Enhancement of Pentose Phosphate Pathway in Vascular Intima from Diabetic Rabbit

TOKUTARO SATO, HISAO SASAKI,* RYOICHIRO WATANABE and KAORU YOSHINAGA

The Second Department of Internal Medicine and *the Second Department of Surgery, Tohoku University School of Medicine, Sendai 980

SATO, T., SASAKI, H., WATANABE, R. and YOSHINAGA, K. Enhancement of Pentose Phosphate Pathway in Vascular Intima from Diabetic Rabbit. Tohoku J. exp. Med., 1988, 155 (1), 97-100 — Activity of pentose phosphate pathway in the intima grown inside vascular prosthesis was studied in alloxan diabetic rabbits. Ratio of $^{14}$CO$_2$ production from 1-$^{14}$C-glucose and 6-$^{14}$C-glucose was 7.8 in the nondiabetic group and 78.9 in the diabetic group. Statistically positive correlation was found between the ratio and the levels of blood glucose or plasma triglyceride. These results suggest that pentose phosphate pathway in the vascular intima is active, and enhanced to a great extent in diabetes.

---

A disruption of the endothelial barrier of the arterial intima increases endothelial permeability, which leads to the proliferation of the smooth muscle cells in the arterial wall (Ross and Glomset 1973). Injury to the endothelium includes hypertension, tissue anoxia, hyperlipidemia and hyperglycemia (Ganda 1980). Mechanism of endothelial injury due to metabolic disturbance is not clear. In this study, activity of pentose phosphate pathway, which is active in the endothelium (Dobrina and Rossi 1983) and one of the main antioxidative mechanisms (Thorburn and Kuchel 1985), was measured in the vascular intima from non-diabetic and diabetic rabbits.

METHODS

Hand-made vascular prosthesis of EPTFE (Gore-tex®), 4×10 mm, were transplanted to left carotid artery of male rabbits weighing 2-3 kg. More than 3 months after the transplantation, diabetes was induced in rabbits by a single intravenous injection of alloxan (100 mg/kg).

Five days after the injection of alloxan, the EPTFE grafts were removed off. Approximately one eighth of the vessel was placed in 5 ml flask containing one ml of Krebs-Ringer bicarbonate buffer with bovine serum albumin (2%) and glucose
(50 mg/100 ml). One hundred microliter of 1-14C-glucose (59 mCi/m mole, 25 μCi/ml) or 6-14C-glucose (58.5 mCi/mmole, 25 μCi/ml) was added to the incubation medium, and incubation was carried out for 2 hr at 37°C under 95% O2 and 5% CO2. Five hundred microliter of 10% trichloroacetic acid was added to drive off the dissolved CO2, which was collected in 0.5 ml of 3N potassium hydroxide in a well above the incubation medium. Radioactivity of 14CO2 was counted by liquid scintillation. Blood glucose was measured by a glucose analyzer (DC-1000, Fuji, Tokyo), and plasma triglyceride by an autoanalyzer. 14C-glucose was purchased from New England Nuclear (Boston, MA, USA).

**RESULTS**

Cumulative results of two different experiments are shown in Table 1. Mean blood glucose levels of the nondiabetic and diabetic groups were 154 and 445 mg/100 ml, and mean plasma triglyceride levels were 255 and 3591 mg/100 ml, respectively. Production of 14CO2 from 1-14C-glucose was 514 dpm/mm² in the

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mg/100 ml)</th>
<th>Plasma triglyceride (mg/100 ml)</th>
<th>Production of 14CO2 (dpm/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-14C-glucose</td>
</tr>
<tr>
<td>Nondiabetic (n = 4)</td>
<td>155 ± 36</td>
<td>225 ± 312</td>
<td>514 ± 292</td>
</tr>
<tr>
<td>Diabetic (n = 7)</td>
<td>445 ± 110</td>
<td>3591 ± 5456</td>
<td>622 ± 530</td>
</tr>
</tbody>
</table>

Values are means ± s.d.

![Fig. 1](image.png)

**Fig. 1.** Correlation between blood glucose level and ratio of 14CO2 produced from 1-14C-glucose and 6-14C-glucose by the vascular intima.
Pentose Phosphate Pathway in Intima

99

...nondiabetic group and 622 dpm/mm² in the diabetic group. That from 6-14C-glucose was 83 and 20 dpm/mm², respectively. Ratio of 14CO₂ produced from 1-14C-glucose and 6-14C-glucose was 7.8±4.1 in the nondiabetic group and 78.9±58.7 in the diabetic group.

As shown in Fig. 1, the blood glucose level and ratio of 14CO₂ produced from 1-14C-glucose and 6-14C-glucose by the intima showed a statistically positive correlation (r=0.660, p <0.05). Statistically positive correlation was also found between the plasma triglyceride levels and the ratio of CO₂ production (r=0.800, p <0.01) (Fig. 2).

DISCUSSION

The pentose phosphate pathway supplies approximately half of the required cytoplasmic NADPH. Increased availability of NADPH may lead to an increased synthesis of fatty acid and cholesterol, or deoxyribose which is needed to sustain the increased rate of cellular proliferation. In the human erythrocytes, flux through the pentose phosphate pathway maintains glutathione in the reduced form as one of the main cellular anti-oxidative mechanisms (Thorburn and Kuchel 1985). During phagocytosis, leucocytes generate large amounts of hydrogen peroxide, for the reduction of which NADPH is used (Tschesche and Macartney 1981). Pentose phosphate pathway is also active in endothelial cells (Dobrina and Rossi 1983).

Changes of activity of pentose phosphate pathway in diabetes are different from tissue to tissue. Activity of glucose-6-phosphate dehydrogenase, the first and the rate-limiting enzyme of the pentose phosphate pathway, is significantly decreased in the liver of diabetic rats (Dessi et al. 1985). However, activity of the
cycle is reported to be enhanced in the lens of diabetic rats (Gonzalez et al. 1986). According to Steer et al. (1985), the activity of the enzymes of the oxidative segment of the pentose phosphate pathway was increased during first 7 days after induction of diabetes.

In the present experiments, pentose phosphate pathway was shown to be active in the intima grown inside the EPTFE grafts, and markedly enhanced in the diabetic group comparing with the nondiabetic group. This increased activity of pentose phosphate pathway in the diabetic intima may be a reaction to overproduction of hydrogen peroxide and/or may lead to sorbitol formation, and may have some relation with diabetic vascular complications through injury to the endothelium.

References