Increased Gene Expression of Glutathione Peroxidase-3 in Diabetic Mouse Heart

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Increased incidence of heart disease is reported in patients with diabetes. To elucidate a molecular profile of expressed genes during the progression of diabetes, a cDNA array was screened in the hearts of mice treated with streptozotocin (200 mg/kg, i.v.). Among the genes investigated, the plasma type glutathione peroxidase, GPX-3, was predominantly up-regulated in diabetic mice compared with control mice. In northern blot analysis, a significant increase in GPX-3 expression was observed as early as 5 d after the induction of hyperglycemia. On day 21, a nearly three-fold induction was demonstrated. Daily administration of insulin (0.2 U/d, s.c.) for 21 d almost completely abolished the increase in GPX-3 mRNA in streptozotocin-treated mice, suggesting that the expression level of the GPX-3 gene was dependent on insulin and serum glucose. Increased GPX-3 may play a significant role in protecting cardiomyocytes from oxidative stress caused by hyperglycemia.

Key words cDNA array; diabetes; glutathione peroxidase-3; heart; mouse

Cardiovascular complications of diabetes are the leading cause of mortality. Coronary artery disease is well known as a frequent complication in diabetic patients, while impaired ventricular performance without coronary lesion has been reported in experimental diabetic animals as well as in diabetic patients.1,2 One of the mechanisms of such functional impairment is the metabolic perturbation of myocytes,3 including the acceleration of the polyol pathway, an alternate route of glucose metabolism.4

On the other hand, the alteration of gene expression under hyperglycemia is another factor involved in the pathogenesis of diabetic cardiomyopathy, which may modulate the cellular metabolism, signal transduction and host defense system. Thus, it is of practical significance to elucidate the differences in gene expression between normal and diabetic subjects. So far, however, the gene expression profile in the diabetic heart has not been well documented. This led us to undertake an investigation of the gene expression profile in experimentally induced diabetic mice using a cDNA array technique. We here report a predominant up-regulation of the plasma type glutathione peroxidase, GPX-3, in the streptozotocin-induced diabetic mouse heart. Since oxidative stress is one of the major causes of diabetic complications,5,6) up-regulation of the GPX-3 gene may be a counter-regulatory response in cardiac tissue to attenuate oxidative insults under hyperglycemia.

MATERIALS AND METHODS

Materials Mouse cDNA macroarray membranes containing 588 known genes were products of Clontech (Palo Alto, CA, U.S.A.). Other reagents were of the highest grade available.

Diabetic Animal Model Male 7-week-old BDF-1 mice fed ad libitum were used in this study. Hyperglycemia was induced by a single administration of streptozotocin (STZ, 200 mg/kg body weight) in the tail vein. The onset of hyperglycemia was verified 3 d after injection of STZ by the mutantorase glucose oxidase method. Insulin (INS, 0.2 U/mouse/d s.c.) was administered daily for 21 d. The animal study was performed according to the ethical standards issued by the Science and International Affairs Bureau of the Japanese Ministry of Education, Science, Sports and Culture.

cDNA Array Analysis Total RNA was extracted from the whole heart using the ISOGEN RNA extraction reagent (Nippon gene Co., Ltd., Tokyo, Japan) according to the manufacturer’s protocol. A pool of mRNA from 5 diabetic or non-diabetic control mice was reverse transcribed, and labeled with [32P]-dCTP according to the manufacturer’s instruction (Clontech). The array membrane was hybridized with [32P]-labeled DNA probes, and the difference in the expression level of each gene between diabetic and control mice was quantified using a BAS2000 Bioimage Analyzer (Fuji Film, Tokyo, Japan). The expression level of each gene was normalized by the levels of glyceraldehydes 3-phosphate dehydrogenase (GAPDH) and β-actin placed on the membrane as internal controls.

Northern Blot Analysis A cDNA probe for the mouse GPX-3 was synthesized by reverse transcription-polymerase chain reaction (RT-PCR) using 5’-ccaaagagcagcagggacgagcag-3’ (sense) and 5’-gagactgtagtcctcggaggtc-3’ (antisense) as primers. Twenty micrograms of total RNA were separated by electrophoresis on a 0.8% agarose/formamide gel, and transferred onto a nylon membrane in 20× sodium chloride/sodium citrate (pH 7.0). The membrane was hybridized with [32P]-labeled cDNA probes, and the level of GPX-3 mRNA was quantified based on the level of GAPDH mRNA.

Statistical Analysis All values were expressed as means ± S.E.M. Group comparisons were analyzed by one-way analysis of variance (ANOVA, Kaleida Graph 3.6 version 3.00, Synergy Software). All groups were analyzed simultaneously with Dunnett’s test. A difference with p<0.05 was considered significant.

