Effects of Nicotinamide and Intravenous Insulin Therapy in Newly Diagnosed Type 1 Diabetes

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OBJECTIVE — To investigate the effect of intravenous insulin therapy combined with nicotinamide in the metabolic control and β-cell function of newly diagnosed type 1 diabetic subjects in comparison with intensive insulin therapy and nicotinamide alone.

RESEARCH DESIGN AND METHODS — A total of 34 newly diagnosed type 1 diabetic patients were included. After the correction of initial metabolic disturbances, subjects were randomly assigned to the following three groups within 72 h after admission: 1) intensive insulin therapy + placebo (C) (n = 12); 2) intensive insulin therapy + nicotinamide, 700 mg three times a day (NIC) (n = 11); and 3) 72-h intravenous insulin followed by intensive insulin therapy + nicotinamide, 700 mg three times a day (NIV) (n = 11). The subjects were monitored for 12 months. GAD, tyrosine phosphatase antibodies, and insulin autoantibodies were measured. C-peptide was measured basally and after 2, 4, 6, 8, and 10 min of 1 mg intravenous glucagon. HbA1c, glucagon, and antibody measurements were determined initially and at 1, 3, 6, 9, and 12 months.

RESULTS — HbA1c values declined to normal after treatment was initiated in all groups and remained not significantly different during the follow-up period. We did not find differences between experimental (NIC and NIV) and placebo (C) groups in terms of β-cell function, considering basal or glucagon-stimulated C-peptide (maximal stimulated C-peptide and area under the curve [AUC] of C-peptide) values during the follow-up period. After pooling data from the NIC and NIV groups (both including nicotinamide) and comparing it with data from the C group, the results remained unchanged. At diagnosis, GAD positivity was observed in 10 of 12, 8 of 11, and 10 of 11 subjects (NS) in the C, NIC, and NIV groups, respectively, and IA2 positivity was observed in 3 of 12, 4 of 11, and 4 of 11 subjects (NS) in the C, NIC, and NIV groups, respectively. Antibody titers displayed a similar behavior in all groups during the follow-up period.

CONCLUSIONS — Our pilot study failed to demonstrate that the addition of 72-h intravenous insulin and nicotinamide to conventional intensive insulin therapy produces any beneficial effect in newly diagnosed type 1 diabetic subjects in terms of β-cell function and metabolic control.

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According to the recent report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, type 1 diabetes encompasses all forms of autoimmune-mediated type 1 diabetes and idiopathic β-cell destruction, finally leading to complete insulin deficiency (1). Several attempts have been made to preserve β-cells from complete destruction by applying experimental therapies in newly diagnosed type 1 diabetes, including insulin and nicotinamide (2–4). The preservation of insulin secretion capacity would allow us to maintain a better metabolic control and reduce the risk not only of acute metabolic disturbances but also of chronic complications.

There are various studies demonstrating the beneficial, although transient, effect of intensive insulin treatment in β-cell function through the induction of β-cell rest (3). Recently, Schnell et al. (5) demonstrated that high-dose intravenous insulin infusion and intensive insulin therapy as initial treatments for newly diagnosed type 1 diabetic subjects, were equally effective in preserving insulin secretion capacity after a 1-year follow-up period.

Nicotinamide has also been tested for its potential benefit in the protection of β-cell destruction in newly diagnosed type 1 diabetic patients (4). Likewise, this drug has been used in intervention trials undertaken to prevent type 1 diabetes in high-risk subjects (6,7). Both types of studies are based on the observation that nicotinamide can protect β-cells from immunologically destructive events (8).

