The Switch from Sulfonylurea to Preprandial Short-Acting Insulin Analog Substitution Has an Immediate and Comprehensive β-Cell Protective Effect in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: Supplementary insulin therapy provides assistance to meal-time insulin secretion in patients with type 2 diabetes and may have protective effects on β-cell function.

Methods: This study explored the immediate effect of supplementary insulin therapy on β-cell function in patients with glimepiride monotherapy (five women, 15 men; 61.8 ± 6.4 years old; body mass index, 31.1 ± 4.4 kg/m²; hemoglobin A1c, 7.0 ± 1.3%). After 1 week of continued glimepiride therapy, the patients were randomized either to continue with their oral treatment or to switch to a fixed-dose supplementary insulin therapy (8 U of insulin aspart subcutaneously before each meal) for another week. Oral glucose tolerance tests (OGTTs) after drug uptake were performed at days 7 and 14, with measurement of glucose, insulin, C-peptide, intact and total proinsulin, glucagon, lactate, free fatty acids, and adiponectin.

Results: Significant reductions from baseline were seen in the supplementary insulin therapy group for the fasting values of insulin (from 13.1 ± 5.1 μU/mL to 10.6 ± 5.2 μU/mL, P < 0.01), intact proinsulin (from 18.3 ± 11.2 pmol/L to 10.3 ± 4.6 pmol/L, P < 0.05), total proinsulin (from 43.3 ± 22.7 pmol/L to 29.7 ± 14.5 pmol/L, P < 0.01), split proinsulin (from 24.9 ± 13.8 pmol/L to 19.4 ± 10.8 pmol/L, P < 0.01), and the degree of β-cell dysfunction (P < 0.05). Also, lower values for intact and total proinsulin and split proinsulin in the OGTT were observed in this group during the OGTT at the end point, while no changes at all occurred in the glimepiride group.

Conclusions: A fixed low-dose preprandial insulin aspart therapy resulted in an overall β-cell protection with an improved fasting β-cell secretion profile already within 1 week. Our study indicates that supplementary insulin therapy might be a reasonable alternative to bedtime basal insulin injections for initiation of insulin therapy in patients with type 2 diabetes.

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About 20% of the U.S. and E.U. population is overweight and is presenting with a measurable insulin resistance. A significant minority of this group will further proceed into development of type 2 diabetes, with an increased risk of cardiovascular events and macrovascular complications. This risk can be partly explained by an impairment of direct insulin action on the endothelial cell with a decrease in nitric oxide production, but an independent contribution can also be assigned to the secretory dysfunction of the β-cell. Earlier studies suggested that elevated proinsulin and disproportionate levels of the des31,32-proinsulin intermediate may be viewed as symptoms of a functionally compromised β-cell, most often arising from the overstimulation of chronic hyperglycemia or therapeutic intervention. When insulin requirements are arriving at a certain threshold, an insufficiency of the cleavage capacities of the proinsulin processing enzymes in the β-cell can lead to an increased secretion of intact proinsulin in addition to the desired insulin molecule. Proinsulin, however, has been demonstrated to be an independent cardiovascular risk factor by stimulating plasminogen activator inhibitor type-1 secretion and blocking fibrinolysis. Elevated endogenous or exogenous proinsulin concentrations are associated with atherosclerosis and cardiovascular mortality. During the past years, specific immunoassays have been developed for measurement of intact (i.e., uncleaved) proinsulin that do not show cross-reactivity to any of the specific or unspecific proinsulin cleavage products found in human plasma. These assays allow a timely assessment of the later-stage qualitative β-cell secretion disorder in type 2 diabetes, and it could be shown that an elevated fasting plasma concentration of intact proinsulin as measured by these assays is a very specific indirect marker for insulin resistance.

