Relation Between Serum 3-Deoxyglucosone and Development of Diabetic Microangiopathy

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OBJECTIVE — 3-Deoxyglucosone (3-DG), a highly reactive intermediate of the glycation reaction, has been suggested to contribute to the development of diabetes complications. To verify this hypothesis, we assessed the relation between serum 3-DG concentrations and the severity of diabetic microangiopathy in diabetic patients.

RESEARCH DESIGN AND METHODS — We conducted a high-performance liquid chromatography assay to determine the serum 3-DG concentrations of 110 diabetic patients with different degrees of severity of diabetic microangiopathy and 57 age-matched control subjects.

RESULTS — The fasting serum 3-DG level in diabetic patients was significantly (P < 0.001) higher than that in control subjects (353 ± 110 vs. 199 ± 53 nmol/l). The 3-DG levels were significantly (P < 0.001) elevated even in the diabetic patients showing normoalbuminuria (n = 62, 322 ± 79 nmol/l) compared with control subjects. The 3-DG levels were further elevated in the patients with microalbuminuria (n = 30, 383 ± 146 nmol/l) and overt proteinuria (n = 18, 410 ± 100 nmol/l) (P = 0.027 and P < 0.001 vs. normoalbuminuria group, respectively). This phenomenon was basically reproduced in a category of retinopathy. Furthermore, the diabetic patients with low nerve conduction velocity showed a tendency to display higher 3-DG levels.

CONCLUSIONS — The present results show that the fasting serum 3-DG level is elevated in diabetic patients and that the patients with relatively higher 3-DG levels were prone to suffer from more severe complications, indicating a possible association of 3-DG with diabetic microangiopathy.

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Prospective studies have revealed that hyperglycemia causes a series of long-term complications, such as microangiopathy, in both type 1 (1) and type 2 (2, 3) diabetic patients. The nonglycemic glycation reaction is believed to contribute to the hyperglycemia-related mechanisms underlying the development of diabetes complications (4, 5). In fact, several studies have demonstrated that the formation of advanced glycation end products (AGEs) is accelerated in diabetic patients (6–13). When modified by AGEs, proteins are known to alter their morphological and functional properties; two examples are the inactivation of enzymes (14) and a decrease in the susceptibility to proteolysis (15), resulting in the deterioration of homeostasis of the tissues. It has also been suggested that interaction between AGEs and cell surface receptors contributes to the development of diabetes complications (16–18).

It is therefore important to clarify the pathway involved in the formation of AGEs. Although the advanced stage of this reaction is very complex due to several possible metabolic pathways, highly reactive dicarbonyl compounds such as 3-deoxyglucosone (3-DG) (6, 19–22) have been identified as important intermediates. In addition, fructose has been indicated as another potential precursor of 3-DG (23, 24). Considering the fact that fructose is generated predominantly in diabetic tissues due to the excessive flux of glucose through the polyol pathway, 3-DG may be a key intermediate linkage between glycation and the polyol pathway. In addition to the action of 3-DG as a potent precursor of AGEs, recent studies have revealed the possibility that 3-DG itself directly affects cell functions (25–27).

Thus, it would be very important to determine the 3-DG level in vivo to address the etiology of diabetic microangiopathy. Despite the difficulty in detecting 3-DG due to its instability, ours (28) and other laboratories (29–32) have been successfully determining levels of 3-DG in blood. Our success in detecting a minute amount of 3-DG in serum was attributable to its derivatization to a quite stable compound by reaction with 2,3-diaminonaphthalene, followed by a high-performance liquid chromatography (HPLC) analysis. Using this method in the present study, we investigated the relation of the serum 3-DG concentration with the degree of diabetic nephropathy, retinopathy, and neuropathy in order to...
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RESEARCH DESIGN AND METHODS

Study subjects

We studied 110 diabetic patients, aged 20–80 years, who were referred to Kobe University Hospital (Kobe, Japan) and National Hyogo Chuo Hospital (Sanda, Japan) for the evaluation and treatment of diabetes. Nine type 1 diabetic patients and 101 type 2 diabetic patients were included. Subjects with elevated serum creatinine concentrations (>115 μmol/l) were excluded from this study to eliminate the possible effect of a deficiency in renal clearance function on the serum 3-DG level. Fasting blood samples for the measurement of the serum 3-DG level were obtained in conjunction with the collection of samples for other routine laboratory studies, according to approved institutional guidelines. The characteristics of the study subjects are shown in Table 1. Each diabetic patient was provided with medical care to achieve glycemic control; 29 patients were treated with dietary modification, 36 patients with oral hypoglycemic drugs, and 45 patients with insulin. Fifty-seven age-matched control subjects were also obtained with informed consent from nondiabetic patients and healthy volunteers at the Kobe University Hospital. None of the nondiabetic control subjects had a past history of renal, ocular, or neurological disease.

