Prostacyclin Production Reduced in Diabetics with Vascular Complications

Tadamitsu Komori, MD, Satoru Fujii, MD, Osamu Nogi, MD, Makoto Ohashi, MD, Osamu Sasakawa, MD, and Kenjiro Yamamoto, MD*

Concentrations of plasma 6-keto-prostaglandin F$_{1\alpha}$ (6-keto-PGF$_{1\alpha}$), a stable metabolite of prostacyclin, were measured by radioimmunoassay before and after 3 min of induced ischemia in 45 diabetics and 23 controls matched for age. In the 45 diabetics, 15 had no vascular complications (group I), 10 had a macroangiopathy (group II), 10 had a microangiopathy (group III) and 10 had both macroangiopathy and microangiopathy (group IV). Plasma levels of 6-keto-PGF$_{1\alpha}$ before forearm ischemia were significantly lower in group IV diabetics than in non-diabetic controls (188 ± 17 pg/ml and 245 ± 14 pg/ml, respectively). After 3 min of ischemia, plasma 6-keto-PGF$_{1\alpha}$ concentrations were increased in control subjects by 34% and by 21% in group I diabetics. In group III diabetics as well as diabetics with atherosclerotic vascular lesions (groups II and IV), no significant change was observed after 3 min of ischemia. These results suggest that impaired vessel wall prostacyclin production may to some extent be responsible for the development of diabetic retinopathy and nephropathy as well as atherosclerotic vascular complications.

Key Words: Diabetes mellitus, Vascular complications, Prostacyclin, Forearm ischemia

In patients with diabetes mellitus, microangiopathies sometimes occur and these patients are highly susceptible to atherosclerosis$^1$ and arterial thrombosis$^2$. Abnormal metabolism of prostacyclin in the vessel wall and thromboxane in platelets may be involved in these vascular complications$^3$. Abnormal platelet functions including hyperaggregability$^4$, increased $\beta$-thromboglobulin release$^5$ and increased synthesis of thromboxane $A_2$ $^6$ occur in patients with diabetes mellitus and these abnormal platelet functions have been implicated in the development of diabetic vascular complications. An additional or alternative explanation may be that production of prostacyclin by the vessel wall is decreased in diabetics. Although the plasma level of prostacyclin has been controversial$^7$-$^10$, decreased release of prostacyclin by the vessel wall in vitro has been observed in tissues from diabetic animals and from patients$^{11}$-$^{15}$. Prostacyclin is released into the circulation in healthy subjects after forearm ischemia$^{16}$. In the present study, we attempted to determine the extent of prostacyclin by the vessel wall, in vivo, after forearm ischemia in diabetic patients. The relationship between the deficiency in prostacyclin production and diabetic vascular complications was given attention.

MATERIALS AND METHODS

Subjects

Twenty-three healthy controls (17 males and...
6 females) and 45 diabetic patients (35 males and 10 females) were studied. The ages were 30–76 with a mean of 48.8 ± 2.6 (±SE) years in healthy controls and 31–72 with a mean of 53.2 ± 1.5 years in diabetic patients, whose the mean duration of diabetes was 11.9 ± 1.2 years, and mean fasting of plasma glucose level was 173 ± 10 mg/dl. Twenty-five were receiving once or twice daily subcutaneous insulin (12~48 unit/day), and 9 were being treated with oral hypoglycemic agents (5 in 500 mg acetohexamide, 4 in 5.0 mg glipenclamide). The diabetics were further separated into four subgroups, as shown in Table 1. Fifteen had no vascular complications (group I), 10 had a macroangiopathy, defined as ischemic heart disease, cerebral vascular disease and/or peripheral vascular disease (group II), 10 had a microangiopathy, defined as proliferative retinopathy and/or nephropathy (group III) and 10 had both macroangiopathy and microangiopathy (group IV). No subjects had taken drugs known to interfere with prostaglandin synthesis or metabolism in the previous 2 weeks (e.g., non-steroidal antiinflammatory drugs and dipyridamole), but insulin and oral hypoglycemic agents were not discontinued.

Collection of Blood Samples

After an overnight fast, 21 guage needle was inserted into an antecubital vein and each subject was asked to lie comfortably on a bed for at least 15 min. Ten ml blood samples were collected. Ischemia of the forearm was obtained by inflating a pneumatic cuff to 30 mmHg above the systolic blood pressure for 3 min. After release the cuff, blood samples were collected within 30 sec.

