High serum concentrations of soluble E-selectin in patients with impaired glucose tolerance with hyperinsulinemia

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Abstract

High serum concentrations of soluble adhesion molecules are present in diabetics, but whether similar levels are present in patients with impaired glucose tolerance (IGT) is unclear. We measured serum concentrations of soluble intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), and sE-selectin in 128 nondiabetic Japanese subjects. The concentrations of sICAM-1, sVCAM-1, and sE-selectin in IGT patients (n = 47) were not different from those in subjects with normal glucose tolerance (NGT; n = 81). IGT patients were subdivided into two groups by the results of 75 g OGTT, those with low- (hypoinsulinemia; n = 23) or high-insulin (hyperinsulinemia; n = 24). The levels of sICAM-1 and sVCAM-1 were not different among NGT and IGT with high-insulin or with low-insulin. However, sE-selectin concentrations were significantly higher in IGT patients with high-insulin than in NGT and IGT with low-insulin (61.1 ± 3.4, 47.1 ± 1.8 and 43.7 ± 3.9 ng/ml, respectively, P < 0.001). Adjustment for age and gender did not influence the results. Serum sE-selectin concentrations correlated significantly with the area under the curve of insulin (AUC insulin), AUC glucose, diastolic blood pressure, and triglyceride levels (r = 0.35, 0.26, 0.18 and 0.21, respectively), and negatively with HDL-cholesterol levels (r = 0.20). Multiple regression analysis showed that AUC insulin was the only independent factor that correlated with sE-selectin levels (P < 0.001). Our results indicate that hyperinsulinemia; insulin resistance may be responsible for the elevation of sE-selectin levels. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Adhesion molecule; E-selectin; Impaired glucose tolerance; Hyperinsulinemia; Insulin resistance

1. Introduction

Adhesion of leucocytes to arterial endothelial cells and subsequent transendothelial infiltration is thought to be an important step in the development of atherosclerosis [1–3]. This process depends on a group of receptors and binding proteins, i.e. adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin [1,2]. Recent studies have reported the presence of high serum concentrations of soluble adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) in patients with type 2 diabetes [4–7]. Moreover, the levels of sE-selectin correlate positively with the degree of hyperglycemia [8–10]. Despite the association between increased soluble adhesion molecules and clinically overt diabetes mellitus, it is not clear whether increased concentrations of soluble adhesion molecules are present in patients with impaired glucose tolerance (IGT).

IGT is often complicated with dyslipidemia, hypertension, and obesity, and is characterized by insulin resistance [11]. Insulin resistance and compensatory hyperinsulinemia may play a central role in the multifaceted clustering syndrome, and accelerate the development of atherosclerosis [11–13]. Such insulin resistance is common in patients with IGT among Pima Indians and Hispanics [14,15]. However, some Japanese patients with IGT do not necessarily show hyperinsulinemia; insulin resistance [16]. Taniguchi et al. [17] reported two subtypes of Japanese patients with IGT; those who are insulin sensitive and others who are insulin resistant. The relationship between concentra-
tions of soluble adhesion molecules and hyperinsulinemia is also not clear at present.

Therefore, we measured sICAM-1, sVCAM-1, and sE-selectin in Japanese patients with IGT with respect to hyperinsulinemia.

2. Research design and methods

A total of 150 Japanese subjects gave their informed consent to participate in this cross-sectional study. All subjects visited the Health Care Center of the Sasebo Chuo Hospital for health checks of lifestyle related diseases (e.g. diabetes, hypertension and hyperlipidemia). Excluded from the study were patients who had been already diagnosed with diabetes mellitus, those with chronic heart or renal failure, ischemic heart disease, stroke, peripheral obstructive artery disease, endocrine disease or intercurrent infections. Each patient completed a medical questionnaire, and physical check up, routine collection of biochemical data, and a 75 g oral glucose tolerance test (OGTT) were performed. Blood pressure was measured with a standard mercury sphygmomanometer after at least 10 min of rest.

A 75 g OGTT was performed after an overnight fast. Subjects ingested carbohydrate equivalent to 75 g of glucose (Tolelan-G, Shimizu, Japan), and blood samples were withdrawn at 0, 30, 60, 90 and 120 min.

Based on the results of OGTT, 22 subjects were found to have diabetes and accordingly were excluded from the study. The remaining 128 subjects were classified into two groups according to their glucose tolerance status as those with normal glucose tolerance (NGT; n = 81) or impaired glucose tolerance (IGT; n = 47). The definition of glucose tolerance was based on the report of the Expert Committee of American Diabetes Association [18]. In this study, patients with impaired fasting glucose were included among those with IGT. IGT patients were subdivided into two classes; IGT with high-insulin and IGT with low-insulin. Area under the curve of insulin during OGTT (AUC_{insulin}) over median level (39.48 nmol min per l) in IGT patients was defined as high-insulin (hyperinsulinemia; n = 24), and AUC_{insulin} below median level was defined as low-insulin (hypoinsulinemia; n = 23). AUC_{glucose} and AUC_{insulin} were calculated by the trapezoidal methods.