Clinical examination

Urine analysis was performed in all diabetic patients. Microalbuminuria and proteinuria were defined as a urinary albumin excretion of ≈30 and ≈300 mg/day, respectively. Only the patients who gave their consent underwent ophthalmological and neurological examinations (99 and 48 patients, respectively). The extent of retinopathy was assessed by ophthalmologists using fundus photography, and it was classified as normal, nonproliferative retinopathy, or proliferative retinopathy. Patients with a history of laser treatment were included in the last category. However, patients who had taken laser treatments for maculopathy were excluded from entry into this study. Electrophysiological data were obtained by a single physician at the clinical laboratory of National Hyogo Chuo Hospital. Sensory nerve conduction velocity (SCV) and motor nerve conduction velocity (MCV) were determined in the sural nerve of 47 patients and peroneal nerve of 46 patients, respectively. F-wave conduction velocity (FCV) in the proximal segment of the peroneal nerve was simultaneously measured in 31 patients.

Measurement of serum 3-DG

The serum 3-DG concentration was determined as previously described (28). Briefly, 2,3-pentanedione was added to serum as an internal standard and deprotonized with 6% perchloric acid, followed by the reaction with 2,3-diaminonaphthalene to derivatize 3-DG to a stable compound. Authentic 3-DG was kindly supplied by Fumitaka Hayase (Meiji University, Kawasaki, Japan) and derivatized simultaneously to be used as a standard. The derivatives in the reaction mixture were extracted using ethyl acetate, dried, and reconstituted with methanol to be subjected to HPLC. The quantitation of 3-DG was performed by calculating the correlation of the peak area with the serum with that of authentic 3-DG.

Table 1—Characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>n (M/F)</th>
<th>Age (years)</th>
<th>Diabetes duration (years)</th>
<th>HbA1c (%)</th>
<th>Serum 3-DG (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>57 (30/27)</td>
<td>54 ± 19</td>
<td>—</td>
<td>—</td>
<td>199 ± 53</td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>110 (64/46)</td>
<td>59 ± 12</td>
<td>12 ± 9</td>
<td>8.0 ± 1.7</td>
<td>353 ± 110</td>
</tr>
<tr>
<td>All patients</td>
<td>110 (64/46)</td>
<td>59 ± 12</td>
<td>12 ± 9</td>
<td>8.0 ± 1.7</td>
<td>353 ± 110</td>
</tr>
<tr>
<td>Severity of nephropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>49 (28/21)</td>
<td>58 ± 13</td>
<td>10 ± 8</td>
<td>7.9 ± 1.5</td>
<td>311 ± 80</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30 (21/9)</td>
<td>59 ± 11</td>
<td>13 ± 8</td>
<td>7.9 ± 1.8</td>
<td>368 ± 104</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>18 (14/4)</td>
<td>59 ± 13</td>
<td>12 ± 7</td>
<td>8.7 ± 1.9</td>
<td>401 ± 140</td>
</tr>
<tr>
<td>Proliferative</td>
<td>23 (12/11)</td>
<td>59 ± 12</td>
<td>17 ± 8</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD.

Figure 1—Relation between HbA1c and serum 3-DG levels in diabetic subjects. No significant correlation was found between the HbA1c and serum 3-DG levels of the 110 diabetic subjects (P = 0.140), since there were several patients whose serum 3-DG levels were markedly deviated from the recent blood glucose control state. Three patients (A–C) showed a serum 3-DG level >500 nmol/l despite the HbA1c <7.0%. Patients A and B had begun to exhibit signs of diabetic microangiopathy despite their short duration of diabetes (3 and 2 years, respectively). Patient C (whose diabetes duration was 20 years) had already developed overt proteinuria and proliferative retinopathy. In contrast, patient D, whose serum 3-DG remained <200 nmol/l regardless of the poor blood glucose control at the time of this study, showed no signs of microangiopathy, with a diabetes duration of 7 years.
peaked height ratio of the 3-DG–derived peak to an internal standard peak.

**Statistical analysis**

The data are expressed as means ± SD. Comparison between groups was performed using one-way ANOVA followed by Benferroni/Dunn multiple comparison test. We used Student’s t test only in the case of comparison between two groups. A P value <0.05 was considered significant.

