Antioxidative enzyme and glutathione S-transferase activities in diabetic rats exposed to long-term ASA treatment

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Abstract

Low-dose acetylsalicylic acid (ASA) treatment is a standard therapeutic approach in diabetes mellitus for prevention of long-term vascular complications. The aim of the present work was to investigate the effect of long-term ASA administration in experimental diabetes on activities of some liver enzymes: glutathione peroxidase (GSHpX), catalase, glucose-6-phosphate dehydrogenase (G6PDH) and glutathione S-transferase (GST). Blood glucose, glycated hemoglobin, as well as plasma ALT and AST activities increased in rats with streptozotocin-induced experimental diabetes. The long-term hyperglycemia resulted in decreased activities of GSHpX (by 26%), catalase (by 34%), GST (by 38%) and G6PDH (by 27%) in diabetic animals. We did not observe increased accumulation of membrane lipid peroxidation products or altered levels of reduced glutathione in livers. The linear correlation between blood glucose and glycated hemoglobin in diabetic animals was distorted upon ASA treatment, which was likely due to a chemical competition between nonenzymatic protein glycosylation and protein acetylation. The long-term ASA administration partially reversed the decrease in GSHpX activity, but did not influence the activities of catalase and GST in diabetic rats. Otherwise, some decrease in these parameters was noted in ASA-treated nondiabetic animals. Increased ASA-induced G6PDH activity was recorded in both diabetic and nondiabetic rats. While both glycation due to diabetic hyperglycemia and ASA-mediated acetylation had very similar effects on the activities of all studied enzymes but G6PDH, we conclude that non-enzymatic modification by either glucose or ASA may be a common mechanism of the observed convergence.

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Introduction

Diabetes mellitus ranks among the main factors in the etiology of vascular diseases. The mechanisms by which high glucose leads to injury of vascular cells and causes functional and structural changes are multifunctional and include increased metabolism of glucose by the sorbitol pathway, nonenzymatic glycation of proteins, glycoxidation reactions and altered production of vasoactive substances such as endothelin, prostanoids and nitric oxide (NO) (Calles-Escandon and Cipolla, 2001; Bujunga et al., 2004). There is substantial evidence that hyperglycemia results in generation of reactive oxygen species (ROS) and increases oxidative stress, thus leading to development of late diabetic complications (Wolff et al., 1991; Anwar and Meki, 2003).

Streptozotocin (STZ), an analog of N-acetylglucosamine, has long been used to generate animal models of type 1 diabetes. Release of nitric oxide, increased glycation of pancreatic proteins and an increased production of reactive oxygen species (ROS) have been proposed as possible causes of STZ-induced pancreatic β-cell damage (O'Brien et al., 1996).

Treatment with acetylsalicylic acid (ASA) is an accepted therapeutic approach for preventing vascular damage in diabetic mellitus. Antiplatelet agents (ASA, paracetamol, ibuprofen) have been documented to exert beneficial effects in the evolution of retinal lesions in early diabetic retinopathy and to protect against cataract (De la Cruz et al., 1997). Anti-
inflammatory and hemostatic (antithrombotic) effects of ASA may be due to the inhibition of cyclooxygenase, increased production of lipoxides, protein acetylation, antioxidative action, etc. (Blakyny and Harding, 1992). In both human and experimental diabetes, ASA has been shown to inhibit protein glycation by acetylation of free protein amino groups (Swamy and Abraham, 1989; Winocour et al., 1992; Swamy-Mrutthinj and Carter, 1999; Watala et al., 2005). In STZ-induced diabetic rats, ASA lowered glucose concentration and attenuated oxidative stress (Blakyny and Harding, 1992; Caballero et al., 2000). ASA was effective in preventing both hemoglobin glycation and aminolevulinic dehydranase inactivation induced by in vitro preincubation with 500 mM glucose (Caballero et al., 1998).

