Temporary Preservation of β-Cell Function by Diazoxide Treatment in Childhood Type 1 Diabetes

EVA ÖRTQVIST, MD, PHD[1]
ELISABETH BJÖRK, MD, PHD[2]
MÅNA WALLENSTEEN, MD, PHD[1]
JOHNNY LUDVIGSSON, MD, PHD[3]
JAN ÅMAN, MD, PHD[4]
CALLE JOHANSSON, MD[5]
GUN FORSANDER, MD, PHD[6]
FREDRIK LINDGREN, MD, PHD[7]
LARS BERGLUND, PHD[8]
MATS BENGTSSON, MD, PHD[9]
CHRISTIAN BERNE, MD, PHD[2]
BENGT PERSSON, MD, PHD[1]
F. ANDERS KARLSSON, MD, PHD[2]

OBJECTIVE — We examined the effect of diazoxide, an ATP-sensitive K^+ channel opener and inhibitor of insulin secretion, on β-cell function and remission in children at clinical onset of type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 56 subjects (21 girls and 35 boys, age 7–17 years) were randomized to 3 months of active treatment (diazoxide 5–7.5 mg/kg in divided doses) or placebo in addition to multiple daily insulin injections and were followed for 2 years.

RESULTS — Diazoxide decreased circulating C-peptide concentrations by ~50%. After cessation of the treatment, basal and meal-stimulated C-peptide concentrations increased to a maximum at 6 months, followed by a decline. Meal-stimulated C-peptide concentration was significantly higher at 12 months (0.43 ± 0.22 vs. 0.31 ± 0.26 nmol/l, $P = 0.018$) and tended to fall less from clinical onset to 24 months in the diazoxide- vs. placebo-treated patients (−0.05 ± 0.24 vs. −0.18 ± 0.26 nmol/l, $P = 0.064$). At 24 months, the meal-stimulated C-peptide concentrations were 0.24 ± 0.20 and 0.20 ± 0.17 nmol/l, respectively. Side effects of diazoxide were prevalent.

CONCLUSIONS — This study demonstrates that partial inhibition of insulin secretion for 3 months at onset of childhood type 1 diabetes suspends the period of remission and temporarily preserves residual insulin production. Further evaluation of the full potential of β-cell rest will require compounds with less side effects as well as protocols optimized for sustained secretory arrest.

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At diagnosis, children with type 1 diabetes display some endogenous insulin production, which in most cases transiently increases after the initiation of insulin therapy (1,2) and subsequently subsides. Two to 3 years after onset of disease, a majority of children have no detectable circulating C-peptide (1,3,4), whereas 11% of adult patients display residual β-cell function >5 years after start of insulin treatment (5). A partially preserved insulin production is associated with a better glycemic control in both children and adolescents (6–8) and may decrease the risk of microvascular complications and severe hypoglycemia in adults with type 1 diabetes (9–12). Therefore, partial preservation of β-cell function represents an attractive goal of therapy for intervention trials (13).

Type 1 diabetes is thought to be due to an autoimmune destruction of insulin-producing β-cells, reflected by the appearance of islet cell autoantibodies. Other researchers and we (14,15) have shown that islet cell autoantigen expression is regulated by the ambient glucose concentration and increases upon stimulation of insulin secretion (16). Moderately elevated glucose concentrations have been shown to sensitize β-cells to destruction by streptozotocin (17) and interleukin-1 (18), and we recently observed that inhibitors of insulin secretion, e.g., diazoxide and a newer potassium channel opener (NNC-118), protected islet β-cells in vitro against the toxic effect of streptozotocin (19). In experimental models of type 1 diabetes (NOD mouse, BB rat), prophylactic insulin treatment prevents or delays the onset of disease (20,21), and insulin treatment can prevent adoptive transfer of disease in the NOD mice (22). In adolescents (6) and adults (23) with recent-onset type 1 diabetes, and in C-peptide–positive young patients with longer duration of disease (9), intensive insulin treatment was associated with improvements of insulin secretion. The prevailing data thus indicate that the destruction of islets is dependent upon the functional activity of the insulin-producing cells.