**RESULTS**

**Serum 3-DG concentrations in control and diabetic subjects**

The serum 3-DG concentrations of the diabetic patients were significantly higher than those of the nondiabetic control subjects (P < 0.001) (Table 1). Principally, there was a tendency for the patients with higher HbA1c levels to show higher 3-DG levels. However, we did not find statistically significant correlation between the HbA1c and serum 3-DG levels in the diabetic patients (P = 0.140), since several patients showed a 3-DG level markedly deviated from the recent blood glucose control state indicated by the HbA1c level (Fig. 1). For example, patient A in Fig. 1 showed a much higher serum 3-DG concentration compared with the value expected from her HbA1c level and already suffered from microalbuminuria and some decrease in nerve conduction velocities, despite short duration (3 years) of diabetes. The other two patients (patients B and C in Fig. 1), whose serum 3-DG levels were >500 nmol/l despite an HbA1c level <7.0%, also showed certain signs of microangiopathy. Patient B (whose diabetes duration was still 2 years) started to show microalbuminuria and nonproliferative retinopathy, while patient C had already developed overt proteinuria (whose diabetes duration was still 2 years) and the serum 3-DG values of each group are summarized in Table 1. A prominent elevation of serum 3-DG levels was observed even in the diabetic patients with normoalbuminuria compared with the control subjects (P < 0.001) (Fig. 2). Significant differences in serum 3-DG concentration were found between the normoalbuminuria and other diabetic groups, such as microalbuminuria and overt proteinuria. The serum 3-DG levels were also significantly (P < 0.001) elevated even in the diabetic patients without any signs of retinopathy. The serum 3-DG levels were significantly increased as retinopathy developed to the nonproliferative or proliferative state.

**Serum 3-DG concentrations and diabetic complications**

The diabetic group was subdivided according to the severity of complications, and the mean value of serum 3-DG significantly increased as retinopathy developed to the nonproliferative or proliferative state (P = 0.008 and P < 0.001 vs. the diabetic patients without retinopathy, respectively). The logistic regression analysis showed that relative risk of any diabetic retinopathy was 2.04 (95% CI 1.26–3.29, P = 0.036) per 100-nmol/l increase in serum 3-DG level.

Regarding neuropathy, we investigated the relation between the serum 3-DG levels and abnormalities in the nerve conduction, including the SCV, MCV, and FCV. We separated the patients by the median value of each conduction velocity value (45.1 m/s for SCV, 40.2 for MCV, and 47.6 for FCV) (Fig. 3). The patients with reduced nerve conduction velocities tended to show higher serum 3-DG levels in each conduction study: a significant difference (P = 0.013) was observed in the MCV. Further analysis revealed that the serum 3-DG concentrations of the patients with a reduction in two or all three of the conduction velocity values (n = 17, 381 ± 107 nmol/l) were significantly (P = 0.035) higher than those of the patients, without any reduction in conduction velocity (n = 9, 291 ± 81 nmol/l). We alternatively conducted a correlation analysis to evaluate overall correlation between NCV measurements and serum 3-DG levels, so that FCV measurement showed significant correlation with 3-DG level (r = 0.392, P = 0.029).

**CONCLUSIONS** — Hyperglycemia has been believed to cause diabetes complications through the glycation reaction, protein kinase C activation, and the polyol pathway, as well as overproduction of reactive oxygen species from mitochondria (33). In fact, these mechanisms are thought to be implicated to each other. Considering the possibility that 3-DG forms from fructose (23,24) via the polyol pathway in addition to glucose-derived glycated proteins, the measurement of this key compound may give more comprehensive information regarding the hyperglycemia-related mechanisms.

In the present investigation, a cause-and-effect relation between the 3-DG and diabetic microangiopathy might be postulated since the elevation of the serum 3-DG concentration exists before the onset of microangiopathy and the degree of severity of the microangiopathy is in part dependent on the serum 3-DG concentra-
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Figure 3—Relation between serum 3-DG level and nerve conduction velocity in diabetic patients. The SCV of the sural nerve (A), the MCV of the peroneal nerve (B), and the FCV of the peroneal nerve (C) were measured in 47, 46, and 31 patients, respectively. When the patients were separated by the median value in each conduction study, the distribution of serum 3-DG levels in the patients with reduced conduction velocities were shifted to higher levels than those of the patients with retained conduction velocity. The patients in the latter category are included in the striped area.