Until now, the combination of both intravenous insulin therapy and nicotinamide has not been evaluated and compared with a conventional intensive insulin therapy. Considering that insulin and nicotinamide act with different and potentially complementary effects, our pilot study aimed to investigate the effect of intravenous insulin therapy combined with nicotinamide in the metabolic control and β-cell function of newly diagnosed type 1 diabetic subjects in comparison with the results obtained with conventional intensive insulin therapy and nicotinamide alone.
Table 1— Clinical characteristics at onset of the study groups

<table>
<thead>
<tr>
<th></th>
<th>C group</th>
<th>NIC group</th>
<th>NIV group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.4 ± 4.8</td>
<td>26.3 ± 6.8</td>
<td>21.6 ± 2.3</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/6</td>
<td>6/5</td>
<td>6/5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 ± 3.9</td>
<td>21.2 ± 2.8</td>
<td>19.2 ± 3.2</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>10.2 ± 2.2</td>
<td>9.4 ± 2.1</td>
<td>9.8 ± 2.0</td>
</tr>
<tr>
<td>Weeks to diagnosis</td>
<td>7.3 ± 2.7</td>
<td>7.1 ± 3.9</td>
<td>4.7 ± 3.3</td>
</tr>
<tr>
<td>H/K/K/A</td>
<td>1/8/3</td>
<td>0/8/3</td>
<td>1/7/3</td>
</tr>
</tbody>
</table>

Data are n or means ± SD. H/K/KA, hyperglycemia/ketosis/ketoacidosis.

RESEARCH DESIGN AND METHODS

Patients
A total of 34 newly diagnosed type 1 diabetic patients admitted to our unit were included in the pilot study. The study protocol was approved by the Hospital Clinic Universitari ethics committee and informed consent was obtained from all of the patients or their parents if needed. Type 1 diabetes was diagnosed according to the National Diabetes Data Group criteria (9). After the correction of initial metabolic disturbances, subjects were randomly assigned to one of the following three groups within 72 h after admission: 1) intensive insulin therapy + placebo for 12 months (control [C] group, n = 12); 2) intensive insulin therapy + nicotinamide, 700 mg three times a day (total daily dose of 2.1 g) for 12 months (NIC group, n = 11); and 3) 72-h intravenous insulin therapy followed by intensive insulin therapy + nicotinamide, 700 mg thrice times a day (total daily dose of 2.1 g) for 12 months (NIV group, n = 11). All patients received a diet adjusted to their age and BMI.

Intravenous insulin therapy
Those subjects assigned to the NIV group were started on a 72-h continuous intravenous insulin infusion using an external and portable pump carrying a syringe with 40 U of regular insulin diluted in 39 ml of a 0.9% sodium chloride solution. Infusion was performed through a peripheral catheter into the antecubital vein. Insulin was started at 0.03 U·kg⁻¹·h⁻¹, and the dosage was adjusted to maintain preprandial blood glucose levels between 3.3 and 5.5 mmol/l, 1-h postprandial levels <8.3 mmol/l, and 2-h postprandial levels <7.2 mmol/l. After the 72-h intravenous insulin treatment, patients were switched to an intensive insulin therapy schedule.

Intensive insulin therapy
The intensive insulin therapy schedule consisted of insulin administered subcutaneously, three or four doses daily as required. Regular insulin was given before meals and NPH insulin was given before dinner or at bedtime. Insulin doses were adjusted in every patient to maintain preprandial glucose levels between 3.9 and 7.0 mmol/l and postprandial glucose levels <10 mmol/l based on four to six daily capillary blood glucose determinations. During the admission period, all patients were included in a 5-day education program for newly diagnosed type 1 diabetic subjects.

Antibody measurements
GAD antibodies (GADAbs), tyrosine phosphatase antibodies (IA2Abs), and insulin autoantibodies (IAA) were measured. GADAbs were determined by a radiobinding assay and were considered positive at >2 U/ml. The assay for GADAb in the second GAD proficiency test achieved 100% sensitivity and 100% specificity. IA2Abs titers were measured using a radioimmunoassay and considered positive at >0.8 U/ml. The interassay and intra-assay coefficient of variation (CV) of IAA determination were 7 and 5%, respectively. The upper limits of normal values for GADAb and IA2Ab were defined by the 99th percentile of antibodies measured in 110 non diabetic subjects without familial history of type 1 diabetes. IAA were measured using a radiobinding method. Upper normal limit (1%) was defined after the analysis of 500 samples from healthy control subjects. Interassay CV for IAA was 12%.