Possibly, the remission phenomenon reflects a reduced destruction of β-cells and/or recovery from "glucotoxicity" (24). In adults with recent-onset disease, we previously observed that 3-month treatment with diazoxide increased residual insulin secretion (25), which was a

From the [1]Department of Woman and Child Health, Astrid Lindgrens Children's Hospital, Karolinska Institutet, Stockholm, Sweden; the [2]Department of Medicine, University Hospital, Uppsala, Sweden; the [3]Department of Pediatrics, University of Linköping, Linköping, Sweden; the [4]Department of Pediatrics, Örebro, Sweden; the [5]Department of Pediatrics, Jönköping, Sweden; the [6]Department of Pediatrics, Falun, Sweden; the [7]Department of Woman and Child Health, Sachs' Children's Hospital, Stockholm, Sweden; the [8]Uppsala Clinical Research Centre, University of Uppsala, Uppsala, Sweden; and the [9]Department of Clinical Immunology, University of Uppsala, Uppsala, Sweden.

Address correspondence and reprint requests to Eva Örtqvist, Astrid Lindgrens Children's Hospital, Q2:05, Karolinska Hospital, S-171 77 Stockholm, Sweden. E-mail: eva.ortqvist@kbh.ki.se.

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Abbreviations: IA-2Ab, IA-2 antibody; ICA, islet cell antibody.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

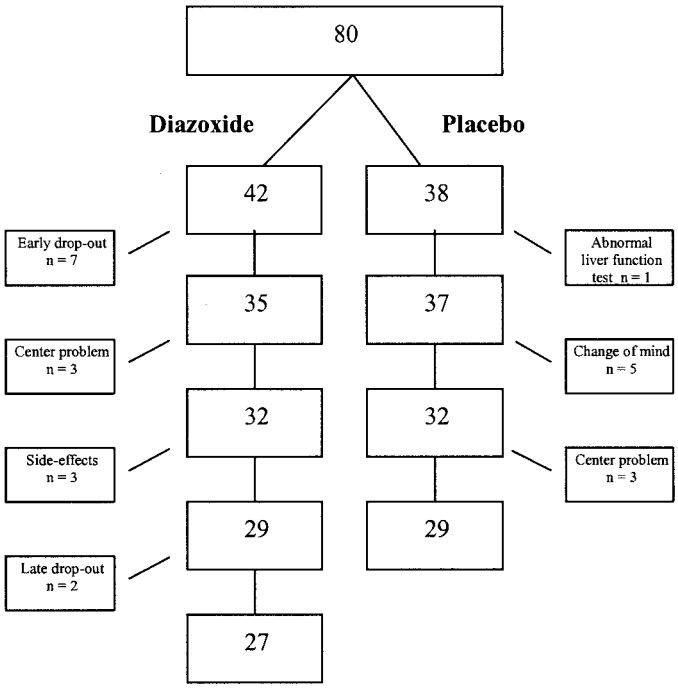

Figure 1—*Flow diagram of children with clinical onset of type 1 diabetes randomized to 3 months of treatment with diazoxide or placebo in addition to multiple daily insulin injections.*

result that prompted an investigation of younger patients.

RESEARCH DESIGN AND METHODS

— Patients were recruited from eight pediatric diabetes centers in central Sweden between November 1995 and March 1998 and were followed for 2 years until March 2000. During these periods, 212 children (119 boys), age 7–17 years old, were diagnosed at the participating centers. Patients and their parents were given oral and written information about the study and an invitation to participate. Children were included within 1 week (median 6 days) of the first insulin injection. Exclusion criteria were endocrine or other chronic diseases requiring regular medication. The protocol was approved by the local ethics committee at each hospital and by the Medical Products Agency, Uppsala, Sweden.

The study had a parallel group design. Primary end points were fasting and stimulated serum C-peptide concentrations at 12 and 24 months. Secondary end points were the changes in basal and stimulated C-peptide from baseline to 12 and 24 months. Calculation of the required sample size was based on a prospective study of 81 new-onset type 1 diabetic children, who had a meal-stimulated C-peptide at 12 months' diabetes duration of 0.20 ± 0.19 nmol/l (E.Ö., B.P., unpublished data). To be able to demonstrate a difference in stimulated serum C-peptide of 0.15 nmol/l, between study groups, at a 5% significance level and with 80% power, 25 patients should be included in each group. An intention-to-treat analysis was considered, i.e., an analysis where all randomized patients are kept in the analysis and the values for the noncompleters are set according to a worst case scenario. As C-peptide production is a complicated function of time, it is difficult to say what the worst case would be at 24 months for a patient that drops out at, e.g., 2 months. Therefore, we choose not to perform an intention-to-treat analysis in this study.

