Effects of Pramlintide on Postprandial Glucose Excursions and Measures of Oxidative Stress in Patients With Type 1 Diabetes

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OBJECTIVE — Oxidative stress has been shown to be increased in the postprandial period in patients with diabetes and has been implicated in the pathogenesis of micro- and macrovascular complications. The aim of this post hoc analysis was to assess the effects of pramlintide, an amylin analog shown to reduce postprandial glucose excursions in patients with diabetes, on markers of oxidative stress in the postprandial period.

RESEARCH DESIGN AND METHODS — In a randomized, single-blind, placebo-controlled, crossover study, 18 evaluable subjects with type 1 diabetes underwent two standardized breakfast meal tests and received pramlintide or placebo in addition to their preprandial insulin. The plasma concentrations of glucose and markers of oxidative stress (nitrotyrosine, oxidized LDL [ox-LDL], and total radical-trapping antioxidant parameter [TRAP]) were measured at baseline and during the 4-h postprandial period.

RESULTS — Compared with placebo, pramlintide treatment significantly reduced postprandial excursions of glucose, nitrotyrosine, and ox-LDL and prevented a decline in TRAP (p < 0.03 for all comparisons). Correlation analyses adjusted for treatment revealed a significant association between postprandial mean incremental area under the curve from 0 to 4 h (AUC0–4 h) for glucose and postprandial mean incremental AUC0–4 h, for each measure of oxidative stress (r = 0.75, 0.54, and −0.63 for nitrotyrosine, ox-LDL, and TRAP, respectively; P < 0.001 for all correlations).

CONCLUSIONS — These findings indicate that the postprandial glucose-lowering effect of pramlintide in type 1 diabetes is associated with a significant reduction in postprandial oxidative stress.

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placebo-controlled, five-way crossover study that examined the effects of pramlintide, administered at various times in relation to a standard breakfast meal, on postprandial plasma glucose in subjects with type 1 diabetes or insulin-using subjects with type 2 diabetes (19,20). For the present report, all patients with type 1 diabetes using human regular insulin (n = 19) were selected and two of the five treatments arms were utilized for this analysis. On separate days and in random order, each subject, in the context of this subanalysis, received either a single subcutaneous injection of pramlintide (60 μg) at t = 0 min (n = 18) or placebo at t = −15 min (n = 19) in relation to a standard breakfast. As determined previously (19), pramlintide administration at 0 min provides optimal reduction in postprandial glucose excursions compared with placebo. On both occasions, subjects received their usual dose of human regular insulin, with similar doses on the two study days (pramlintide 7.2 ± 0.9 vs. placebo 7.6 ± 1.0 units [means ± SE]), at t = −30 min. In addition, plasma concentrations of glucose and markers of oxidative stress (nitrotyrosine, oxidized LDL [ox-LDL], and total radical-trapping antioxidant parameter [TRAP]) were measured at baseline and during the 4-h interval subsequent to a standard meal (30% of daily caloric requirements; 55%/15%/30% of kcal from carbohydrate/protein/lipid, respectively) during each visit.

**Assays**

Nitrotyrosine plasma concentrations were assayed by enzyme-linked immunosorbent assay, as previously described (21). TRAP was evaluated as previously described (22). Ox-LDL was assessed by the Oxidized LDL Enzyme-Linked Immunosorbent Assay (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions.

**Statistical analysis**

A total of 18 subjects completing the study were considered sufficient to provide comparisons of postprandial responses between pramlintide and placebo treatment groups. Baseline demographic data were presented as means ± SD for continuous variables (age, duration of diabetes, BMI, weight, and A1C) and tallied for categorical variables (sex and race).

For each evaluable subject, the incremental (baseline-corrected) plasma concentrations of glucose, nitrotyrosine, ox-LDL, and TRAP were used to calculate areas under the curve from 0 to 4 h (AUC0–4 h) using the linear trapezoidal method. The incremental mean plasma concentration profiles (means ± SE) of the analytes were calculated and are presented graphically by treatment. Statistical significance was noted by pairwise comparisons between the placebo and pramlintide groups based on mixed-effects models, with treatment as a fixed effect and subject within-treatment sequence as a random effect. Least square means (and their SEs) for comparing the least square means between treatment groups are presented. Associations between the incremental AUC0–4 h for plasma glucose and the incremental AUC0–4 h for each marker of oxidative stress were studied using Pearson correlation analyses adjusted for treatment.

Safety evaluations were based on reports of treatment-emergent adverse events, clinical laboratory evaluations (hematology, serum chemistry, and urinalysis), vital signs, physical examinations, and electrocardiograms in all subjects.

**RESULTS** — Nineteen subjects with type 1 diabetes (14 men and 5 women; age 37 ± 16 years; duration of diabetes 22 ± 12 years; 11 white, 1 black, and 7 Hispanic; A1C 9.3 ± 1.7%; and BMI 26.8 ± 3.8 kg/m²) were studied. Of the 19 subjects randomized, 18 were considered evaluable (1 subject excluded due to deviation from study dosing procedure).

