Addition of insulin glargine vs. NPH insulin to metformin results in a more efficient postprandial β-cell protection in individuals with type 2 diabetes

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Aim: Postprandial release of intact proinsulin (IP) is an independent marker for β-cell dysfunction in patients with type 2 diabetes. This open-label, parallel-group, two-arm, pilot study compared the β-cell protective effect of adding insulin glargine (GLA) vs. NPH insulin to ongoing metformin.

Material and methods: Overall, 28 insulin-naive type 2 diabetes subjects (mean ± SD age, 61.5 ± 6.7 years; diabetes duration, 9.8 ± 6.5 years; HbA1c, 7.1 ± 0.5%; BMI, 30.7 ± 4.3 kg/m²) treated with metformin and sulfonylurea were randomized to add once-daily GLA or NPH at bedtime. At baseline and after 3 months, subjects received a standardized breakfast, lunch and dinner, with pre- and postprandial blood sampling to measure plasma IP, total insulin and blood glucose (BG).

Results: Insulin dose after 3 months was comparable in both groups (GLA vs. NPH: 23.6 ± 13.4 vs. 23.3 ± 12.7; p = NS). Both treatments significantly reduced fasting BG levels (GLA: 158 ± 19 to 121 ± 23 mg/dl; NPH: 156 ± 34 to 119 ± 29 mg/dl; both p < 0.01 vs. baseline). Fasting and postprandial BG levels did not differ between groups. IP levels decreased in both groups (p < 0.05 at all timepoints). Although IP release after breakfast did not differ between treatments, GLA induced a greater reduction in IP release after lunch (p = 0.08) and dinner (p = 0.04). Total plasma insulin levels did not differ between groups.

Conclusions: Adding basal insulin to metformin reduces postprandial β-cell load. While GLA and NPH had comparable effects at breakfast, GLA reduces β-cell stress more effectively at dinner, and with a trend at lunch, most probably because of its longer lasting pharmacodynamic profile.

Keywords: beta cell stress, insulin glargine, intact proinsulin, NPH insulin

Introduction

Increasing β-cell stress with subsequent failure to release sufficient amounts of biologically active insulin is thought to precede the deterioration of blood glucose control in individuals with type 2 diabetes treated with oral agents [1,2]. Type 2 diabetes is classically monitored by measurement of laboratory markers, such as HbA1c, glucose, lipids, body mass index and blood pressure. In addition to these traditional laboratory markers, the measurement of intact proinsulin (IP) levels may provide pursuing information with regard to β-cell function and disease stage [3,4].

In addition to the much weaker glucose-lowering activity, the increasing IP levels have been reported to be associated with a substantial increase in cardiovascular risk and are now considered to be an independent cardiovascular risk marker in subjects, with and without disturbed glucose metabolism [5–7]. In individuals with type 2 diabetes, the increasing insulin requirements, owing to insulin resistance or excess β-cell stimulation by sulfonylurea, result in β-cell overload with impaired processing of IP into insulin and C-peptide [8,9]. On the contrary, recent studies have indicated that the early introduction of insulin treatment, even at low doses that are insufficient to restore blood glucose control can reduce β-cell stress, improve endogenous insulin processing and might contribute to an improvement in the overall cardiovascular risk profile by reducing circulating IP levels [10–12].

In individuals with type 2 diabetes, a combination of oral antidiabetic drugs (OAD) and basal insulin is commonly initiated after OAD treatment has failed to achieve sufficient metabolic control. In these subjects, basal insulin is usually given in the evening to suppress hepatic gluconeogenesis overnight, while OADs are continued to provide sufficient blood glucose control during the day. Recent studies comparing the long-acting insulin analogue glargine (GLA) vs. NPH insulin in combination with metformin revealed the advantages of insulin GLA, owing to its flat and long-lasting pharmacodynamic profile in patients with type 2 diabetes [13].
The goal of the recent study was to investigate the effect of basal insulin treatment, by adding insulin GLA or NPH insulin to metformin, on postprandial β-cell function in patients with type 2 diabetes using a combination of metformin and sulfonylurea.