Initially, 80 children were randomized to double-blind treatment with either diazoxide (Avondale, Rathdrum, Ireland) (5–7.5 mg/kg body wt, given in divided doses three time daily with meals [$n = 42$]) or placebo ($n = 38$). Early dropouts were due to change of mind in the first week and refusal to participate to difficulties with swallowing of the capsules, unpleasantness caused by the blood sampling, or stress related to the recent diagnosis of diabetes (Fig. 1). Two centers were unable to participate throughout the study, and their patients were excluded. Altogether, the 56 patients (21 girls and 35 boys) from six centers who completed the study were examined at inclusion and at the 1-, 2-, 3-, 6-, 9-, 12-, 18-, and 24-month follow-up. The children were treated with three to six daily injections of human insulin or insulin lispro at meals and basal NPH insulin one to two times daily. All subjects performed home blood glucose monitoring.

At inclusion, a thorough physical examination, including blood pressure and evaluation of pubertal development, was done together with analysis of hemoglobin, blood cell counts, glycemic control, electrolytes, liver enzymes, thyroid hormones, and antibodies against gliadin and endomysium. The fasting venous blood samples, before medication, were repeated at 1, 2, 3, and 6 months in the study. A standardized meal test was used for analysis of blood glucose, fasting, and stimulated serum C-peptide at 1, 2, 3, 6, 12, 18, and 24 months' duration of diabetes, with the exception of 8 diazoxide- and 12 placebo-treated children at 18 and 24 months, who were only sampled in the fasting state. HbA_{1c}, insulin requirements, and longitudinal growth were recorded at each visit. Autoantibodies to GAD (GAD65Ab) and protein tyrosine phosphatase (IA-2 antibody [IA-2Ab]) were determined throughout the study. Islet cell antibodies (ICAs) and HLA type were analyzed at baseline.

Serum C-peptide concentrations were measured by radioimmunoassay (Delphia, Wallac, Sweden) with a lowest detection level of 0.01 nmol/l. All samples from a given individual were analyzed in the same assay run. After omission of morning insulin, venous blood for C-peptide measurements was drawn in the fasting state and 90 min after the start of a standardized meal containing 20% of the calculated daily caloric intake (1). Insulin was then injected after the last blood sample.

ICAs were determined by indirect immunofluorescence assay, autoantibodies to GAD65 by a radioimmunoassay (Mercodia, Uppsala, Sweden), and autoantibodies to the tyrosine phosphatase–like protein IA-2 by an immunoprecipitation assay (26).

HbA_{1c} was analyzed at each center and HbA_{1c} values calibrated against the Swedish standard high-performance liquid chromatography method (MonoS) (reference value for nondiabetic subjects

Table 1—*Clinical characteristics at baseline and at 1 and 2 years in children with type 1 diabetes treated with diazoxide* (n = 27) *or placebo* (n = 29)