Assessment of pancreatic β-cell function
A glucagon test was performed in the absence of hypoglycemia in the previous 48 h and only when fasting blood glucose values were between 5.0 and 8.0 mmol/l. Plasma C-peptide measurements were performed basally and after 2, 4, 6, 8, and 10 min of the intravenous injection of 1 mg of glucagon. C-peptide was determined using a radioimmunoassay (limit of detection 0.033 nmol/l; intra-assay CV 2.6%; interassay CV 4.4%) and a commercially available kit (Bick Santeg, Dietzenbach, Germany). Basal and maximally stimulated C-peptide and the area under the curve (AUC) were used as β-cell function parameters during the glucagon test.

Follow-up period
Patients were visited by the same team every 2 weeks during the first 3 months and at 6, 9, and 12 months. At each visit, weight, daily insulin dose, and hypoglycemic episodes were recorded and patients were again instructed in glucose goals. Glucagon and antibody measurements were determined initially and at 1, 3, 6, 9, and 12 months. HbA₁c was determined by chromatography (high-performance liquid chromatography, HA 8121; Menarini Diagnostici, Firenze, Italy) at the same intervals (normal range 3.4–5.5%).

Statistical analysis
Results are presented as means ± SD. An analysis of variance considering repeated measures, with time and treatment as covariates, was used for multiple comparisons. A P value <0.05 was considered statistically significant. All statistical calculations were performed by the Statistical Package for Social Science. The empiric power of our study (considering the comparison of the maximal stimulated C-peptide, month 1) was calculated using a bootstrap process STATA (Stata Statistical Software, Release 6.0; StataCorp, College Station, TX).

RESULTS — A total of 34 subjects (median age and range for each group were as follows: C group, 23.5 [16–32]; NIC group, 29.0 [16–35]; NIV group, 22.0 [17–25]) were studied. The clinical characteristics of the three experimental groups are shown in Table 1. During the 72-h intravenous insulin treatment period, those subjects in the NIV group received on average a 16% higher dose of insulin than those included in the C and NIC groups (0.73 ± 0.2, 0.75 ± 0.2, and 0.85 ± 0.3 U/kg body weight for the C, NIC, and NIV groups, respectively). As shown in Fig. 1, during the follow-up period, the three experimental therapy groups received comparable insulin doses.
On average, HbA1c values declined toward normal shortly after treatment was initiated in all groups. They were not significantly different at any time during the follow-up period (Fig. 2). None of the patients included in the protocols (C, NIC, NIV) experienced severe hypoglycemic episodes requiring assistance during the follow-up period. After 12 months of treatment, BMI increased similarly in all three groups.

Fasting C-peptide (C group, 0.2 ± 0.17; NIC group, 0.24 ± 0.12; and NIV group, 0.19 ± 0.10 nmol/l), maximal-stimulated C-peptide (C group, 0.39 ± 0.21; NIC group, 0.45 ± 0.18; and NIV group, 0.35 ± 0.16 nmol/l), and AUC of stimulated C-peptide (C group, 3.3 ± 1.8; NIC group, 3.9 ± 1.7; and NIV group, 3.1 ± 1.3 nmol/l) were comparable at basal time in the three groups. We could not find differences between experimental (NIC and NIV groups) and placebo (C group) groups in terms of β-cell function, considering basal or glucagon-stimulated C-peptide values during the follow-up period (power 62.9%, IC 59.8–65.9). After pooling the data from the NIC and NIV groups (both including nicotinamide) and comparing with those from the C group, the results remained unchanged. In Fig. 3, the follow-up of maximal stimulated C-peptide values is shown.

At diagnosis, GAD positivity was observed in 10 of 12, 8 of 11, and 10 of 11 subjects (NS) in the C, NIC, and NIV groups, respectively, and IA2 positivity was observed in 3 of 12, 4 of 11, and 4 of 11 subjects (NS) in the C, NIC, and NIV groups, respectively. Antibody titers displayed a similar behavior in all groups during the follow-up period, including IAA.