Endogenous and exogenous antioxidants, as well as the enzymes of antioxidative defense and phase 2 of detoxication metabolism, are crucial for tissue protection under oxidative and electrophilic stress (Cao and Li, 2004). Glucose-6-phosphate dehydrogenase (G6PDH), the key enzyme of the pentose phosphate pathway, plays a significant role in providing NADPH and maintaining the normal ratio of [GSH]/[GSSG], thus ensuring a normal redox state in cells (Sies, 1999). It was suggested that glutathione metabolism and glutathione S-transferase (GST) distribution in tissues of diabetic rats may be crucial in the etiology, pathology, and prevention of diabetes (Raza et al., 2004a).

The aim of the present study was to investigate the effects of long-term low- and high-dose ASA treatment on the activities of antioxidative enzymes (glutathione peroxidase and catalase), glucose-6-phosphate dehydrogenase and the conjugating enzyme glutathione S-transferase, in streptozotocin-induced diabetes in rats.

**Materials and methods**

**Chemicals**

5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman’s reagent), reduced glutathione (GSH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), NADP, i-erythro-hydroperoxide (EHP) and glucose-6-phosphate disodium salt hydrate were from Sigma-Aldrich (St. Louis, MO, USA). 1-Chloro-2,4-dinitrobenzene (CDNB) and streptozotocin (Streptozocin) were from Fluka Chemie AG (Buchs, Switzerland). Lysine acetylsalicylate (Laspal) was purchased from Le Plessis-Robinson (France). All other reagents were of analytical grade and were purchased from Reachim (Moscow, Russia). All the solutions were made with water purified in the Milli-Q system.

**Animal model**

The investigations were performed using 84 albino male Wistar rats (150–180 g). A standard balanced diet and tap water ad libitum were provided. ASA was given intraperitoneally (i.p.) as a saline solution of Laspal. The animals were adapted to a 12-h light/dark cycle for 1 week and were divided into two groups. The first group (24 animals) received physiological saline (i.p.). The second group (72 animals) was injected with a single dose of streptozotocin (60 mg/kg, i.p.), dissolved in 0.01 M citrate buffer, pH 4.5, immediately before use. Three days later, blood glucose levels were determined in whole blood samples. The rats injected with STZ were considered diabetic if their fasting blood glucose was > 200 mg/dl (Blood Glucose Sensor Electrodes, Medisense, Abbot Laboratories, Bedford, UK). Because this study was performed along with another one, devoted to the effects of long-term hyperglycemia and ASA treatment on blood platelet reactivity and platelet prostaglandin metabolism, we used the same protocol of ASA administration: low and high ASA doses differed in their efficacy at hampering thromboxane generation and platelet reactivity (Watala et al., 2006). Some nondiabetic animals were injected daily with physiological saline solution (i.p.) and kept as ASA-ununtreated control, whereas the remaining nondiabetic rats (from now on referred to as “control+50 mg ASA”) were given daily injections of 50 mg ASA/kg body weight. All animals diagnosed as diabetic were further divided into five subgroups: the first subgroup was injected daily with physiological saline (the group “diabetes”), the second subgroup received daily 5 mg ASA/kg b.w. (“diabetes + 5 mg ASA/day”), the third was given 5 mg ASA/kg every second day (“diabetes + 5 mg ASA/2 days”), the fourth received daily 50 mg ASA/kg b.w. (“diabetes + 50 mg ASA/day”) and the last subgroup was treated with 50 mg ASA/kg b.w. every second day (“diabetes + 50 mg ASA/2 days”).

The experiment was started seven days after the STZ injection and rats were sacrificed after 9 weeks (1 week of acute diabetes and 8 weeks of chronic diabetes) of ASA (or saline) administration. Blood samples were drawn by an abdominal aorta puncture into tubes containing heparin (50 µg/ml). The livers and hearts were excised immediately, dried with paper filter, weighed, snap-frozen in liquid nitrogen and stored at −80 °C until used.