Parameters	Baseline		1 year		2 years	
	Diazoxide	Placebo	Diazoxide	Placebo	Diazoxide	Placebo
Age (years)	11.3 ± 2.6	11.6 ± 2.6	—	—	—	—
Sex (boys/girls)	18/9	17/12	—	—	—	—
HbA_{1c} (%)	10.3 ± 2.1	10.0 ± 2.6	6.3 ± 1.3	6.9 ± 1.7	7.1 ± 1.6	7.3 ± 1.8
Insulin dose (units/kg$^{-24\ h}$)	1.09 ± 0.51	1.10 ± 0.40	0.71 ± 0.32	0.80 ± 0.26	0.85 ± 0.29	1.00 ± 0.25
GAD65Ab*	18/27	19/29	14/26	17/29	16/27	14/28
IA-2Ab†	21/27	18/29	17/27	13/29	14/27	12/29
ICA‡	24/27	25/29	ND	ND	ND	ND
C-peptide (nmol/l)						
Fasting	0.17 (0.12/0.22)	0.16 (0.11/0.20)	0.27 (0.20/0.34)	0.21 (0.16/0.26)	0.18 (0.12/0.24)	0.14 (0.10/0.19)
Stimulated	0.29 (0.18/0.39)	0.30 (0.21/0.38)	0.43 (0.35/0.52)§	0.31 (0.21/0.41)	0.24 (0.15/0.34)‖	0.20 (0.11/0.29)¶
Baseline to 1 year						
Δ fasting			0.10 (0.03/0.17)	0.05 (0.00/0.10)		
Δ stimulated			0.14 (0.03/0.26)	0.02 (−0.09/0.12)		
Baseline to 2 year						
Δ fasting					0.01 (−0.04/0.06)	−0.01 (−0.06/0.04)
Δ stimulated					−0.05 (−0.17/0.07)	−0.18 (−0.31/−0.04)

Data are mean ± SD and, for C-peptide concentrations, 95% CI. Antibodies, positive/total number analyzed; ND, not determined. *GAD65Ab, antibodies of the 65 kDa isoform of GAD; †IA-2Ab, antibodies of the protein tyrosine phosphatase–related IA molecule; ‡ICA, islet cell antibodies; §P = 0.018; ‖n = 19; ¶n = 17.

<5.2%) (27). Diazoxide was determined in serum by the assistance of Novo Nordisk (Bagsværd, Denmark). HLA-DQB1 typing was performed by PCR-SSP (sequence-specific primer) (28) or by direct sequencing of exon 2.

Statistical analysis

The distribution of the end points was examined with Shapiro-Wilk's test. When a W value from this test was ≥0.95, the distribution was considered normal. When the W value was <0.95, a logarithmic transformation was performed. The two groups were then compared using the unpaired Student's t test in which a two-sided P value of <0.05 was considered a statistically significant result.

RESULTS

Patient characteristics, C-peptide concentrations

Patient characteristics are shown in Table 1. Islet antibodies (GAD65Ab and/or IA-2Ab and/or ICA) were present in all but two cases. The frequencies of GAD65Ab and IA-2Ab tended to decline during the study, with no difference between the diazoxide- and placebo-treated groups. At inclusion, ketonuria was observed in 37 of the 56 patients and basal and meal-stimulated C-peptide above the limit of detection in 54 of 56 and 55 of 56 case subjects, respectively. The C-peptide concentrations among the patients varied at inclusion as well as during the study period (Fig. 2). Two children, one in each treatment group, had low C-peptides throughout the investigation. When the diazoxide-treated group was compared with placebo at 1, 2, and 3 months, the basal and stimulated C-peptide concentrations, determined 8 h after dosing, were lowered on average by 50, 45, and 24% and 54, 51, and 31%, respectively. In the placebo group, there were, in general, transient rises in C-peptides after the start of insulin therapy, whereas in the diazoxide-treated group, the pattern was different, and the C-peptide values were suppressed in most cases during the period of active treatment. The meal-stimulated/fasting C-peptide ratio in the placebo group was 2.22 (range 0.90–5.80) during the 3 months and 1.90 (0.86–4.00) in the diazoxide-treated group.