Plasma glucose was measured in duplicate with an automatic analyzer (Kyoto-Daichikagaku, Kyoto, Japan) by the glucose oxidase method. Intra- and interassay coefficients of variations (CVs) were 1.5%. Immuno-reactive insulin was measured by a commercial radioimmunoassay (RIA) kit (Shionogi, Osaka, Japan). The intra- and interassay CVs were less than 5%. Total cholesterol and triglyceride were measured by enzymatic methods (Kokusai Shiyaku, Kobe, Japan). HDL-cholesterol was determined after isolation by the precipitation method (Kyowa, Tokyo, Japan). CVs for intra- and interassay in total cholesterol, triglyceride, and HDL-cholesterol were less than 3%.

Serum concentrations of sICAM-1, sVCAM-1, and sE-selectin were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abington, UK). The intra- and interassay CVs were less than 6%.

3. Statistical analysis

Unpaired data were analyzed by the χ^2 test or Student’s t-test. Multiple comparisons were conducted by one-way analysis of variance (ANOVA) with post hoc Bonferroni test. Analysis of covariance (ANCOVA) and contrasts were used to compare data after adjustment for other variables. The relationship between sE-selectin concentrations and other variables was estimated by Pearson’s correlation coefficients. To determine the independent factor(s) related to sE-selectin concentrations, we performed the multiple regression analysis. Data were presented as means ± SE. Differences were considered statistically significant at P < 0.05. Statistical analysis was performed using Statview 4.5 or Super ANOVA (Abacus, Berkeley, CA) software package.

4. Results

4.1. Clinical characteristics

Table 1 shows the clinical characteristics of patients with NGT and IGT. Patients with IGT were significantly older than NGT subjects and had a higher blood pressure, fasting plasma glucose, and serum triglyceride concentrations. However, body mass index, the percentage of smokers among each group, fasting insulin concentrations, and total- and HDL-cholesterol concentrations were comparable between IGT and NGT. When patients with IGT were subdivided into two groups based on the levels of insulin, patients with high-insulin showed significant fasting and post glucose challenge hyperinsulinemia. This result suggested the presence of insulin resistance in patients with high-insulin.

4.2. Serum concentration of soluble adhesion molecules

Table 2 shows the serum levels of sICAM-1, sVCAM-1, and sE-selectin in subjects with NGT and patients with IGT. The mean levels of soluble adhesion molecules were slightly higher in patients with IGT than in NGT, but these differences did not reach statis-
tential significance. We also investigated the effects of hyperinsulinemia/insulin resistance on the serum concentrations of soluble adhesion molecules. Soluble ICAM-1 levels in NGT, IGT with low-insulin, and IGT with high-insulin were comparable by ANOVA ($P = 0.308$, Table 2). Soluble VCAM-1 levels in IGT with high-insulin tended to be higher than in NGT and IGT with low-insulin albeit insignificantly ($P = 0.174$, Table 2). On the other hand, sE-selectin levels in IGT with high-insulin were significantly higher than in NGT and IGT with low-insulin albeit insignificantly ($P = 0.20$, Table 2). Adjustments for age and gender by ANCOVA did not influence these results.

4.3. Relationship between sE-selectin and other variables

In the simple correlation coefficients, age, body mass index, AUC$_{\text{glucose}}$, AUC$_{\text{insulin}}$, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, and HDL-cholesterol were entered as independent variables. Serum concentrations of sE-selectin significantly correlated with AUC$_{\text{glucose}}$ ($r = 0.26$, $P = 0.003$), AUC$_{\text{insulin}}$ ($r = 0.35$, $P < 0.001$), diastolic blood pressure ($r = 0.18$, $P = 0.042$), and triglyceride ($r = 0.21$, $P = 0.018$). The levels of sE-selectin also significantly but negatively correlated with HDL-cholesterol ($r = -0.20$, $P = 0.021$). The correlation between AUC$_{\text{insulin}}$ and sE-selectin levels is depicted in Fig. 1. To determine the independent factor(s) related to sE-selectin levels, we performed multiple regression analysis by entering AUC$_{\text{glucose}}$, AUC$_{\text{insulin}}$, diastolic blood pressure, triglyceride, and HDL-cholesterol as independent variables. Multiple regression analysis identified AUC$_{\text{insulin}}$ as the only independent factor that determined sE-selectin levels ($\beta = 0.353$, $F = 17.98$; $P < 0.001$).

5. Discussion

In the present study, we demonstrated the presence of high serum concentrations of sE-selectin in Japanese

table 1

<table>
<thead>
<tr>
<th>Clinical characteristics of the subjects studied</th>
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<tbody>
<tr>
<td>NGT*</td>
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<tr>
<td>All IGT subjects</td>
</tr>
<tr>
<td>Number (male/female)</td>
</tr>
<tr>
<td>Age (year)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
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<tr>
<td>Smoking n (%)</td>
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<tr>
<td>Fasting plasma glucose (mmol/l)</td>
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<tr>
<td>Fasting insulin (pmol/l)</td>
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<td>AUC$_{\text{glucose}}$ (mmol min per l)</td>
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<tr>
<td>AUC$_{\text{insulin}}$ (mmol min per l)</td>
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<tr>
<td>Total cholesterol (mmol/l)</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
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</table>

* NGT, normal glucose tolerance; IGT, impaired glucose tolerance. AUC, area under the curve during OGTT. Data are $n$, means ± SE, or $n$ (%).
* $P < 0.05$ versus NGT.
† $P < 0.05$ versus IGT-hyperinsulinemia (−).