After plasma was removed by centrifugation, the erythrocytes were washed three times with cold phosphate buffered saline (PBS), pH 7.4 and hemolyzed. Livers were homogenized in cold 1.15% KCl solution (1:3, w/v), and liver homogenates were centrifuged at 12,000×g for 30 min to isolate the postmitochondrial hepatocyte fraction for biochemical measurements. To determine GST activity in the isolated cytosolic fraction, the postmitochondrial supernatant was further centrifuged at 105,000×g for 90 min.

**Biochemical measurements**

The postmitochondrial hepatocyte fraction was used for analysis of membrane lipid peroxidation and reduced glutathione (GSH) content. GSH was assayed with Ellman’s reagent, using the molar absorption coefficient ε_{412} = 1.36 · 10⁴ M⁻¹ cm⁻¹ (Ellman, 1959). The accumulated products of membrane lipid peroxidation (thiobarbituric acid-reactive substances, TBARS) were monitored assuming that the molar absorption coefficient ε_{436} = 1.56 · 10⁵ M⁻¹ cm⁻¹ (Stocks and Dormandy, 1971). A stable form of glycated hemoglobin (GHB), containing 1-deoxy-1-(N-valyl)fructose, and the activities of the
serum marker enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicating the degree of hepatoysis, were assayed using reagent sets (Pliva-Lachema a.s., Brno, Czech Republic). Protein content was measured by the method of Lowry et al. (1951).

The activity of cytosolic GST was measured employing the method of Habig et al. (1974). The reactions were carried out in the presence of 10 to 30 μg protein, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) (as GST substrate), 1 mM reduced glutathione and 100 mM sodium-phosphate buffer, pH 7.5, at 30 °C in the final volume of 3 ml. A complete assay mixture without the enzyme was used as a control. The conjugation of CDNB with glutathione was monitored at 340 nm, using the molar absorption coefficient of 9600 M⁻¹ cm⁻¹ and expressed as μmol GSH-CDNB conjugate/min/mg sample protein.

The activity of GSH-Px in the postmitochondrial fraction was determined according to the method of Martinez et al. (1979). The reaction mixture contained 0.1 M Tris–HCl, pH 8.0, 1 mM EDTA, 12 mM sodium azide, 2 mM t-BHP and 4.8 mM GSH (as cosubstrates of GSH-Px). The reaction was started by addition of the sample and was stopped by 0.2 ml 25% TCA after 10 min incubation at 37 °C. The reaction plot was linear at this time interval. The protein concentration in the reaction mixture was 40 μg/ml; the reaction volume was 1 ml. The activity was measured as the amount of GSH oxidized in the GSH peroxidase reaction, using Ellman’s reagent.

The method of Aebi (1984) was used to measure catalase activity. The reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H₂O₂) to 0.65 ml of 50 mM potassium phosphate buffer and 50 μl (15 μg protein) of the sample. The H₂O₂ decomposition was monitored at 240 nm, 37 °C, for 3 min. The catalase activity was expressed as micromoles of H₂O₂ consumed per minute per milligram of sample protein using the molar absorption coefficient of 36 M⁻¹ cm⁻¹.

Glucose-6-phosphate dehydrogenase activity was measured as described by Costa Rosa et al. (1995). The assay mixture contained 100 mM Tris–HCl, 5 mM MgCl₂, 0.5 mM NADP⁺, 1 mM glucose-6-phosphate at pH 7.6. Enzyme activity was assayed by following the rate of NADPH production at 340 nm and 30 °C. The protein concentration was 50 μg/ml.