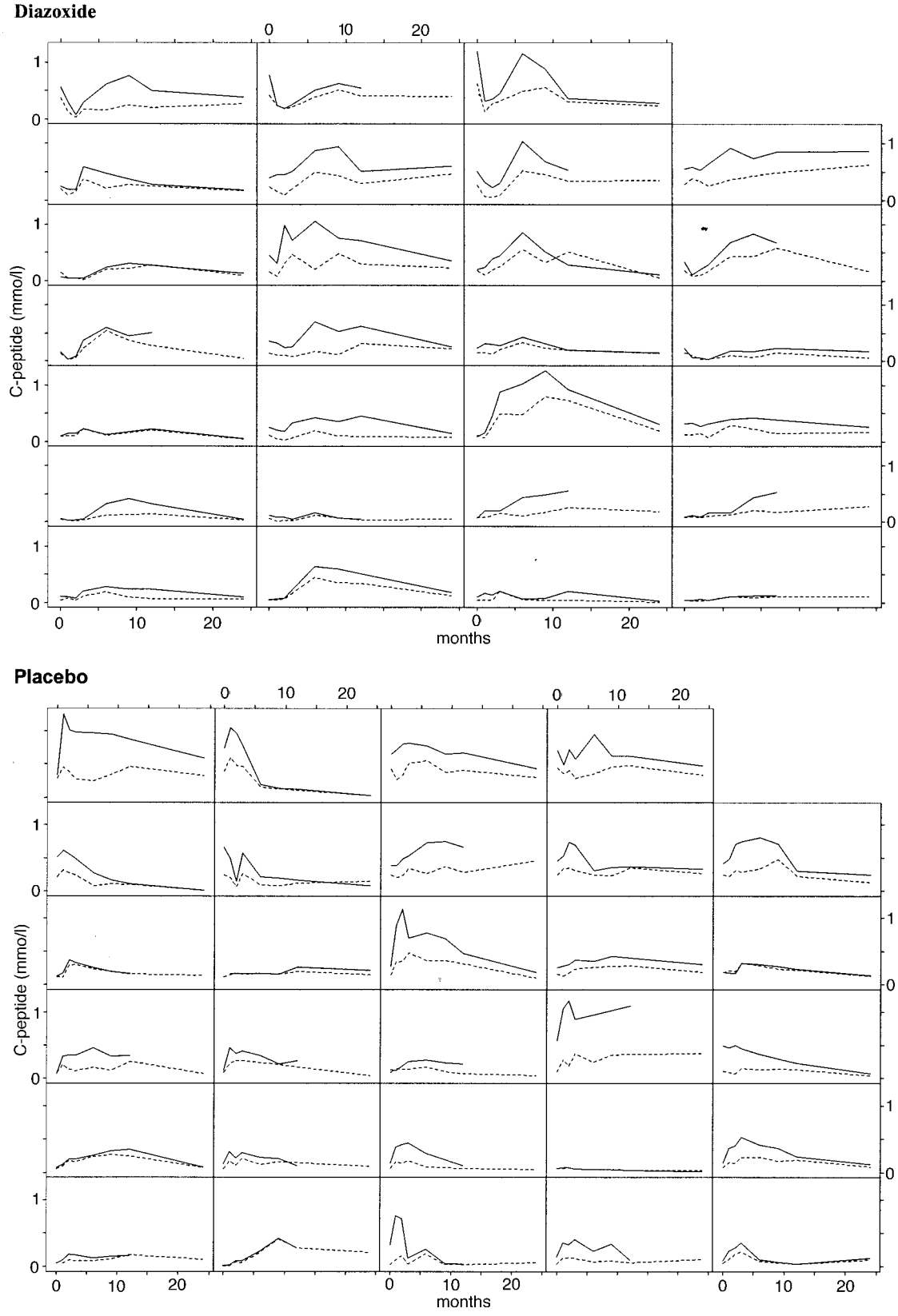

Figure 2—*Fasting (broken line) and meal-stimulated (solid line) C-peptide concentrations over 24 months in 56 children with new-onset type 1 diabetes treated with diazoxide* (upper panel, n = *27) and placebo* (lower panel, n = *29) for 3 months after diagnosis. The data were sorted by fasting C-peptide at baseline from bottom left to top right.*