Table 2

<table>
<thead>
<tr>
<th>Serum concentrations of soluble ICAM-1, VCAM-1, and E-selectin in subjects with NGT and patients with IGT*</th>
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<tbody>
<tr>
<td>NGT</td>
</tr>
<tr>
<td>All IGT subjects</td>
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<tr>
<td>sCAM-1 (ng/ml)</td>
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<tr>
<td>sVCAM-1 (ng/ml)</td>
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<td>sE-selectin (ng/ml)</td>
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</tbody>
</table>

* NGT, normal glucose tolerance; IGT, impaired glucose tolerance. Data are means ± SE.
* $P < 0.05$ versus NGT.
† $P < 0.05$ versus IGT with low-insulin.
patients with IGT with hyperinsulinemia. Our results also showed that sE-selectin levels were not elevated in IGT patients with hypoinsulinemia. Further analysis showed that serum sE-selectin concentrations correlated with AUC_inulin, AUC_glucose, blood pressure, triglyceride, and negative with HDL-cholesterol.

Recent studies have demonstrated the presence of significantly high concentrations of sICAM-1, sVCAM-1, and sE-selectin in patients with overt diabetes [5–7]. In the present study, sICAM-1 and sVCAM-1 levels in Japanese IGT patients were not elevated, and the rise in sE-selectin levels was dependent on the presence or absence of hyperinsulinemia. Therefore, it seems that elevation of sICAM-1 and sVCAM-1 may appear only after the full manifestation of diabetes [19,20].

Other recent studies have shown that high levels of soluble adhesion molecules, especially sICAM-1 and sVCAM-1, were strongly associated with established atherosclerosis such as coronary artery, carotid artery, and peripheral artery [6,21–23]. The subjects in our present study lacked clinical evidence of ischemic heart disease, stroke, and peripheral obstructive artery disease. Thus, it is likely that the normal levels of sICAM-1 and sVCAM-1 noticed in the present study in patients with IGT were due to the inclusion criteria used in the present study.

It is well known that E-selectin is expressed only on activated endothelial cells, and mediates the initial ‘rolling’ of leucocytes on the endothelium [1,3,4]. The presence of high concentrations of sE-selectin in the serum reflects activation of the endothelium and is a manifestation of the initial stage of atherosclerosis [1,3,4]. Accordingly, early activation of endothelium may be present in patients with IGT, which represents a prediabetic stage. Similar high concentrations of sE-selectin have been reported in other patients with prediabetic conditions such as former gestational diabetes mellitus or first degree relatives of type 2 diabetes mellitus [24,25].

Several studies have investigated the relationship between adhesion molecules and hypertension or dyslipidemia [26–28]. In our present study, sE-selectin levels correlated with elevated AUC_{glucose}, elevated AUC_{insulin}, high blood pressure, elevated triglyceride concentrations, and low HDL-cholesterol. These conditions are strongly related to insulin resistance [11–13,29]. Our study showed that only hyperinsulinemia is an independent factor related to elevated sE-selectin. These results suggest that hyperinsulinemia/insulin resistance may be a basic factor related to endothelial activation. Another possible cause of elevated sE-selectin levels is endothelium damaged by insulin resistant state [4]. In the present study, we have not measured markers of endothelial cell damage, like von Willebrand factor [1,21], so we are unable to confirm the suggestion that elevated sE-selectin levels reflect activation or injury of the endothelium.

How does insulin resistance increase the expression of E-selectin on the endothelium? While the exact mechanisms are unknown at present, recent studies demonstrated a blunted endothelium-dependent vasodilation in the insulin resistant state [30]. This phenomenon was also associated with low nitric oxide (NO) release from the endothelium [30,31]. Furthermore, NO limits endothelial activation and reduces the expression of E-selectin in vitro [32]. Therefore, high serum concentrations of sE-selectin in insulin resistance may be, at least in part, related to low NO levels.

Agents that improve insulin resistance are reported to decrease serum levels of sE-selectin [28,33]. For example, Cominacini et al. [33] reported that the treatment with troglitazone resulted in decrease of sE-selectin levels in patients with type 2 diabetes. Ferri et al. [28] reported that the treatment with enalapril, an angiotensin converting enzyme inhibitor, decreased sE-selectin concentrations in patients with essential hypertension. Another study has also shown that intensive insulin treatment reduced sE-selectin levels in patients with type 2 diabetes [34]. These results suggest that improvement in insulin resistance may be associated with a concomitant decrease in sE-selectin levels.

In conclusion, we demonstrated in the present study the presence of high concentrations of sE-selectin in Japanese patients with IGT with hyperinsulinemia (suggesting an insulin resistant state). This may be associated with a premature atherogenesis in insulin resistance syndrome.
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References