**Research Design and Methods**

Thirty insulin-naive subjects with type 2 diabetes treated with a combination of metformin and sulfonylurea were randomized to receive treatment with insulin GLA or NPH insulin at bedtime along with 1000 mg metformin twice daily. The doses of metformin and sulfonylurea were to be stable over the last 3 months before inclusion in the study. Further inclusion criteria for study participation were an HbA1c level between 6.5 and 8.5%, and an intact fasting proinsulin level between 7 and 20 pmol/l. Subjects were excluded if they had been treated with insulin, peroxisome proliferator-activated receptor-γ agonists, glinides or glucosidase inhibitors within the last 4 weeks prior to the screening visit. All other concomitant treatments were kept stable during the study. After the initiation of basal insulin therapy, both insulin treatments were titrated to reach a target fasting glucose level of 100 mg/dl (5.6 mmol/l).

At baseline (before insulin treatment) and 3 months after the initiation of insulin treatment, the subjects visited the study site in the morning after an 8-h overnight fast for a test meal day consisting of three standardized meals. For this test meal day, the subjects were hospitalized in a comfortable environment and an intravenous cannula for blood sampling was placed into a large antecubital or forearm vein. The subjects received a standardized breakfast at 08:00 hours (434 kcal, 26.7 g protein, 15 g fat, 48 g carbohydrates), a standardized lunch at 12:00 hours (642 kcal, 48 g protein, 25 g fat, 53 g carbohydrates) and a standardized dinner at 18:00 hours (427 kcal, 18 g protein, 23 g fat, 55 g carbohydrates). Blood samples were collected before the test meals and 60 and 120 min after food intake to measure plasma glucose, insulin and IP levels.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethical committee. All subjects gave a written, informed consent.

**Laboratory Analysis**

All laboratory measurements were analysed at the Institute for Clinical Research and Development (ikfe GmbH, Mainz, Germany). Blood samples were centrifuged and maintained at −20 °C until analysis. Plasma glucose concentrations were determined by the glucose dehydrogenase method (Super GL, RLT, Möhnesee-Delecke, Germany). Insulin was measured by a chemiluminescence assay (Invitron, Monmouth, UK), which shows a high cross-reactivity with insulin GLA and NPH Insulin. Therefore, the plasma insulin levels given in the study represent the total insulin plasma level comprising endogenous and exogenous insulin. Intact proinsulin was measured using an enzyme-linked immunosorbent assay (LincoResearch, St Charles, MO, USA) and HbA1c was determined by high-performance liquid chromatography (Menarini Diagnostics, Neuss, Germany).

**Safety**

Adverse events experienced by subjects during the study were documented by the investigator at each visit.

**Subjects Sample Size Considerations and Statistical Analysis**

No clinical information was available for the primary endpoint: the effects of basal insulin supplementation on postprandial IP secretion. Therefore, this study was designed as a pilot study, without confirmatory sample size consideration. The number of participating subjects was estimated based on a previous study investigating the effect of low-dose prandial insulin supplementation on postprandial IP levels [12]. Enrolment of 30 subjects was considered appropriate to obtain data of at least 10 evaluable subjects per treatment arm for the full analysis set.

Results are presented using descriptive summary statistics. All measurements are presented as means ± standard deviation (SD). For the postprandial time course of IP levels, the area under the curve (AUC) was calculated according to the trapezoidal rule. Statistical comparison between baseline and at 3 months of insulin treatment, and between the two treatment groups were performed using the Student’s t-test (paired and unpaired as appropriate). A two-tailed p < 0.05 was considered statistically significant.

**Results**

Thirty subjects were randomized (15 individuals in each study arm). One subject in the NPH insulin group terminated the study by withdrawing informed consent and did not receive any follow-up investigation. One subject in the insulin GLA group was excluded owing to abnormal IP levels. In total, 28 subjects (14 subjects in each group) were included in the per-protocol analysis. Baseline demographic data and clinical characteristics of the two groups are presented in Table 1.

Slight but non-significant differences between both study groups were found for age and duration of diabetes. No relevant differences in concomitant medication were found between the two groups.