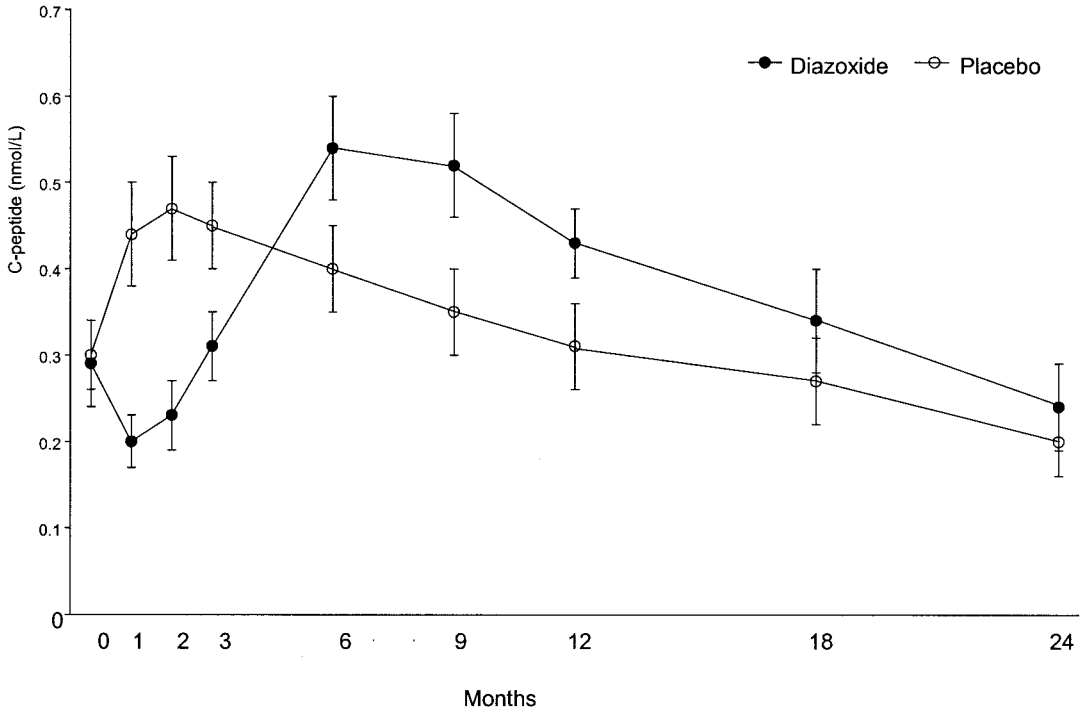

Figure 3—*Meal-stimulated C-peptide concentrations (mean ± SE) in diazoxide (●)- and placebo (○)-treated groups from study start and during 24 months of follow-up (E.Ö., B.P., unpublished data).*

Mean C-peptide pattern

At the end of the diazoxide treatment (3 months of the study), the basal and stimulated mean C-peptide concentrations were 0.18 ± 0.13 and 0.31 ± 0.21 nmol/l and increased to maximum values 6 and 3 months later (0.29 ± 0.19 and 0.54 ± 0.33 nmol/l), respectively. Subsequently, the mean values of basal and stimulated C-peptide concentrations were reduced and reached the values similar to the 3-month time point at 24 and 18 months, respectively. In the placebo group, basal and stimulated C-peptide values at the start of the study (0.16 ± 0.12 and 0.30 ± 0.23 nmol/l) increased to maximum values 3 and 2 months later (0.24 ± 0.12 and 0.47 ± 0.32 nmol/l) and returned to values similar to the baseline after 24 and 12 months, respectively. The meal-stimulated C-peptide concentrations (Fig. 3) were higher in the diazoxide-treated group compared with placebo at 12 months ($P = 0.0177$) but not at 24 months. The decline in stimulated serum C-peptide from clinical onset to 24 months tended to be less in the diazoxide-treated patients (−0.05 ± 0.24 vs. −0.18 ± 0.26 nmol/l, $P = 0.064$).

Insulin doses, glycemic control

Daily insulin doses were adjusted according to the patient's self-monitoring of blood glucose. The doses were similar in the two groups at inclusion (Table 1) and did not differ during the drug treatment period (e.g., at 2 months 0.63 ± 0.34 and 0.59 ± 0.21 units/kg$^{-24\ h}$ in the diazoxide- and placebo-treated groups, respectively). Long-term glycemic control and insulin requirement did not differ between the diazoxide and placebo groups (Table 1).

Diazoxide, adverse effects

Median serum levels of diazoxide (8 h after last dosing) were 50, 43, and 51 μg/ml at 1, 2, and 3 months, respectively. One patient at 2 months and three patients at 3 months had no detectable diazoxide in serum. Side effects were seen in 26 of the 35 subjects exposed to diazoxide. The most common adverse effect was increased hair growth on the face, arms, legs, and back, which disappeared in all instances within 6 months after termination of the treatment. Two patients stopped treatment after 2 and 2.5 months due to increased hair growth, but continued in the study. Severe edema during the first week occurred in two patients who discontinued the study. Four patients were given a thiazide diuretic during the treatment period. There were no changes in electrolytes or blood pressure. One boy had an increased eosinophil count in tests at 2 and 6 months (29). One boy discontinued the study after 2 months due to a short-term rash of unexplained origin. No side effects were reported in the placebo group.

