patient. (– – – –), Patients with placebo; (———), patients with ketotifen. Patient symbols according to letters used in Table 1: (◇), patient A; (◆), patient B; (▼), patient C; (▽), patient D; (●), patient E; (△), patient F; (■), patient G; (□), patient H; and (▲), patient I.*

performed using the SAS software package (SAS Institute, Cary, NC).

RESULTS— The IVGTT results of all patients are given in Fig. 1. For follow-up analysis, patients were divided into two groups, those who developed IDDM (n = 7) (Fig. 1*A*) and those who remained normoglycemic during further follow-up at 14 and 54 months (n = 2) (Fig. 1*B*). No difference could be detected in 1 + 3-min insulin values between the verum and placebo group (β = 0.80). Considering the 95% confidence interval for the observed difference between the verum and placebo groups, it is most unlikely that a clinically significant difference between the groups would have been detected, even with large numbers of patients. In all patients who later developed diabetes, a significant ($P < 0.05$) decline in FPIR occurred independent of treatment until manifestation of diabetes. Discontinuation of the test drug had no effect on 1 + 3-min insulin values ($P < 0.05$). Fasting blood glucose levels increased from 4.7 ± 0.8 mM at the beginning of the study to 5.4 ± 1.2 mM at the manifestation of diabetes at the end of the study ($P < 0.05$). The 2 patients who did not progress to diabetes showed major fluctuations of the FPIR (Fig. 1*B*); 1 of the 2 patients received placebo.

HbA_1 levels are given in Fig. 2. FPIR correlated negatively to HbA_1 levels ($r = -0.33$, $P < 0.02$).

ICA titers did not change significantly during the study. IAAs were positive in 3 patients only; their titer fluctuated. No correlation was noted between the decline in FPIR and ICA or IAA titers in patients with high or low titers. Serum TNF-α was elevated (>100 pg/ml) in 4 of 5 patients but did not change during the study. Serum β_2-microglobulin and CRP levels were within the normal range in all patients and remained constant. The following complaints were reported: fatigue (1 patient on verum, 2 patients on placebo), nausea (1 placebo-treated patient), increased appetite (1 verum-treated patient). The medication did not have to be interrupted in any of the subjects. All symptoms were resolved immediately after planned discontinuation of the study medication.

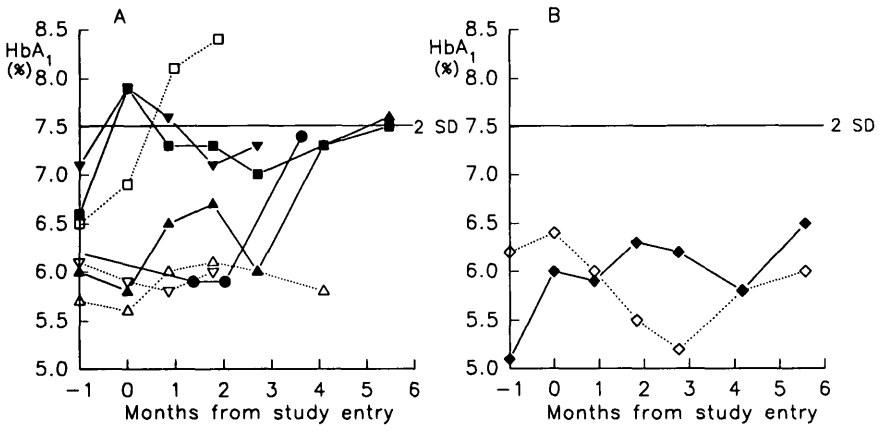

Figure 2—*HbA_1 values during the ketotifen study for patients who developed diabetes (A) and those who did not develop diabetes (B). Each symbol represents one patient. (– – –), Patients with placebo; (———), patients with ketotifen. Patient symbols according to letters used in Table 1: (◇), patient A; (◆), patient B; (▼), patient C; (▽), patient D; (●), patient E; (△), patient F; (■), patient G; (□), patient H; and (▲), patient I.*

CONCLUSIONS— The data show that the administration of ketotifen to patients at a late prediabetic state was neither able to restore nor preserve the FPIR to intravenous glucose.

The dosage of 2–4 mg ketotifen daily corresponded to maximum doses given in allergic diseases (12), and in the animal model, 5 mg/kg body weight of pizotifen was able to prevent insulitis and macrophage infiltration (11). The failure of ketotifen to protect β-cell function may have several reasons. First, the intervention may have started too late. In the animal experiments, the drug was administered before the onset of insulitis, whereas the patients received ketotifen in a late stage before manifestation of clinical diabetes. Ketotifen may not be able to inhibit or reverse peri-insular edema at a stage where physical damage of the islet vasculature may have occurred. Second, vasoactive amines have been shown to reduce peri-insular edema in animal models (8–10); however, a relevance of this finding in human diabetes has not yet been shown. Because vascular leakage in the presence of otherwise intact endothelia should quickly be inhibited by ketotifen, a beneficial effect should have been observed within a few weeks. Because our study was unable to show a preservation of β-cell function, we assume that histamine-dependent reversible endothelial changes do not play an important role in advanced islet inflammation in humans. Antiallergic intervention with common doses of ketotifen in late prediabetes thus cannot be encouraged.

Markers of specific islet autoimmunity (ICA, IAA) did not change during the study period. To assess changes in immunological disease activity we measured several markers (TNF-α, CRP, β2-microglobulin) of general inflammatory processes as well. None of these markers revealed significant changes during the study period. Therefore, we conclude that no evidence exists for an "immunological burst" immediately before the diagnosis of the disease.

This notion is supported by our observation of a slow and steady deteri-

oration of β-cell function as measured by glucose-induced insulin secretion in the patients presented herein. No evidence was found for accelerated deterioration of β-cell function in the last months before the diagnosis of IDDM. On the other hand, the 2 patients who did not develop diabetes had major fluctuations in their FPIR to intravenous glucose. In accordance with the reported high variability of IVGTT results in normal individuals (18), these data show that a prognosis of the course of individual progression to IDDM based on a single IVGTT in ICA^+ individuals is not possible. Our data suggest that the manifestation of clinical diabetes is a slow process and that the point of diabetes manifestation can hardly be distinguished from late prediabetes by the currently available tests of insulin secretion or immunological activity. In contrast to clinical practice, manifestation of IDDM does not appear to be a sharply defined incident, even when assessed by sensitive methods of islet β-cell function and immune reactivity.

In conclusion, we found no evidence for an effect of an antiedematous therapy with ketotifen on insulin secretion in late prediabetes. The progression to insulin dependency seems to be a slow process not predictable by single IVGTT values, and no evidence exists for an accelerated loss of islet β-cell function or increased immunological activity immediately before the diagnosis of insulin dependency.

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