Protection of β-cell function by appropriate insulin substitution is a generally accepted treatment philosophy in patients with type 2 diabetes. However, there is no general consensus about the overall best treatment approach to achieve this goal. Some authors favor a basal insulin substitution as the best option to initiate insulin therapy. Oral therapy supported by basal insulin substitution is a currently strongly promoted alternative regimen (e.g., glimepiride with insulin glargine). Other groups vote for a preprandial insulin substitution, e.g., with short-acting insulin analogs as the best way of insulin introduction. They argue that β-cell protection should start at the time point of most prevalent β-cell stress, which is represented by the postprandial state. However, information is still lacking about the effect of the latter approach on overall β-cell function and about the time periods required to achieve β-cell protection by this kind of therapy.

We performed this prospective randomized parallel study in order to assess the immediate effect of a preprandial insulin substitution with a short-acting insulin analog in comparison with sulfonylurea treatment on preprandial and postprandial β-cell secretion as assessed by several direct and indirect β-cell dysfunction markers before and after an oral glucose challenge in patients with type 2 diabetes.

**PATIENTS AND METHODS**

This study was approved by the responsible Ethics Committee of the State of Rheinland-Pfalz, Germany. After giving written informed consent, 20 patients with type 2 diabetes were included into the study. All patients had been well controlled on glimepiride (Amaryl®, Sanofi-Aventis, Berlin, Germany) monotherapy (mean dose, 2.6 ± 1.2 mg) for at least 3 months prior to the study, and all were kept on this therapy for another 7 days. At baseline (day 7), an oral glucose tolerance test (OGTT) was performed in the morning after the patients had taken their antidiabetes medication. Blood samples were taken at time points -15, 0, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min for measurement of glucose, insulin, C-peptide, intact proinsulin, adiponectin, and free fatty acids. Thereafter, the patients were randomized either to continue with their glimepiride treatment or to switch to monotherapy with preprandial insulin substitution with a fixed dose of 8 units of insulin aspart (NovoRapid®.
Novo Nordisk, Mainz, Germany) before each major meal. The demographic data of both treatment groups are given in Table 1 [five women, 15 men; 61.8 ± 6.4 years old; disease duration, 6.7 ± 9.3 years; body mass index (BMI), 31.1 ± 4.4 kg/m²; glycosylated hemoglobin (HbA1c), 7.0 ± 1.3%]. After 7 days of treatment (day 14), the OGTT experiment was repeated. On this occasion, both treatments were applied immediately before the experiment. Thereafter, the study was terminated.

Laboratory measurements

A standard laboratory glucose oxidase reference method was used for measurement of blood glucose levels (SuperGL, Ruhrtal Labortechnik, Delecke-Möhnese, Germany). Insulin was measured using a chemiluminescence immunoassay that shows 100% cross-reactivity with insulin aspart (MLT Insulin, MLT, Cardiff, UK). Intact insulin and total proinsulin were analyzed by means of specific enzyme-linked immunoassay methods (Linco Research, St. Charles, MO). As published previously, the intact proinsulin assay has no cross-reactivity to des31,32 proinsulin. C-peptide, glucagon, and adiponectin were measured by radioimmunoassay (all tests from Linco). Free fatty acids were measured with colorimetry (Wako, Neuss, Germany), and lactate was determined from capillary blood by means of the SuperGL. Proinsulin split products were calculated by subtracting the intact from the total proinsulin values. Based on the assay specificities, the difference represents the levels of the des32,33 split product and its unspecified cleavage derivatives.

Assessment of insulin resistance and β-cell dysfunction

Next to assessment of insulin resistance by intact proinsulin secretion as published previously, homeostatic model assessment (HOMAIR) score calculation was applied as a second measure for insulin resistance analysis in patients with normal β-cell function (i.e., normal intact proinsulin values). The estimate of insulin resistance by HOMAIR score was calculated with the following formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5. As described by Hedblad et al., patients with HOMAIR score values exceeding the 75th percentile of a population without diabetes (i.e., 2.0) were considered to have insulin resistance.

