IJC Metabolic & Endocrine 4 (2014) 70–72

Contents lists available at ScienceDirect

IJC Metabolic & Endocrine

journal homepage: http://www.journals.elsevier.com/ijc-metabolic-and-endocrine

# Vascular injury is improved by pre-meal glulisine-based bolus insulin therapy in type 2 diabetic patients

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ARTICLE INFO

*Article history:*
Received 9 July 2014
Accepted 24 July 2014
Available online 3 August 2014

*Keywords:*
sRAGE
Oxidative stress
Vascular damage
Glulisine
MCP-1
DTSQ

The pathological role of the non-enzymatic modification of proteins by reducing sugars, a process that is known as glycation (also called the "Maillard reaction"), has become increasingly evident in various types of diseases [1–5]. The early glycation products undergo progressive modification over time *in vivo* to the formation of irreversibly cross-linked, heterogeneous fluorescent derivatives termed "advanced glycation end products (AGEs)" [1–5]. There is accumulating evidence that activation of the receptor for AGEs (RAGE) evokes oxidative and inflammatory reactions, thereby contributing to vascular complications in diabetes [2–5]. Further, recently, soluble form of RAGE (sRAGE) has been indentified and could reflect tissue RAGE expression in diabetes [6]. Moreover, prospective studies have shown that higher levels of sRAGE are associated with incident of cardiovascular disease or all-cause mortality in diabetic subjects [6,7]. These findings suggest that sRAGE might be a biomarker of cardiovascular disease in diabetes and a potential therapeutic target for intervention.

Postprandial hyperglycemia is of greater importance in cardiovascular disease in patients with impaired glucose tolerance or diabetes [8]. It is associated with endothelial dysfunction and increased intima-media thickness as well as a higher prevalence of atherosclerotic plaques of the common carotid arteries in these subjects [8]. Since multiple daily pre-meal short-acting insulin injections can decrease postprandial glycemic excursions, it would be a promising strategy for preventing vascular injury in diabetes. However, which type of short-acting insulin therapy is best for reducing sRAGE levels and improving patient satisfaction remains unclear. To address the issue, we compared clinical effects of glulisine-based bolus insulin therapy with those of other short-acting insulin in type 2 diabetic patients.

This was a prospective, open-label, 24-week study. 26 consecutive type 2 diabetic outpatients seen in the clinic of Sapporo City General Hospital whose blood glucose levels were uncontrolled (HbA1c >6.2%) by pre-meal injections of bolus short-acting insulin (aspart (n = 9), lispro (n = 13) and regular human insulin (n = 1)) for at least 8 weeks were enrolled in this study. All patients were assigned to replace short-acting insulin by glulisine. Some part of subjects was enrolled in our previous glargine plus glulisine study [9]. Doses of glulisine were titrated to reach and maintain target glycemic goals defined as postprandial glucose levels of 160 mg/dL or lower. During the study period, subjects were instructed not to change their lifestyles and to continue taking the same dose of any concomitant oral drugs. We excluded any patients with inflammatory, neoplastic disorders, or those who had a recent (<3 months) cardiovascular disease. Three patients dropped out of the study during the assessment due to colon cancer, stroke and poor adherence to treatment. Finally 23 patients were evaluated at baseline and 24 weeks of follow-up. All participants gave informed consent to participate in this study. The Ethical Committee for the Clinical Research of Sapporo City General Hospital approved this study.

Blood pressure (BP) was measured in the sitting position using an upright standard sphygmomanometer. Blood was drawn after 12-hour fasting in the morning, and blood chemistries, including sRAGE and monocyte chemoattractant protein-1 (MCP-1), were measured as described previously [9]. Urinary albumin excretion (UAE) levels were measured by immunoturbidimetry assay (Hitachi LABOSPECT 008, Tokyo, Japan). Patient satisfaction was assessed with Diabetes Treatment Satisfaction Questionnaire (DTSQ) [10]. DTSQ score assesses total diabetes treatment satisfaction with higher scores indicating higher satisfaction. Perceived frequency of hyperglycemia and hypoglycemia is also

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http://dx.doi.org/10.1016/j.ijcme.2014.07.006