tion. Furthermore, we found that the development of diabetes complications was accelerated in the patients with an extremely high level of serum 3-DG (i.e., >500 nmol/l) and that they tended to suffer multiple and severe complications. In addition to subjects A–C in Fig. 1, some of the patients with high 3-DG levels showed a tendency to have more severe complications for their duration of diabetes, even if their HbA1c levels were not high (data not shown). On the contrary, patients with low 3-DG seemed to be relatively resistant to the development of the complications. However, even the subjects with modest elevation of 3-DG developed complications over a longer period of time, suggesting that duration of diabetes should also be taken into account when estimating the effect of 3-DG. Nevertheless, the present logistic regression analysis revealed that serum 3-DG level contributed to the development of both nephropathy and retinopathy more significantly than duration of diabetes. Thus, we had an impression that the serum 3-DG level may be a useful marker to predict the prognosis of microangiopathy, rather than a marker to reflect the current status of the complications. In other words, intensive prevention might be required to delay the progression of diabetes complications in the subjects with high 3-DG levels.

The potential mechanisms of 3-DG action in vivo presumably consist of both direct and indirect effects via AGE formation. Concerning the latter mechanism, 3-DG is well known to form AGEs much more efficiently than glucose. For example, the cross-linking potency of 3-DG in protein polymerization was ~10 times higher than that of glucose (34). These findings indicate that AGE formation is exponentially accelerated when glucose is converted to 3-DG. Immunohistochemical studies also showed 3-DG–derived AGEs, such as pyrraline (6) and imidazolone compound (35), accumulated in diabetic angiopathy lesions. In addition, recent studies have revealed that 3-DG itself has direct effects on cell functions (25–27). For example, Che et al. (27) reported that highly reactive dicarboxyls, including 3-DG, might induce intracellular oxidative stress by attacking the active sites of antioxidant enzymes, leading to the expression of heparin-binding epidermal growth factor–like growth factor in the aortic smooth muscle cells of rats. Thus, a longer exposure of a high level of 3-DG to cells in vivo might be responsible for some of the disorders observed in diabetic subjects. This may be supported by the present finding that diabetic patients with relatively higher 3-DG levels were prone to suffer from more severe complications.

The present study did not reveal a statistically significant correlation between serum 3-DG and HbA1c levels because of the presence of some patients with deviated 3-DG levels. This result is inconsistent with the previous report (32), showing a significant correlation between plasma 3-DG and HbA1c levels in 27 diabetic patients. We also believed that the levels of Amadori products, such as HbA1c, contribute to 3-DG level in part, since these compounds are potent precursors of 3-DG. However, 3-DG can even form through other pathways, including the polyol pathway. In this relation, Hamada et al. (36) reported the beneficial effect of epalrestat, an aldose reductase inhibitor, on the reduction of erythrocyte 3-DG content in diabetic patients, supporting the potential involvement of the polyol pathway in the formation of 3-DG in vivo. Therefore, activity of the enzymes in this pathway may also influence the 3-DG levels independent of glycemic level. Furthermore, it is also known that 3-DG is metabolized to a relatively inert species by several enzymes such as aldehyde reductase (37) and oxaldehyde dehydrogenase (38). Considering that several milligrams of 3-DG are formed in the body per day, as estimated by Baynes et al. (29), this detoxification system is important to regulate the 3-DG level. It therefore appears that the level of serum 3-DG is determined not only by its formation, but also by the ability to metabolize it in vivo. The result of our additional analysis for correlation between glucose and 3-DG concentrations in 43 subjects \( r = 0.459, P = 0.002 \) supports that glycemic status partly predicts 3-DG level but not completely. Takahashi et al. (14) reported the possibility that this detoxification system itself might be deteriorated due to glycation of the enzymes. Consequently, a “vicious circle” is established as follows: the elevation of 3-DG levels induced by hyperglycemia in turn inactivates these enzymes by modifying them, resulting in the retention of a high level of 3-DG in diabetic subjects. In addition,
Beisswenger et al. (39) recently indicated that a postprandial increase in the 3-DG level was correlated with postprandial glycemic excursions, but not with HbA_{1c} level. This implies that 3-DG metabolism differs among the diabetic patients with a similar HbA_{1c} level. The possible individual differences in the metabolism of 3-DG may in part account for the incomplete correlation between HbA_{1c} and serum 3-DG levels observed in the present study. Furthermore, we found that the patients whose 3-DG concentrations were relatively higher compared with the values expected from their HbA_{1c} levels were likely to show a faster progression of complications. This suggests that an elevation of 3-DG level is more closely related to the complications in insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 28:103–117, 1995