**Statistical analysis**

Data for 10–12 rats in each group were presented as a mean ± S.D. for the normally distributed parameters or as a median and interquartile range for data showing departures from normality. Data that showed the right-skewed distribution and met the remaining criteria of normal distribution were transformed logarithmically and analyzed with the relevant parametric tests. The analysis of outliers was performed based on the estimates of Cook’s distances and 2.5σ deviation. The nested design of hierarchical one-way ANOVA was used to test for the simultaneous effects of group (control vs. diabetic) and pharmacological treatment (ASA). We used the standard Student’s t-test for the comparison of raw and transformed data showing no departures from normality (according to Shapiro–Wilk’s test), and the non-parametric Mann–Whitney U-test for the remaining variables. One-way ANOVA and the post hoc Tukey test were employed for detailed multiple comparisons (more than two groups). Non-parametric analysis of variance (Kruskal–Wallis test) and the post hoc all-pairwise comparisons Conover–Inman test were used as the inference tests for data that departed from normality. Spearman’s rank correlation (Rₛ) (for testing a null hypothesis of independence between two variables) or Kendall’s rank correlation (τ) (for testing the strength of a dependence between variables) were used to assess simple associations (Zar, 1999; Armitage et al., 2002). Unless stated otherwise, the significance of all analyzed differences was estimated using two-sided inference tests.

**Results**

Effects of diabetes and ASA treatment on biochemical and morphometric parameters

Blood glucose concentration significantly increased in the streptozotocin-induced diabetic rats compared to control animals, and plasma hyperglycemia was not affected by ASA treatment at either dose (Table 1). As expected, diabetic rats showed significantly elevated levels of glycosylated hemoglobin. The intake of ASA slightly decreased the level of hemoglobin glycosylation in diabetic, but not in nondiabetic rats (Fig. 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Blood glucose, body, liver and heart weights in non-treated and ASA-treated non-diabetic and streptozotocin-diabetic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood glucose [mg/dl]</td>
</tr>
<tr>
<td>Control</td>
<td>85 (77, 91)</td>
</tr>
<tr>
<td>Control ASA 50 mg/day</td>
<td>82 (76, 144)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>474 (415, 530)*</td>
</tr>
<tr>
<td>Diabetes ASA 5 mg/day</td>
<td>440 (372, 509)</td>
</tr>
<tr>
<td>Diabetes ASA 5 mg/2 days</td>
<td>397 (344, 436)</td>
</tr>
<tr>
<td>Diabetes ASA 50 mg/day</td>
<td>401 (345, 466)</td>
</tr>
<tr>
<td>Diabetes ASA 50 mg/2 days</td>
<td>346 (324, 377)</td>
</tr>
</tbody>
</table>

Data, presented as median (IQ, UQ), represent values at the termination of experiment (9 weeks of experimental diabetes).

*p<0.001, **p<0.05 vs. control non-treated rats (one-sided inference tests).

***p<0.05 vs. diabetic non-treated rats, each of other diabetic groups.
A linear correlation between blood glucose (x) and non-enzymatically glycosylated hemoglobin (y) was observed, when considering these parameters in all the animal groups (y = 0.0083x + 1.586, \( R^2 = 0.61 \); Fig. 2A), as well as in diabetic animals without ASA administration (y = 0.122x - 0.532, \( R^2 = 0.74 \)). In diabetic rats given ASA ("diabetes + ASA" groups), this linearity was distorted (y = 0.0058x + 2.872, \( R^2 = 0.21 \); Fig. 2B).

At the end of the experiment (after 9 weeks of hyperglycemia), the diabetic animals showed lack of weight gain (Table 1), and these fluctuations were not affected by ASA administration, in either control or diabetic animals. The relative liver and heart weights were considerably higher in diabetic animals compared to control rats (Table 1).

We revealed a significantly decreased TBARS accumulation in liver tissue of the diabetic rats in comparison with that of the control rats (0.080, 0.040–0.080 nmol/mg protein vs. 0.100, 0.088–0.110 nmol/mg protein, \( p < 0.01 \)). The ASA treatment brought the TBARS level in liver tissue of diabetic rats to lower values (0.070, 0.048–0.095 nmol/mg protein in rats treated with 5 mg/day ASA, and 0.070, 0.058–0.090 nmol/mg protein in animals treated with 50 mg/day ASA, NS). TBARS accumulation in livers from control rats was also slightly reduced upon ASA treatment, although the decrease was not statistically significant (0.080, 0.055–0.098 nmol/mg protein). The tissue GSH level did not change under diabetic conditions (25.6–23.0–27.5 nmol/mg protein in control vs. 29.1, 25.4–33.1 nmol/mg protein in diabetic rats, NS), and ASA administration did not affect this parameter in either control (25.6, 21.8–26.1 nmol/mg protein, NS) or diabetic animals (29.5, 28.3–31.7 nmol/mg protein and 28.8, 26.4–30.4 nmol/mg protein in rats given 5 mg or 50 mg ASA a day).

