Variations of 1-Deoxyglucose (1,5-Anhydroglucitol) Content in Plasma from Patients with Insulin-Dependent Diabetes Mellitus

Shigetake Yoshioka, Shizuko Saitoh, Chiyoko Negishi, Tomoo Fujisawa, Akihiko Fujimori, Osamu Takatani,\(^1\) Mitsuo Imura,\(^2\) and Masuo Funabashi\(^2\)

The concentration of 1-deoxyglucose (1,5-anhydroglucitol) in plasma from patients with insulin-dependent diabetes mellitus was measured by gas–liquid chromatography with an all-glass capillary column. Twenty-one plasma samples were obtained from 21 patients before insulin therapy, and 34 more from 13 patients receiving insulin therapy. 1-Deoxyglucose was generally not detectable in plasma of diabetic patients before they received insulin; it was measurable in the patients who had received insulin, although its concentration was low compared with that of healthy subjects. We therefore suggest that the absence of 1-deoxyglucose in plasma is one of the markers of metabolic states of diabetes, perhaps reflecting a disturbed function of carbohydrate metabolism; its presence in plasma within a normal range may reflect the better control of diabetic patients.

Additional Keyphrases: chromatography, gas–liquid chromatography, capillary monitoring therapy

1-Deoxyglucose (1,5-anhydroglucitol), a glucose metabolite originally discovered in Polyagalaceae (1), has been stated to be present in lower concentration in plasma of diabetic patients receiving insulin than in healthy persons (2).

In 1979, we confirmed (3–5) the presence of 1-deoxyglucose by using gas–liquid chromatography (GLC)\(^3\) and GLC-MS with an all-glass capillary column, and suggested that it played some important role in carbohydrate metabolism.

In this paper, we discuss the variations of 1-deoxyglucose content in plasma from diabetic patients before and during their treatment with insulin.

Materials and Methods

Materials

Subjects and samples: We obtained 21 plasma samples from 21 patients with insulin-dependent diabetes mellitus (IDDM) before insulin therapy, and 34 plasma samples from 13 patients with IDDM during insulin therapy. Each sample was taken shortly before breakfast. The samples from patients receiving insulin therapy were taken after more than six months of treatment with insulin. All of the patients were more than two years old when diagnosed at the National Defense Medical College Hospital; diagnosis was by the criteria of the National Diabetes Data Group (USA) (6).

Apparatus: We used a GLC (Model 6AMPtrF) equipped with an automatic calculator (Chromatopac C-R1A), a capillary column holder (Model CHL-4M), and a solventless injection system (Model SVL-6), all from Shimadzu Seisakusho Co., 160 Tokyo, Japan. The GLC-mass spectrometer (GLC-MS), Model 6020, was equipped with an all-glass capillary column, the solventless injection system, and a Model R-111 recorder, all from Shimadzu Seisakusho Co.

Chemicals: Absolute ethanol, pyridine, and other chemical reagents were all purchased from Wako Pure Chemicals Co., 130 Tokyo, Japan. Pyridine was distilled twice and stored at 4 °C until use. Trimethylsilyl (TMS) reagents (trimethylchlorosilane and hexamethyldisilazane) were obtained from Gasukuro-Kogyo Co., 160 Tokyo, Japan. The gases (H2, He, and N2) were 99.9999% pure, and were obtained from Nihon-Sanso Co., 105 Tokyo, Japan.

Procedures

Sample preparation: We prepared the samples as reported previously (4, 5), deproteinizing with 99.9% ethanol, evaporating the supernates under reduced pressure, and binding with TMS-groups.

GLC procedure: In determining concentrations of 1-deoxyglucose, we used adonitol as the internal standard. The sample volume injected was 1 μL; N2 was the carrier gas, at a flow rate of 50 mL/min (0.25 mL/min in the column). The temperature of both the detector and the sample injection port was 240 °C. The detector was a hydrogen flame ionization detector. The 0.25 mm i.d. × 20 m column, coated with silicone OV-101 and used as a coated-wall, open-tube column, was set at 160 °C initially, then linearly programmed to 200 °C at 0.5 °C/min. The split ratio, sensitivity, and range were 1:180, 10^3 MI, and 0.002 or 0.004 V, respectively. The sensitivity was such that 0.5 mg of 1-deoxyglucose per liter could be determined in a 0.1-mL initial sample of plasma.

GLC-MS procedure: To identify 1-deoxyglucose in plasma, we used a 0.25 mm × 15 mm all-glass, coated-wall, open-tube column, coated with silicone OV-101. The sample volume injected was 1 μL. The flow rates of the carrier gas and the scavenger gas (He) were 30 and 18 mL/min, respectively. For mass-spectrometer electron-impact analysis, the temperatures of the injector, separator, and ion source were 240, 230, and 250 °C, respectively. The electric energy was 70 eV, and the electric current was 100 μA. The acceleration high voltage was 3.5kV, and the scanning was for 3 e. The range of masses scanned was m/e 10–700.