Measurement of Plasma 6-keto-PGF1α

Plasma 6-keto-PGF1α, which is stable metabolite of prostacyclin, was determined according to Okahara et al.16 Briefly, blood samples were collected in heparinized plastic syringes and transferred into siliconized tubes containing EDTA-2Na (2 mg/ml) and sodium meclofenamate (10 μg/ml). The plasma was obtained after the centrifugation at 5,000 rpm for 20 min. Tritium-labelled 6-keto-PGF1α (1,000 count/min, 120 Ci/mmol, New England Nuclear, Boston) was added to 3.0 ml of plasma to determine the recovery rate. The mixture was initially extracted with petroleum ether (9 ml) to remove neutral lipids, followed by extraction with the solvent (9 ml) consisting of ethylacetate, isopropanol and 0.1 N HCL, 3:3:1 by volume. The organic phase was dried under N2 gas at 55°C, and with silicic acid column chromatography (0.6 g, 100 mesch) separated into three groups and a mixture of benzene (B), ethylacetate (E) and methanol (M) was used for the developing solution. Prostaglandin (PG) A2 and metabolites of PGE2 were eluted in 8 ml of B:E (60:40), PGE2 and thromboxane B2 in 18 ml of B:E:M (60:40:2) and PGF2α and 6-keto-PGF1α in 6 ml of B:E:M (60:40:20). The dried material of 6-keto-PGF1α fraction was used for radioimmunoassay. The antiserum of 6-keto-PGF1α was kindly provided by Ono Pharmaceutical Company. This antiserum cross-reacted

<table>
<thead>
<tr>
<th>N</th>
<th>Age (yrs)</th>
<th>M : F</th>
<th>Duration (yrs)</th>
<th>FBS (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>23</td>
<td>48.8 ± 2.6 (30 - 76)</td>
<td>17 : 6</td>
<td>11.9 ± 1.2</td>
</tr>
<tr>
<td>Diabetics</td>
<td>45</td>
<td>53.2 ± 1.5 (31 - 72)</td>
<td>35 : 10</td>
<td>11.9 ± 1.2</td>
</tr>
<tr>
<td>Group I</td>
<td>15</td>
<td>49.3 ± 2.7 (31 - 68)</td>
<td>11 : 4</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>62.3 ± 3.6 (43 - 72)</td>
<td>9 : 1</td>
<td>13.4 ± 2.6</td>
</tr>
<tr>
<td>Group III</td>
<td>10</td>
<td>48.2 ± 1.9 (34 - 59)</td>
<td>6 : 4</td>
<td>13.7 ± 2.2</td>
</tr>
<tr>
<td>Group IV</td>
<td>10</td>
<td>56.0 ± 1.3 (50 - 65)</td>
<td>9 : 1</td>
<td>18.8 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Table 2. Plasma 6-keto-PGF₁α concentration before and after induced ischemia

<table>
<thead>
<tr>
<th></th>
<th>Plasma 6-keto-PGF₁α concentration (pg/ml)</th>
<th>A/B ratio</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before ischemia</td>
<td>After ischemia</td>
</tr>
<tr>
<td>Controls</td>
<td>245 ± 14</td>
<td>324 ± 24*</td>
</tr>
<tr>
<td>Diabetics</td>
<td>235 ± 13</td>
<td>240 ± 16+</td>
</tr>
<tr>
<td>Group I</td>
<td>243 ± 21</td>
<td>289 ± 30</td>
</tr>
<tr>
<td>Group II</td>
<td>248 ± 34</td>
<td>235 ± 34+</td>
</tr>
<tr>
<td>Group III</td>
<td>242 ± 24</td>
<td>213 ± 28+</td>
</tr>
<tr>
<td>Group IV</td>
<td>188 ± 17+</td>
<td>181 ± 18+</td>
</tr>
</tbody>
</table>

Values are means ± SE. B; before ischemia, A; after ischemia
*significant difference before and after ischemia (p < 0.01)
+significant difference from controls (p < 0.01).

with PGF₁α (6.0%), 6, 15-diketo-PGF₁α (0.4%), 6, 15-diketo-13, 14-dihydro-PGF₁α (0.3%) and PGF₂α (5.0%). Fifty percent of inhibition of 6-keto-PGF₁α represents approximately 150 pg used in the present experiment. Recovery of the initially added ^3H-6-keto-PGF₁α was about 55%.

Statistical significance was determined by Student’s paired or non-paired t-test.

**RESULTS**

As shown in Table 2, plasma 6-keto-PGF₁α concentration before ischemia was not significantly different in 3 groups of diabetics and the controls. In group IV diabetics, however, it was significantly lower than in the controls.

After forearm ischemia, plasma 6-keto-PGF₁α concentration was significantly increased in healthy controls by 34%, but no significant change was observed in groups II, III and IV diabetics. In group I diabetics, 3 min of ischemia induced an increase in plasma 6-keto-PGF₁α concentration by 21%, albeit not statistically significant.