As shown in Table 1, HbA1c at the start of the study was comparable between the two groups. A slight, but non-significant reduction in HbA1c was observed with both treatments. At the end of the study, the HbA1c levels were comparable between both treatment groups (insulin GLA: 6.9

**Table 1. Clinical characteristics of the study subject.**

|                         | Insulin GLA + metformin | NPH insulin + metformin |
|-------------------------|-------------------------|-------------------------|
| N                       | 14                      | 14                      |
| Males/females           | 12/2                    | 10/4                    |
| Age (years)             | 66.9 ± 6.2              | 58.0 ± 8.5              |
| Body mass index (kg/m²) | 30.0 ± 3.7              | 31.5 ± 4.9              |
| Duration of diabetes (years) | 11.6 ± 7.5          | 8.6 ± 4.7               |
| HbA1c (%)               | 7.1 ± 0.6               | 7.1 ± 0.4               |

GLA, glargine.
null
In addition, elevated IP levels have been shown to predict coronary atherosclerosis [18] and the risk of cardiovascular events in subjects with and without diabetes [5,6,19,20]. Furthermore, we recently reported a postprandial increase in IP levels in non-diabetic subjects with increased cardiovascular risk [7]. In a clinical study, proinsulin administration for at least one year was associated with an increased rate of cardiovascular events [21]. Therefore, our findings of a marked overall decrease in fasting and postprandial IP levels after the initiation of basal insulin treatment might have important implications not only for metabolic control, but also for cardiovascular risk reduction in individuals with type 2 diabetes.

Insulin GLA is a long-acting human insulin analogue with a longer time–action profile and no pronounced peak of action when compared with NPH insulin [22]. In a recent study, treatment with insulin GLA in combination with OADs achieved better postprandial metabolic control when compared with NPH insulin in combination with OADs [13]. In our study population, no significant differences in glucose control were observed between the two insulin formulations. On the contrary, treatment with insulin GLA in combination with metformin revealed a more pronounced reduction in postprandial release of IP after lunch and dinner when compared with NPH insulin. Owing to the shorter time–action profile of NPH insulin, the rate of insulin release from the subcutaneous tissue depot is more rapid, which exhausts the supply more quickly and, thus, requires earlier endogenous insulin release. The greater demand on β cell will become evident, particularly after a meal, when the requirements for insulin are high. In individuals with type 2 diabetes who have barely compensated β-cell function, this will lead to a greater release of IP from the exhausted β cells [23–25]. Despite comparable glucose control, the prolonged pharmacodynamic profile of insulin GLA results in stronger β-cell protection, lasting over 24 h.

The comparable total plasma insulin levels found in both treatment groups indicate that, in NPH insulin-treated subjects, enhanced endogenous insulin release was able to compensate for the shorter time–action profile of NPH insulin, thereby keeping blood glucose levels comparable between the study groups. Nevertheless, the greater demand on the β cells in NPH insulin-treated subjects will be followed by an increase in the release of IP, particularly after meals, as observed in our study.

A potential limitation of our findings is that this was an exploratory pilot study to evaluate the protective effects on the β cell by initiating basal insulin therapy with metformin in individuals with type 2 diabetes, pretreated with OADs (sulfonylurea in combination with metformin). Further studies are needed to investigate if the effect of basal insulin supplementation will translate into longer β-cell survival or a reduction in cardiovascular risk. In addition, our study only compared once-daily application of insulin GLA, with once-daily injection of NPH insulin. Most probably, NPH insulin administered twice daily will result in postprandial IP levels that more closely match those achieved with once-daily insulin GLA.

In conclusion, the initiation of basal insulin in combination with metformin results in an overall improvement of β-cell function, as indicated by a reduction in the fasting and postprandial release of IP from the β cells. Because of the protracted pharmacokinetic profile of insulin GLA compared with NPH insulin, treatment with insulin GLA may offer more prolonged β-cell preservation when compared with NPH insulin applied once daily.

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**Conflicts of Interest**

Prof. Dr. Andreas Pfützner and Prof. Dr. Thomas Forst received unrestricted research grants and speaker fees from Sanofi-aventis. Dr. Martin Larbig is an employee of Sanofi-aventis. Dr. Cloth Hohberg, Senait Forst, Dr. Stepahn Diesel,
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