Results

A control plasma (from a six-year-old boy) showed an obvious peak of 1-deoxyglucose (Figure 1A). Nineteen of the 21 samples taken from diabetic subjects before insulin therapy showed no peak at the retention time for 1-deoxyglucose (Figure 1B). The peak observed after insulin therapy was identified as originating from 1-deoxyglucose, on the basis of its chromatographic retention time (Figure 1C), and
was confirmed by GLC-MS to be the TMS-derivative of 1-deoxyglucose (Figure 2). 1-Deoxyglucose in plasmas from patients before insulin therapy was measurable in only two samples: 0.5 and 0.6 mg/L, respectively; 1-deoxyglucose in plasmas from patients after insulin therapy was not detected in only one sample (Table 1). The precision of determination of 1-deoxyglucose in low concentration was shown in Table 2. The mean contents of 1-deoxyglucose in plasma of control subjects who are more than one month old range from 12.3 to 33 mg/L (5).

Figure 3 shows the time course of the appearance of 1-deoxyglucose in plasma obtained from a typical patient with IDDM before and after several months of insulin therapy. The clinical data for this patient are briefly given in the legend to Figure 3.

From these findings, we concluded that 1-deoxyglucose is generally absent or low in plasmas from patients with IDDM before insulin therapy and generally present in various amounts after insulin therapy.

Table 1. Variation of 1-Deoxyglucose in Plasma from Patients with IDDM, before and after Insulin Therapy

<table>
<thead>
<tr>
<th>No. of samples/cases</th>
<th>1-Deoxyglucose concn, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min/max</td>
</tr>
<tr>
<td>Before insulin</td>
<td>21/21</td>
</tr>
<tr>
<td>After insulin</td>
<td>34/13</td>
</tr>
</tbody>
</table>

The difference between the two groups of patients was statistically significant, by Student's t-test (p = 0.05).

Table 2. Precision of GLC Determination of 1-Deoxyglucose in Low Concentration

<table>
<thead>
<tr>
<th>1-Deoxyglucose concn, mg/L</th>
<th>Prepared</th>
<th>Mean found</th>
<th>SD</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10.6</td>
<td>0.2</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.2</td>
<td>0.07</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>0.08</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.98</td>
<td>0.12</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

n = 6 at each concentration.

Fig. 2. Comparison of the mass spectra from the peak shown in Fig. 1 C (lower) and from a standard solution of 1-deoxyglucose (upper); both spectra totally coincide.
Discussion

The metabolic states of diabetes mellitus have been evaluated by monitoring plasma concentrations of various markers of sugar metabolism, such as glucose and (or) glycohemoglobin ($A_1$ or $A_{1C}$). These markers usually increase in plasma from diabetic patients who have insufficient control of their diabetes. In contrast, according to our studies, 1-deoxyglucose is not detectable in plasma from diabetics before insulin therapy, but increases in plasma after diabetic control with insulin has been started. These findings are, to our knowledge, the first report in human diabetics, although similar findings have been reported for experimental diabetes in rats (7). The detection limit for 1-deoxyglucose by our method is 0.5 mg/L in plasma, at which concentration there is no measurable chromatographic peak. We therefore estimate the content of this analyte in diabetics who have never been treated with insulin to be 0.5 mg/L or less. In the plasmas from diabetic patients after insulin therapy, the concentrations of 1-deoxyglucose were 10 mg/L or less. In our previous study (6), plasma 1-deoxyglucose contents in apparently healthy subjects, older than two years, were 10 mg/L or more.

Although attempts to achieve satisfactory metabolic control in diabetic patients have advanced in recent years, through the introduction of various regimens of insulin therapy, long-term control studies show that the rates of mortality and vascular complications in diabetics are still considerably high (8).

According to our studies, the diabetic patients, even those in satisfactory diabetic control in terms of plasma concentrations of glucose or glycohemoglobin, showed less 1-deoxyglucose in their plasma than did nondiabetics. This suggests that procedures for diabetic control are still not well developed, and that the determination of plasma glucose and (or) glycohemoglobin may not necessarily be sufficient to evaluate the metabolic states of diabetes.

Although we do not know how to control the diabetic patients to maintain the concentration of plasma 1-deoxyglucose within a normal range, or how its plasma concentration relates to various diabetic states, the compound may have a close correlation with carbohydrate metabolism. However, further studies are needed to clarify whether the measurement of plasma 1-deoxyglucose will be a useful marker for sugar metabolism and lead to better control of diabetes. In particular, it may be interesting to study whether the low 1-deoxyglucose concentration exerts some influence on diabetic symptoms, or whether keeping the concentration within a normal range can prevent diabetic symptoms and complications.

We thank Professor F. Iwanami for his valuable advice. This study was partly supported by a research grant from Morinaga-Hoshikai Foundation, Japan.

References