The analysis of HOMAIR values in conjunction with the intact proinsulin values allows us to classify β-cell dysfunction as described previously. In brief, insulin-sensitive patients with normal quantitative insulin secretion but a lack of the first-phase insulin response would be classified as stage I. When insulin resistance is developing, the β-cell may effectively counteract this phenomenon by increased secretion of active insulin (stage II). However, the cell may sooner or later reach the level of saturation of the processing capacity, and intact proinsulin is secreted in an increasing manner (stage IIIa). While this contributes to an increased cardiovascular risk, the increasing demand for insulin may finally conclude in a complete exhaustion of β-cell secretion (stage IIIb).

Statistical analysis

The analysis of efficacy is based on the intention-to-treat population, which consists of all patients who were treated and provided complete assessment of the laboratory parameters at baseline and at the end point of the study. All analyses were performed in an exploratory sense with appropriate parametric and non-parametric methods. Changes from baseline were evaluated by using analysis of covariance models with treatment groups as factor and baseline values as covariate. The difference between treatment groups was assessed by using t test statistics for the hypot-
esis that treatment group is a relevant factor in the model. All values of $P < 0.05$ were interpreted as statistically significant.

**RESULTS**

All patients completed the protocol and were included into the efficacy analysis. Both treatments were well tolerated. In particular, no hypoglycemic episode was observed in any patient during the observation period.

There was no difference for any of the observation parameters between the treatment groups before and during the OGTTs at baseline, when both groups were on glimepiride treatment. As expected, no changes in the fasting morning values or in the OGTT results were reported from the group continuing glimepiride therapy at the end point after 1 week of continuous treatment.

The fasting morning values for all observation parameters were not significantly different at baseline. They are given in Table 2, and the percent changes from baseline for both treatment groups are given in Figure 1. A significant reduction in the fasting morning values was seen for intact and total proinsulin, split proinsulin, insulin, and the HOMA$_{IR}$ score in the insulin aspart group. At the end point, these values were significantly different between the treatment groups, since no such changes could be reported from the glimepiride group. Mean blood glucose values obtained by self-measurement of blood glucose during the 1-week treatment period were comparable between the groups.

Comparable results were also seen for glucose and insulin concentrations and glucose area under the concentration–time curves during the oral glucose challenges in both treatment groups at the end point as shown in Figure 2. At baseline in both groups and at the end point in the glimepiride group, a fivefold increase of the intact proinsulin levels after 2 h indicated increased $\beta$-cell stress during the OGTT. A significantly lower $\beta$-cell secretion activity could be observed in the insulin group at the end point as demonstrated by lower intact and proinsulin and split proinsulin values throughout the entire experiment (Fig. 3).