**CONCLUSIONS** — Our pilot study failed to demonstrate that the addition of 72-h intravenous insulin therapy and 1-year nicotinamide to conventional intensive insulin therapy produces any beneficial effect in newly diagnosed type 1 diabetic subjects in terms of β-cell function and metabolic control.

Insulin has been considered potentially useful in the prevention of islet destruction (10). This assumption is based on the concept that suppression of β-cell function may render insulin-producing cells less susceptible to immunological mediated destruction because of lower expression of putative type 1 diabetes autoantigens on resting β-cells that are therefore less immunogenic (11). Accordingly, insulin prophylaxis prevents diabetes development in the BB rat and NOD mouse models of human type 1 diabetes (10,12). Nicotinamide has also been investigated as a tool to protect islets cells from destruction, diminishing the incidence of type 1 diabetes (13). This beneficial action is related to its capacity counteracting the deleterious effect on islets of free radicals, especially nitric oxide, and some cytokines (14,15). In our study, we combined 1-year nicotinamide with a 72-h intravenous insulin period of treatment shortly after the clinical onset of type 1 diabetes, looking for synergistic and/or additive effects of both therapeutic tools.

Intravenous high-dose insulin treatment during 2 weeks was reported as effective in improving β-cell function when compared with conventional insulin therapy in newly diagnosed adolescent type 1 diabetic subjects (3). More recently Schnell et al. (5) failed to demonstrate in adult patients that high-dose intravenous insulin infusion, as initial treatment of newly diagnosed type 1 diabetic subjects, was related to a better preservation of β-cell function than that obtained by an intensive insulin therapy during the first year of treatment. In our study, despite those subjects in the NIV group who received on average a 16% higher dose of insulin (72-h intravenous insulin period) than those included in the C and NIC groups (0.73 ± 0.2, 0.75 ± 0.2, and 0.85 ± 0.3 U/kg body weight in the C, NIC, and NIV groups, respectively), this amount of insulin was far from that used by Shah et al. (3) using the biostator for 2 weeks than that used by Schnell et al. (5). We did not measure urinary C-peptide excretion during the intravenous insulin period in any subject to elucidate whether the 72-h intravenous insulin period provided a significant repose in β-cell function. Nevertheless, because of its CV, changes in this parameter have a restrained
value in illustrating β-cell rest (16). In absolute terms, we can conclude that our results after a 1-year follow-up period are in agreement with those derived from the study by Schnell et al., considering that we could not demonstrate differences between all three experimental therapies either in terms of metabolic control nor in preserving insulin secretion capacity. However, it should be pointed out that β-cell function of our group of subjects at diagnosis was on average 50% lower than that observed by Schnell et al., ketoacidosis being the clinical diagnosis at onset in 9 of 34 subjects. Patients lacking C-peptide are known to respond poorly to therapies addressed to β-cell function at diagnosis (17,18). We did not use C-peptide at diagnosis as an inclusion criteria, but only one subject (NIV group) lacked a detectable C-peptide when included. On the other hand, and despite basal C-peptide at onset of the disease, after the 1-year follow-up, glucagon-stimulated C-peptide concentration was on average equivalent or better than that obtained in trials using other therapeutic approaches, including nicotinamide and cyclosporine, whichever group the patients were allocated to (4,19,20).

During the follow-up period, we could not demonstrate any difference in terms of autoantibody profile in the three experimental groups. Thus, none of these treatments either modulated or induced autoantibody-mediated immune mechanisms against β-cells when our selected experimental therapies were applied after clinical onset of type 1 diabetes. This resembles those studies aimed at preventing type 1 diabetes in high-risk subjects by making preventive attempts in presumable earlier stages of the disease, where autoantibodies also remained mostly unchanged (21,22).

In conclusion, intravenous insulin + nicotinamide, or nicotinamide alone, added to conventional intensive insulin therapy was as effective as the latter alone in preserving β-cell function and metabolic control during the first year after the onset of type 1 diabetes.

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Nicotinamide and intravenous insulin at onset of type 1 diabetes

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