Fig. 1 shows the relationship between age and ratio of plasma 6-keto-PGF₁α before and after ischemia. The vascular response of prostacyclin production to a transient ischemia was reduced with age in the control subjects (30~39 years old; 1.64 ± 0.18, 40~49; 1.50 ± 0.22, 50~59; 1.28 ± 0.23, 60~; 1.15 ± 0.11). This tendency was also seen in diabetic patients and in those over 40 years of age, the ratio (40~49; 1.02 ± 0.23, 50~59; 0.98 ± 0.06, 60~; 0.92 ± 0.05) was significantly lower than that of age-matched controls.

Plasma concentration of 6-keto-PGF₁α and ratio of 6-keto-PGF₁α before and after ischemia were not related to the duration or treatment of the diabetes or to the plasma level of glucose, cholesterol and triglycerides, respectively.

**DISCUSSION**

The group IV diabetic patients with severe vascular complications showed a reduction of plasma 6-keto-PGF₁α concentration before fore-
arm ischemia, in comparison with the control subjects. In the other three groups of diabetics, the circulating level of 6-keto-PGF$_{1\alpha}$ differed little from the controls. In the literature, the concentration of plasma 6-keto-PGF$_{1\alpha}$ in diabetic patients was found to be low$^{7,8}$, normal$^9$ and high$^{10}$, as compared with age-matched control subjects. The discrepancy of these findings may be due to the difference of the severity of vascular lesions, drugs for treatment and duration of diabetes. Davis et al.$^{17}$ reported that there was great day-to-day variability in plasma 6-keto-PGF$_{1\alpha}$ concentration within subjects, yet such would not account for by intraassay and interassay variations. Therefore, the involvement of prostacyclin in diabetic vascular complications cannot be validly determined only by a single measurement of circulating level of 6-keto-PGF$_{1\alpha}$.

Prostacyclin is generated in animal circulation$^{18}$, in isolated organs and in fragments of the arterial and venous wall from humans$^{19}$ and laboratory animals$^{20}$. Moreover, Neri Serineri et al.$^{19}$ reported that prostacyclin release into the circulation in healthy subjects after 3 min of forearm ischemia and the rise of plasma prostacyclin may be due to increased production in forearm blood vessels$^{20}$. Therefore, our present study was undertaken to investigate the ability of the vessel wall in diabetic patients to produce prostacyclin after forearm ischemia. We found that after 3 min of ischemia, plasma 6-keto-PGF$_{1\alpha}$ concentration was significantly increased in healthy controls, by 34%, but not so in diabetics. It seems that the vascular response of prostacyclin production to a transient ischemia is reduced with age in control subjects and this tendency was also observed in diabetic patients and over 40 years old, the responses were significantly lower than that of age-matched controls.

In group I diabetics, plasma 6-keto-PGF$_{1\alpha}$ concentration was increased by 21% after forearm ischemia, albeit not significant statistically. No significant change was observed in groups II, III and IV diabetics. Thus, the decreased prostacyclin production by the vessel wall in vivo was found in not only diabetic patients with macroangiopathy but also in those with microangiopathy. Prostacyclin generation by the vascular wall is reduced either in experimental atherosclerosis$^{21}$ and human atherosclerotic lesions$^{22,23}$. Our present data on groups II and IV are consistent with these findings.

Webster et al.$^8$ reported that although plasma levels of 6-oxo-PGF$_{1\alpha}$ were significantly decreased in diabetic patients with proliferative retinopathy, forearm ischemia produced an increase in plasma 6-oxo-PGF$_{1\alpha}$ and they stated that in these patients the forearm blood vessels are able to produce prostacyclin, in response to a transient ischemia. Contrary to their findings, our data showed that in group III diabetics, there was no increased concentration of plasma 6-keto-PGF$_{1\alpha}$ after forearm ischemia and plasma levels before ischemia were not different from age-matched controls. None of the group III patients had clinical evidence of atherosclerotic vascular diseases, but it may be in a latent phase and would account for the reduction in prostacyclin production after forearm ischemia. On the other hand, Harrison et al.$^{11}$ reported that in experimental diabetic rats, the production of prostacyclin from the renal cortex, tissues which develop microangiopathy, was decreased, thereby suggesting that impaired vessel wall prostaglandin metabolism may play a role in development of diabetic microangiopathy. Moreover, it has been reported that in diabetic patients with microangiopathy, there is a thickening of capillary basement membranes in muscle$^{24}$ and skin$^{25}$ as well as in renal glomeruli. Therefore an alternative explanation for our results is that in patients with microangiopathy, there is a reduced production of prostacyclin after ischemia due to diabetic vascular changes. An abnormality in prostacyclin metabolism in the vessel wall may relate to diabetic microangiopathy.

REFERENCES

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