The stratification of the patients of the two treatment arms into the different stages of $\beta$-cell secretion at baseline and the end point is provided in Figure 4. It can be seen that the switch from glimepiride to preprandial insulin aspart therapy shifted 50% of the patients back from stage IIIa into the pathophysiologically earlier stage II, while $\beta$-cell function was more deteriorated with glimepiride. This difference between the groups reached the level of statistical significance despite the small size of the cohorts and the very short observation period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glimepiride</th>
<th>Insulin aspart</th>
</tr>
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<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>164 ± 43 (147)</td>
<td>171 ± 44 (161)</td>
</tr>
<tr>
<td>Insulin ($\mu$U/mL)</td>
<td>12.9 ± 5.9 (11.2)</td>
<td>14.2 ± 4.9 (15.0)</td>
</tr>
<tr>
<td>C-peptide (pmol/L)</td>
<td>933 ± 412 (827)</td>
<td>959 ± 261 (899)</td>
</tr>
<tr>
<td>Proinsulin (pmol/L)</td>
<td>16.3 ± 8.4 (13.4)</td>
<td>15.1 ± 6.4 (13.2)</td>
</tr>
<tr>
<td>Intact</td>
<td>38.4 ± 14.7 (36.8)</td>
<td>37.5 ± 10.6 (33.1)</td>
</tr>
<tr>
<td>Total</td>
<td>22.1 ± 8.4 (22.3)</td>
<td>22.5 ± 6.4 (23.2)</td>
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<tr>
<td>Split</td>
<td>6.0 ± 2.8 (5.4)</td>
<td>5.9 ± 2.9 (5.8)</td>
</tr>
<tr>
<td>Adiponectin (mg/L)</td>
<td>18.3 ± 2.9 (18.3)</td>
<td>19.2 ± 3.0 (19.7)</td>
</tr>
<tr>
<td>Lactate (mg/dL)</td>
<td>0.59 ± 0.12 (0.58)</td>
<td>0.57 ± 0.16 (0.52)</td>
</tr>
<tr>
<td>Glucagon (pg/mL)</td>
<td>90.7 ± 28.9 (84.6)</td>
<td>89.3 ± 21.5 (79.4)</td>
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<tr>
<td>HOMA</td>
<td>11.5 ± 4.8</td>
<td>12.7 ± 3.3</td>
</tr>
<tr>
<td>Glucagon (pg/mL)</td>
<td>156 ± 31 (140)</td>
<td>159 ± 23 (149)</td>
</tr>
<tr>
<td>Adiponectin (mg/L)</td>
<td>13.1 ± 5.1 (11.1)</td>
<td>10.6 ± 5.2 (10.4)$^a$</td>
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<td>Lactate (mg/dL)</td>
<td>930 ± 352 (829)</td>
<td>876 ± 422 (627)</td>
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<tr>
<td>Glucagon (pg/mL)</td>
<td>18.3 ± 11.2 (12.7)</td>
<td>10.3 ± 4.6 (9.6)$^b$</td>
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<tr>
<td>Adiponectin (mg/L)</td>
<td>43.3 ± 22.7 (36.6)</td>
<td>29.7 ± 14.5 (28.5)$^a$</td>
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<tr>
<td>Lactate (mg/dL)</td>
<td>24.9 ± 13.8 (23.0)</td>
<td>19.4 ± 10.8 (19.5)$^a$</td>
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<tr>
<td>Glucagon (pg/mL)</td>
<td>8.7 ± 5.2 (8.4)</td>
<td>8.3 ± 4.5 (7.5)</td>
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<tr>
<td>Adiponectin (mg/L)</td>
<td>17.0 ± 2.7 (16.5)</td>
<td>15.8 ± 4.5 (15.1)</td>
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<tr>
<td>Lactate (mg/dL)</td>
<td>0.55 ± 0.24 (0.50)</td>
<td>0.48 ± 0.11 (0.48)</td>
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<tr>
<td>Glucagon (pg/mL)</td>
<td>85.2 ± 22.6 (78.2)</td>
<td>82.9 ± 29.9 (79.8)</td>
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<td>Adiponectin (mg/L)</td>
<td>11.5 ± 4.8</td>
<td>12.2 ± 6.7</td>
</tr>
<tr>
<td>Lactate (mg/dL)</td>
<td>9.6 ± 6.3$^b$</td>
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Data are mean ± SD values (median).

$^a$P < 0.01, $^b$P < 0.05 versus baseline.
DISCUSSION

This study was performed to investigate the immediate and short-term effects of a switch from sulfonylurea to supplementary insulin therapy with a low dose of short-acting insulin aspart on β-cell function during glucose challenge experiments in patients with well-controlled type 2 diabetes. In comparison with a continued sulfonylurea monotherapy, an improved qualitative secretion profile was observed not just during the experiments but also for the fasting morning secretion pattern, indicating a sustained effect of prandial β-cell protection also during the night.

The switch from oral agents to insulin therapy is a crucial step in the treatment of type 2 diabetes. As mentioned in the Introduction, different approaches for a simple initiation of insulin therapy in patients with type 2 diabetes have been discussed in the scientific literature that support either prandial or basal insulin delivery. Pathophysiological considerations may vote for an initial prandial insulin delivery: meal-related secretion is considered to be the most challenging and stressful situation for the β-cells. The β-cell may still be able to secrete correctly processed insulin between the meals, even in the stages of impaired glucose tolerance and type 2 diabetes and during the night. The glucose challenge during the meal usually exhausts the cleavage capacity of the insulin-processing enzymes, and increasing amounts of intact proinsulin are secreted in this period.6,7 Proinsulin, however, has only 10–20% of the glucose-lowering effect of insulin, but a comparable adipogenetic activity.19 In addition, proinsulin is considered to be an independent cardiovascular risk factor.10,12,13,16 Because of the minor but evident glucose-lowering effect, patients with severe β-cell dysfunction and high proinsulin output may still have sufficient glucose-lowering capacity to avoid the diagnosis of diabetes mellitus in an OGTT experiment. In consequence, β-cell dysfunction and proinsulin secretion are not consequently correlated with diabetes duration,14,15,33 and proinsulin secretion may even precede the onset of clinically overt type 2 diabetes.34