CONCLUSIONS

— In the present study, the diazoxide regimen partially suppressed endogenous insulin production, delayed the remission phenomenon, and transiently enhanced the residual β-cell function. This indicates that the β-cell loss is influenced by the secretory process and lends support to trials with β-cell rest (30). Previously, inhibition of β-cell activity has been examined in short-term trials with intensive insulin treatment (6), octreotide (a somatostatin analog inhibiting insulin release) (31), or diazoxide (a potassium channel opener inhibiting insulin release). Two weeks of intravenous intensive insulin treatment was associated with significant improvement in C-peptide at 1 year (6). Three

weeks of treatment with octreotide in 10 children with new-onset diabetes resulted in higher glucagon-stimulated C-peptide concentrations at 6 and 12 months compared with control patients (31). In 1976, Greenwood et al. (32) administered diazoxide to 10 diabetic subjects for 7 days and reported on increased stimulated insulin responses. Previously, we performed a randomized study of 3 months of diazoxide treatment in young adults and observed long-term preservation of residual insulin secretion. In the present study, few of the children achieved stimulated C-peptide concentrations in the range of healthy control subjects (basal C-peptide 0.50 ± 0.14 nmol/l and stimulated 1.16 ± 0.39 nmol/l, $n = 57$, mean age 12 years, E.Ö., B.P., unpublished data). The placebo group of young adults (25) reached a fasting serum C-peptide concentration of 0.25 ± 0.04 nmol/l in comparison with 0.21 ± 0.13 nmol/l in the children of the present study. The diazoxide-treated adult patients achieved a fasting serum C-peptide at 12 months of 0.40 ± 0.04 nmol/l compared with 0.27 ± 0.17 nmol/l in the children, illustrating that the children had less benefit from diazoxide treatment compared with the adults. At a follow-up of a mean duration of 4 years, the latter still showed higher C-peptide concentrations in the diazoxide-treated group compared with placebo (E.B., C.B., F.A.K., unpublished data). Remarkably, in the present as well as in the previous study, the diazoxide treatment was not associated with increased insulin doses. This finding deserves further investigation and may reflect an increase in insulin sensitivity during active treatment.

Children compared with adults appear to have a more aggressive autoimmune destructive process with shorter periods of symptoms before referral to hospital (33) and lower C-peptide levels at diagnosis. Age also affects the association with HLA, as the high-risk genotypes are more frequent in young patients (33). In the present study, we did not observe any association between HLA-DQB1 genotypes and response to treatment (data not shown). In children and adolescents, most studies have found a relationship between the early loss of β-cell function and young age (1,4,34,35) and regarding the magnitude of insulin resistance during growth and the course of puberty (7,36). In the present study, several of the adolescents had high HbA_{1c} already at the 1-year follow-up.

The administration of diazoxide was accompanied by easily recognized side effects. This may have revealed a treatment alternative to patient, family, and physician, which in turn may have biased the outcome. The mean insulin doses after cessation of treatment tended to be lower in the diazoxide group, however, arguing against the use of a more intensive insulin treatment in the patients who received diazoxide.

In conclusion, the results demonstrate that inhibition of endogenous insulin secretion through ATP-sensitive K^+ channel opening at the onset of clinical type 1 diabetes in children and adolescents has the capacity to preserve residual β-cell function over several months and postpone the remission phenomenon. The suppression of β-cell function was partial and not always achieved, as reflected by various levels of C-peptide during the treatment period. The high rate of adverse, albeit mild, side effects and the less positive results compared with adults makes diazoxide treatment a less attractive alternative in treatment of children. New ATP-sensitive K^+ channel openers, with higher specificity (37) and less side effects, added on to optimal insulin treatment with glucose control, or in combination with new immunosuppressive regimens, should be of interest for a further evaluation of the full potential of β-cell rest to preserve residual insulin secretion in early type 1 diabetes.

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