**Table 1**
Clinical characteristics.

| Parameters | Baseline | 24 weeks after replacement | p-Value |
|---|---|---|---|
| Number | 23 | 23 | – |
| Male/female (number) | 12/11 | 12/11 | – |
| Age (years old) | 59.4 ± 12.3 | – | – |
| Duration of diabetes (years) | 19.0 ± 8.3 | – | – |
| Retinopathy/nephropathy (number) | 14/10 | 14/10 | – |
| Body weight (kg) | 69.3 ± 14.0 | 70.0 ± 14.5 | 0.063 |
| Plasma glucose (mg/dL) | 172.0 ± 92.8 | 151.9 ± 64.1 | 0.243 |
| HbA1c (%) | 8.1 ± 1.5 | 7.8 ± 1.6 | 0.100 |
| GA (%) | 23.7 ± 4.6 | 22.0 ± 4.5 | $p < 0.05$ |
| Systolic BP (mm Hg) | 126.0 ± 9.6 | 123.8 ± 8.6 | 0.280 |
| Diastolic BP (mm Hg) | 74.5 ± 9.6 | 73.2 ± 8.6 | 0.354 |
| AST (IU/L) | 21.8 ± 6.9 | 22.4 ± 9.7 | 0.765 |
| ALT (IU/L) | 21.5 ± 10.4 | 21.5 ± 10.0 | 0.979 |
| BUN (mg/dL) | 16.3 ± 4.3 | 16.7 ± 4.7 | 0.449 |
| Creatinine (mg/dL) | 0.8 ± 0.3 | 0.9 ± 0.3 | $p < 0.01$ |
| Total cholesterol (mg/dL) | 188.1 ± 37.3 | 185.6 ± 37.8 | 0.672 |
| Triglycerides (mg/dL) | 148.1 ± 93.7 | 134.3 ± 93.3 | 0.317 |
| HDL (mg/dL) | 54.3 ± 17.0 | 53.8 ± 16.0 | 0.858 |
| sRAGE (pg/mL) | 935.2 ± 322.4 | 832.2 ± 350.7 | $p < 0.05$ |
| MCP-1 (pg/mL) | 406.3 ± 255.0 | 356.7 ± 248.2 | $p < 0.01$ |
| UAE (mg/g creatinine) | 126.5 ± 248.5 | 140.0 ± 320.6 | 0.720 |
| DTSQ | | | |
| Score | 4.4 ± 1.3 | 4.7 ± 1.1 | $p < 0.01$ |
| Hyperglycemia | 3.5 ± 1.6 | 2.9 ± 1.4 | $p < 0.05$ |
| Hypoglycemia | 2.0 ± 1.6 | 1.6 ± 1.2 | $p < 0.05$ |
| Basal insulin (glargine) (number) | 18 | 18 | – |
| Bolus insulin (U/day) | 11.9 ± 9.6 | 13.1 ± 13.0 | 0.302 |
| Bolus insulin (aspart/lispro/regular) (number) | 9/13/1 | 0/0/0 | – |
| Bolus insulin (U/day) | 27.0 ± 11.4 | 27.2 ± 11.4 | 0.266 |
| Medication | | | |
| Hypertension (number) | 11 | 11 | – |
| Dyslipidemia (number) | 12 | 12 | – |

HbA1c; glycated hemoglobin, GA; glycoalbumin, BP; blood pressure, AST; aspartate aminotransferase, ALT; alanine aminotransferase, BUN; blood urea nitrogen, HDL-C; high-density lipoprotein-cholesterol, sRAGE; soluble form of RAGE; MCP-1; monocyte chemoattractant protein-1, UAE; urinary albumin excretion, DTSQ; diabetes treatment satisfaction questionnaire.

evaluated by DTSQ. All values were presented as mean ± standard deviation. Paired *t*-test was performed for comparisons between baseline and post-glulisine treatment. Correlations between changes in glycoalbumin (GA), sRAGE, MCP-1, or DTSQ score from baseline (ΔGA, ΔsRAGE, ΔMCP-1, or ΔDTSQ score) and other clinical variables were determined by a linear regression analysis. To determine independent determinants of ΔGA, ΔsRAGE, ΔMCP-1, or ΔDTSQ score, multiple stepwise linear regression analysis was performed. Intra- and inter-assay coefficients of variation were 0.7 and 0.7% for HbA1c, 0.9 and 0.7% for GA, 5.7 and 7.7% for sRAGE, 5 and 5.1% for MCP-1, and 0.7 and 2.5% for UAE, respectively [9]. All statistical analyses were performed with the use of the SPSS system. Statistical significance was defined as $p < 0.05$.

Demographical data of the subjects are presented in Table 1. Eighteen patients already received basal glargine therapy. Although bolus and basal insulin doses were almost unchanged before and after the glulisine replacement, switching to glulisine for 24 weeks significantly decreased GA, sRAGE, MCP-1 and perceived frequency of hyperglycemia and hypoglycemia, while it increased DTSQ score and serum creatinine values (Table 1). As shown in Table 2, multiple stepwise regression analysis showed that Δplasma glucose, ΔHbA1c and Δtriglycerides were independent determinants of ΔGA ($R^2 = 0.802$). Furthermore, there was an inverse and independent correlation of ΔGA with ΔDTSQ ($R^2 = 0.196$) (Table 2). ΔsRAGE was a sole independent determinant of ΔMCP-1, even after adjustment for confounders such as age, sex, and change of GA, HbA1c, or plasma glucose.