RESULTS

cDNA Array Analysis of the Heart Isolated from Diabetic Mice A significant increase in the serum glucose level was observed at 5 d after STZ administration, while a significant decrease in body weight was demonstrated at 21 d
after administration (Table 1). No difference in the heart to body weight ratio was observed during this period. At 21 d after the induction of hyperglycemia, the cDNA array analysis was performed using RNA preparations obtained from diabetic or non-diabetic mouse hearts. As shown in Fig. 1, several candidate genes of which expression levels appeared to decrease under hyperglycemia were: transferrin receptor protein (p90; CD71), KDR/flk1 vascular endothelial growth factor tyrosine kinase receptor (VEGFR2) and retinoic acid receptor β-2 (β2-RAR). Conversely, candidate genes of which expression levels appeared to increase were: vascular endothelial growth factor receptor 1 (VEGFR1), HSP84, interleukin-6 receptor beta chain, glutathione S-transferase Mu1, nucleoside diphosphate kinase B, ubiquitin-conjugating enzyme, cathepsin D, cathepsin L and glutathione peroxidase-3 (GPX-3). Among these genes, we focused on an antioxidant enzyme, GPX-3, since augmented oxidative stress is implicated in the pathogenesis of cardiac dysfunction.7–9)

**Increased Expression of the GPX-3 Gene in the Heart under Hyperglycemia** To confirm the augmented expression of GPX-3 gene in the diabetic mouse heart, northern blot analyses were carried out using total RNA isolated from STZ-induced diabetic mice. As shown in Fig. 2, a significant increase in GPX-3 gene expression was observed as early as 5 d after STZ administration. The increased level of the GPX-3 transcript remained up to 3 weeks after the induction of hyperglycemia. A maximal induction of about 3-fold was observed at this time point.

The diabetogenic effects of STZ include liberation of toxic amounts of nitric oxide, NO, leading to the rapid destruction of pancreatic β-cells.10 To clarify whether the decrease was due to the irrelevant effects of STZ on blood insulin or glucose level, we examined the effect of insulin administration on the GPX-3 gene expression. Insulin treatment significantly restored the serum glucose level and body weight of STZ-treated mice (Table 2). The increase in GPX-3 expression in the heart of diabetic mice was almost completely abolished by the administration of insulin (Fig. 3), while insulin itself did not affect GPX-3 expression in the control
The effect of insulin on body weight, heart weight and serum glucose in STZ-induced diabetic mice (21 d)

<table>
<thead>
<tr>
<th></th>
<th>Body wt. (g)</th>
<th>Heart wt. (mg)</th>
<th>Heart wt. /body wt.×10⁶</th>
<th>Serum glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.7±0.7</td>
<td>137.7±9.8</td>
<td>5.34±0.28</td>
<td>219.2±5.8</td>
</tr>
<tr>
<td>STZ</td>
<td>21.0±0.6*</td>
<td>119.5±11.9</td>
<td>5.66±0.44</td>
<td>710.7±26.9#</td>
</tr>
<tr>
<td>Control+INS</td>
<td>27.4±0.6</td>
<td>127.3±13.0</td>
<td>4.67±0.51</td>
<td>238.2±8.2</td>
</tr>
<tr>
<td>STZ+INS</td>
<td>24.4±0.6*</td>
<td>125.7±8.1</td>
<td>5.14±0.29</td>
<td>443.9±85.3*</td>
</tr>
</tbody>
</table>

The values represent means±S.E.M. (n=6). ∗p<0.05 vs. control, #p<0.05 vs. STZ.

Fig. 3. Effects of Daily Administration of Insulin (INS) on GPX-3 Gene Expression in the STZ-Induced Diabetic Mouse Heart

Mice were sacrificed 21 d after STZ injection. The GPX-3/GAPDH ratio is expressed relative to that of the control. The values represent means±S.E.M. (n=6). ∗p<0.05 vs. control; #p<0.05 vs. STZ.

non-diabetic mice. These results suggested that the expression of the GPX-3 gene was dependent on the deficiency of insulin and increased blood glucose level.

DISCUSSION

The alteration of gene expression by hyperglycemia results in the modulation of the cellular metabolism, signal transduction and host defense system. In this study, we identified the increased expression of the GPX-3 gene in the heart of STZ-induced diabetic mice using a cDNA array. The administration of insulin to STZ-induced diabetic mice completely abolished the GPX-3 induction while insulin alone neither induced hyperglycemia nor affected GPX-3 expression in the heart non-diabetic mice. These results suggest that hyperglycemia plays a key role in the up-regulation of GPX-3 gene expression. An increase in GPX-3 expression was demonstrated in a type 1 diabetic model induced by STZ administration. The result raised the intriguing question whether GPX-3 expression is also up-regulated in type 2 diabetic subjects. As the increase in GPX-3 was one of the major events in the heart under hyperglycemia, GPX-3 gene expression may be induced in a similar fashion in type 2 diabetic models as well. It, however, needs to be confirmed by further studies.

REFERENCES