**Postprandial oxidative stress**

At baseline, plasma glucose concentrations were similar between the pramlintide and placebo groups (pramlintide 9.5 ± 0.77 mmol/l, placebo 9.5 ± 0.82 mmol/l). As previously reported (19), prandial administration of pramlintide, as an adjunct to regular insulin, prevented the initial rise in postprandial plasma glucose and significantly reduced the overall glucose excursion observed with regular insulin alone (placebo) (Fig. 1A). Mean incremental glucose AUC0–4 h values (mean ± SE) were −0.6 ± 2.5 mmol · l⁻¹ · h⁻¹ for pramlintide and +11.0 ± 2.9 mmol · l⁻¹ · h⁻¹ for placebo (P = 0.001).

**Postprandial oxidative stress**

At baseline, plasma concentrations of nitrotyrosine (pramlintide 7.1 ± 0.5 nmol vs. placebo 7.2 ± 0.6 nmol [means ± SE]), ox-LDL (pramlintide 54.5 ± 2.9 units/l vs. placebo 56.8 ± 2.4 units/l), and TRAP (pramlintide 341.0 ± 27.2 μmol/l vs. placebo 337.8 ± 22.2 μmol/l) were similar between the pramlintide and placebo treatment groups (Fig. 1).

For the pramlintide treatment group compared with placebo, AUC0–4 h values were significantly altered for all oxidative stress markers tested, with reductions of >100% for plasma nitrotyrosine and ox-LDL and >100% preservation in TRAP (Fig. 1). For nitrotyrosine, mean incremental AUC0–4 h values (means ± SE) were 0.07 ± 1.3 mmol · l⁻¹ · h⁻¹ for pramlintide compared with 5.3 ± 2.2 mmol · l⁻¹ · h⁻¹ for placebo (P = 0.014). For ox-LDL, mean incremental AUC0–4 h values were −4.3 ± 3.4 units · l⁻¹ · h⁻¹ with pramlintide compared with 19.4 ± 6.0 units · l⁻¹ · h⁻¹ with placebo (P = 0.002). Mean incremental AUC0–4 h values for TRAP were −0.79 ± 73.4 μmol · l⁻¹ · h⁻¹ for pramlintide compared with −200 ± 89.4 μmol · l⁻¹ · h⁻¹ for placebo (P = 0.021).

**Safety and tolerability**

As previously reported (19), pramlintide treatment was generally well tolerated and there was no evidence of cardiovascular, pulmonary, hepatic, or renal toxicity or of drug-related idiosyncratic side effects associated with its use. There were no severe hypoglycemia events and no serious adverse events. Mild to moderate hypoglycemia (placebo 16% vs. pramlintide 28%) and mild nausea (placebo 11% vs. pramlintide 17%) were the most frequent treatment-emergent adverse events.

**CONCLUSIONS** — Previous studies have demonstrated that pramlintide, administered at mealtime in conjunction with regular insulin, reduced the early
postprandial rise and overall postprandial glucose excursion in subjects with type 1 or type 2 diabetes (15–18). Results from the present analysis in subjects with type 1 diabetes demonstrate that the postprandial glucose-lowering effect of pramlintide is accompanied by significant improvements in markers of oxidative stress.

Following injections of insulin alone (placebo), meal-induced increases in plasma glucose concentrations were associated with concomitant generation of oxidative stress, evidenced by a marked postprandial rise in plasma nitrotyrosine and ox-LDL, as well as antioxidant consumption, reflected by reduced TRAP values (Fig. 1). In contrast, postprandial excursions of nitrotyrosine and ox-LDL were significantly reduced and TRAP was significantly preserved with pramlintide treatment compared with placebo (Fig. 1). Notably, initial elevations in postprandial plasma concentrations of each oxidative measure observed with placebo were essentially prevented, and plasma concentrations of these markers remained within a narrow range throughout the testing period. Pronounced effects of pramlintide on postprandial oxidative stress were demonstrated by reductions of >100% in plasma nitrotyrosine and ox-LDL and a >100% preservation in plasma TRAP. These findings, observed with three independent markers, present a consistent and conclusive profile of pramlintide-mediated improvement in oxidative status. Nitrotyrosine is a suitable marker of peroxynitrite and nitrosative stress–related damage and increases during acute hyperglycemia (21). Interestingly, nitrotyrosine has recently been identified as an independent risk factor for cardiovascular disease (CVD) (23). Measurement of ox-LDL, which is considered more atherogenic than native LDL, is a widely used indicator of free radical activity (8). TRAP is a global measure of the antioxidant capacity of plasma, which takes into account concentrations of antioxidants as well as their synergy (7). Therefore, results from this study demonstrate that preprandial administration of pramlintide is accompanied by conditions favoring protection of antioxidant capacity (TRAP) and systemic reductions in atherogenic and cytotoxic agents (ox-LDL and nitrotyrosine) (8,21) in the postprandial period.