We found here that switching from short-acting insulin to glulisine for 24 weeks significantly improved GA in type 2 diabetic patients. Moreover, Δplasma glucose, ΔHbA1c and Δtriglycerides were independently correlated with ΔGA, and decreased ΔGA was a sole determinant of the improvement of patient satisfaction evaluated by DTSQ score. Since GA is a more useful biomarker for postprandial glycemic excursions in type 2 diabetic subjects [11], glulisine might improve GA and patient satisfaction partly by ameliorating postprandial metabolic derangement due to its rapid absorption with a shorter duration of action compared with other short-acting insulin such as aspart, lispro, and regular human insulin [9]. In this study, switching to glulisine also decreased serum levels of sRAGE and MCP-1. Furthermore, ΔsRAGE and ΔMCP-1 were independently correlated with each other in stepwise regression analysis (data not shown). MCP-1, a CC chemokine, plays an important role in the early phase of atherosclerosis by initiating monocyte recruitment to the vessel wall, and its expression is elevated in human atherosclerotic plaques [12]. Given the active involvement of

**Table 2**
Univariate and multivariate stepwise regression analysis for the determinants of ΔGA and ΔDTSQ score.

| | For ΔGA | | | | For ΔDTSQ score | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | |
| Parameters | β | p | β | p | β | p | β | p |
| ΔBody weight (kg) | −0.470 | $p < 0.05$ | | | 0.150 | 0.494 | | |
| ΔPlasma glucose (mg/dL) | 0.681 | $p < 0.01$ | 0.295 | $p < 0.05$ | −0.340 | 0.112 | | |
| ΔHbA1c (%) | 0.786 | $p < 0.01$ | 0.618 | $p < 0.01$ | −0.421 | $p < 0.05$ | | |
| ΔGA (%) | – | – | | | −0.482 | $p < 0.05$ | −0.482 | $p < 0.05$ |
| ΔSystolic BP (mm Hg) | 0.127 | 0.563 | | | −0.124 | 0.574 | | |
| ΔDiastolic BP (mm Hg) | 0.213 | 0.329 | | | 0.011 | 0.959 | | |
| ΔAST (IU/L) | 0.057 | 0.796 | | | −0.321 | 0.136 | | |
| ΔALT (IU/L) | 0.136 | 0.535 | | | −0.354 | 0.098 | | |
| ΔBUN (mg/dL) | 0.005 | 0.981 | | | −0.145 | 0.508 | | |
| ΔCreatinine (mg/dL) | −0.559 | $p < 0.01$ | | | 0.115 | 0.601 | | |
| ΔTotal cholesterol (mg/dL) | 0.029 | 0.896 | | | −0.044 | 0.842 | | |
| ΔTriglycerides (mg/dL) | 0.466 | $p < 0.05$ | 0.306 | $p < 0.01$ | −0.161 | 0.464 | | |
| ΔHDL (mg/dL) | −0.119 | 0.590 | | | −0.141 | 0.522 | | |
| ΔsRAGE (pg/mL) | 0.061 | 0.782 | | | −0.091 | 0.679 | | |
| ΔMCP-1 (pg/mL) | 0.066 | 0.766 | | | 0.204 | 0.351 | | |
| ΔUAE (mg/g creatinine) | −0.011 | 0.959 | | | −0.070 | 0.752 | | |
| ΔDTSQ score | −0.482 | $p < 0.05$ | | | – | – | | |

$R^2 = 0.802$ for ΔGA.
$R^2 = 0.196$ for ΔDTSQ score.

RAGE in MCP-1 overexpression and vascular injury in diabetes [13], our present study suggests that pre-meal injections of bolus insulin glulisine might be superior to other short-acting insulin in controlling post-prandial hyperglycemia, improving patient satisfaction, and protecting against vascular damage in type 2 diabetes partly by suppressing RAGE activation.

## Disclosure

Dr. Yanagisawa, Dr. Fukami, and Dr. Yamagishi have received honoraria such as lecture fees from Sanofi, Novo Nordisk, and Eli Lilly.

## Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

## Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research (B) and by the MEXT-Supported Program for the Strategic Research Foundation atPrivate Universities from the Ministry of Education, Culture, Sports, Science and Technology (MEXT (S0901050)) (to S.Y.).

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