There is no doubt that sulfonylurea drugs are effective in lowering blood glucose! It appears,
however, questionable and remains to be explored whether treatment of type 2 diabetes patients with these β-cell secretion-stimulating agents is optimally addressing the underlying β-cell secretion disorder. While almost all patients had reached the HbA1c target value (<7.0%) with glimepiride in our patient population, a five- to sixfold elevation of intact proinsulin was seen in this group after 2 h during the OGTT experiment. This phenomenon was significantly improved after 1 week of low-dose preprandial insulin aspart therapy. Still, a
smaller increase of intact proinsulin was also seen in the insulin group. We believe that an adaptation of the insulin dose to the real needs of the individual patients would have avoided this rise in level of the insulin precursor molecule in the peripheral blood. The intention of our study, however, was to demonstrate the β-cell protective effect of a simplified prandial insulin regimen (without any further BMI- or meal-related dose adaptation) that supports endogenous insulin secretion without leading to an increased risk of hypoglycemia. Therefore,
the dose was intentionally chosen to be presumably too low in many cases for safety reasons. Despite this suboptimal approach, the prandial insulin analog therapy led to a significant release of β-cell stress before and during glucose uptake. The comparable glucose excursions at baseline and the end point in both treatment groups are in our opinion an indication that the selected insulin dose only served as a buffer for the β-cell secretion and had no additional antihyperglycemic effect. It has to be acknowledged that both the removal of the sulfonylurea drug and the initiation of exogenous insulin will have contributed to the β-cell protective effect; however, the experimental design of this study, planned to mimic the routine treatment situation, does not allow the quantification of each contribution.

One argument for starting insulin therapy with basal insulin is the expected β-cell protective effect of this approach during the night, which has in the past been associated with an increased hypoglycemic risk because of the suboptimal time-action profile of basal NPH insulin. Since the introduction of the long-acting insulin analog glargine, the concept of basal insulin substitution in addition to oral treatment with glimepiride has become more attractive to primary care physicians in many countries. It has to be mentioned, however, that this concept has not been shown to address meal-related β-cell dysfunction. When planning the experiment, we expected to see an immediate short-term supportive effect of external short-acting insulin analog delivery on endogenous β-cell secretion during the glucose challenge experiments. The additional finding of significantly lower fasting morning values, however, indicates an improved overnight β-cell function that could not necessarily be predicted after only 1 week of prandial therapeutic intervention. A significant number of patients could be reversed from stage III to stage II of our β-cell dysfunction staging, thus demonstrating a beneficial and prolonged β-cell protective effect of supplementary insulin therapy in comparison with sulfonylurea monotherapy. Because of a lacking treatment arm with insulin glargine, our study is not able to determine whether basal insulin substitution or prandial insulin delivery is the better choice for the β-cell to start insulin therapy. Further questions

FIG. 4. Stratification of the patients into the stages of β-cell dysfunction according to Pfützner et al. at baseline and the end point (no patient was in stage I or stage IIIb).
β-CELL PROTECTIVE EFFECT

raised by this pilot study, e.g., whether the β-cell protective effect is sustained over a longer treatment period or how the results would be influenced by a combination therapy of sulfonylurea with insulin, need also to be clarified in future studies.

However, our results demonstrate that supplementary prandial insulin therapy with a fixed dose of a short-acting insulin analog has a fast positive short-term effect on β-cell dysfunction and may be a simple but safe and effective method for initiation of insulin therapy in patients with type 2 diabetes.

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