Additional findings show that the extent of postprandial glycemia is significantly correlated with levels of oxidative stress. A significant relationship between postprandial plasma glucose levels and plasma concentrations of nitrotyrosine, ox-LDL, or TRAP was found, lending further support to the concept that hyperglycemia...
Cemia promotes proatherogenic conditions. Significant associations were also observed between placebo-corrected changes in postprandial glycemia and changes in postprandial concentrations of each oxidative stress marker (data not shown). Thus, postprandial improvements in oxidative stress with pramlintide were proportional to pramlintide’s postprandial glucose-lowering effects.

Several studies have suggested that postprandial glucose is a stronger predictor of CVD than fasting glucose in patients with diabetes (8); however, the overall importance of glycemic control in delaying macrovascular disease in type 1 diabetes remains poorly understood (24). Currently, most epidemiological and clinical trial data on diabetes and CVD have resulted from the study of type 2 diabetes. However, causes for the increased incidence and earlier onset of CVD in patients with type 1 diabetes are still unknown. Much of the risk in patients with type 1 diabetes has been attributed to diabetes-related factors such as established renal impairment and proteinuria rather than more conventional factors (25). The question remains whether known risk factors and their impact in type 2 diabetes will be similar to those for patients with type 1 diabetes.

The potential role of increased oxidative stress in the development of diabetes complications is currently of great interest. Under hyperglycemic conditions, oxidative stress is largely a consequence of unrestrained production of superoxide anions, which impact multiple biochemical pathways leading to endothelial dysfunction, impaired insulin sensitivity, and other forms of metabolic stress (i.e., vascular inflammation, abnormal energy expenditure) (3–5). In the case of type 1 diabetes, enhanced levels of oxidative stress have been observed regardless of diabetes duration, extent of metabolic control, or overt complications (26–28). In addition, in a study of possible correlation between oxidative stress and insulin requirements in subjects with recent-onset type 1 diabetes, oxidative injury was identified as a factor in β-cell dysfunction (29). Therefore, reductions of oxidative stress in this patient population may have a lasting benefit on glycemic control as well as on long-term prognosis.

Several recent clinical trials in subjects with type 2 diabetes support this hypothesis. Data from the prospective Study to Prevent Noninsulin-Dependent Diabetes (STOP-NIDDM) trial demonstrated that treatment with acarbose, a compound that specifically reduces postprandial hyperglycemia, in subjects with impaired glucose tolerance resulted in risk reductions of 36% in the development of diabetes (30), 34% in the development of new cases of hypertension, and 49% in cardiovascular events (31).
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subgroup of patients, acarbose treatment was also associated with a significant decrease in the progression of intima-media thickness, an accepted surrogate for atherosclerosis (32). Furthermore, in a recent meta-analysis of subjects with type 2 diabetes, a significant reduction in cardiovascular events was demonstrated with acarbose treatment, even after adjusting for other risk factors (33).

More recently, two insulin secretagogues, repaglinide and glyburide, were evaluated for their effects on carotid intima-media thickness and markers of systemic vascular inflammation in subjects with type 2 diabetes (34). Reductions in carotid intima-media thickness, observed in 52% of diabetic subjects receiving repaglinide and in 18% of those receiving glyburide, were associated with changes in postprandial, not fasting, hyperglycemia (34). Therefore, a growing body of evidence strongly indicates that treatment of postprandial hyperglycemia may have a positive effect on the development of CVD.

While postprandial hyperglycemia has been implicated in the generation of oxidative stress, such oxidative changes have been linked to other cardiovascular risk factors that may accompany hyperglycemia in the postprandial period. Among these are hypertriglyceridemia and other qualitative lipoprotein abnormalities (35). Previous studies have shown that addition of pramlintide to existing insulin regimens leads to reductions in postprandial triglyceride concentrations in patients with type 1 diabetes in addition to improvements in postprandial glycemic excursions (36). Delineating the relative contributions of hyperglycemia and lipid abnormalities in the generation of postprandial oxidative stress has been an ongoing challenge; however, some studies indicate that postprandial hypertriglyceridemia and hyperglycemia have independent but cumulative effects (37,38). Since triglyceride levels were not measured in this study, the specific contribution, if any, of pramlintide’s postprandial lipid-lowering effects to reductions in oxidative stress remains unclear. However, given that hyperlipidemia presents an additional cardiovascular risk factor, the potential effect of pramlintide in lowering postprandial hyperlipidemia is an interesting prospect that warrants further examination. Finally, the theoretical possibility that pramlintide itself has antioxidant properties was not explored in this study.

In conclusion, results from this study demonstrate that the reduction in postprandial glucose excursions achieved with the addition of pramlintide to regular insulin in type 1 diabetes is accompanied by significant reductions in postprandial oxidative stress. These findings, which merit further investigations, suggest that pramlintide treatment may have a positive impact on long-term diabetes complications through attenuation of hyperglycemia-related oxidant injury.

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References