We observed an elevation of the hepatocyte enzyme activity in diabetic rat blood plasma: ALT (1.2-fold, 0.35, 0.21–0.64 kat/l in diabetic vs. 0.30, 0.21–0.36 kat/l in control, NS) and AST (1.3-fold, 0.47, 0.40–0.55 kat/l in diabetic vs. 0.35, 0.29–0.38 kat/l in control, \( p < 0.03 \)). The ASA administration at either dose increased ALT activity (0.56, 0.42–0.66 kat/l and 0.57, 0.37–0.77 kat/l by 5 mg/day ASA and 50 mg/day ASA, respectively, NS) and did not change AST activity in blood plasma of the diabetic animals (0.44, 0.41–0.47 kat/l and 0.50, 0.38–0.63 kat/l, respectively, by 5 mg/day ASA and 50 mg/day ASA, NS). Likewise, the long-term ASA administration to the control animals did not affect the levels of the hepatic enzymes ALT (0.24, 0.18–0.24 kat/l, NS) and AST (0.32, 0.29–0.40 kat/l, NS).

**Effects of diabetes and ASA treatment on antioxidant enzymes and enzymes of phase 2 and pentose-phosphate pathway**

We observed significantly decreased activities of the antioxidant enzymes, glutathione peroxidase (1.3-fold) and catalase (1.5-fold) (Fig. 3A,B), in liver tissue from diabetic rats. The long-term administration of ASA partially reversed the decrease in GSHPx activity, but did not change the catalase activity in the diabetic animals. Moreover, ASA treatment at the 50 mg/kg dose slightly decreased GSHPx and catalase activities in the livers of control animals (Fig. 3A,B).

There was a statistically significant decrease in GST activity in the cytosolic postmitochondrial fraction of diabetic liver tissue in diabetic compared to nondiabetic rats. The ASA treatment of diabetic rats did not reverse the effect of diabetes, but similar ASA treatment of control rats led to decreased GST activity (Fig. 3C).

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**Fig. 1.** Glycated hemoglobin (GHB) in nondiabetic (control) and diabetic rats treated with 5 or 50 mg (gray or black boxes) ASA/kg every day (mg/day) or every second day (mg/2d). For all diabetic rats \( p < 0.05 \) in comparison with control value.

**Fig. 2.** Relationship between fasting blood glucose and glycated hemoglobin (GHB): (A) for all animals, including both nondiabetic (triangles) and diabetic rats (circles); determination coefficient \( R^2 = 0.61 \) means that fasting blood glucose contributes in 61% to the level of glycated hemoglobin; (B) for diabetic animals treated with 5 (open circles) or 50 mg (black circles) ASA/kg every day; determination coefficient \( R^2 = 0.21 \) means that fasting blood glucose contributes in merely 21% to the level of glycated hemoglobin.
G6PDH activity in the livers of the diabetic rats was about 27% lower as compared to that of control animals, but this difference was not statistically significant. Following ASA administration, we observed an increase in liver G6PDH activity in both control and diabetic rats (Fig. 3D).

**Discussion**

The published literature contains a confusing array of data concerning the effects of diabetes on the activities of antioxidant defense enzymes in different tissues (Anwar and Meki, 2003; Perlevski et al., 2003; Uluslu et al., 2003; Yavuz et al., 2003; Latha and Pari, 2004; Raza et al., 2004b). Our present report adds some new evidence on the experimental diabetes-mediated distortions in antioxidative defense systems, pointing out that the oxidative stress ongoing in diabetes may be tissue-specific and depend upon diabetes duration.

In our present study, long-term STZ-induced diabetes markedly affected the activities of GSHPx and catalase, the activity of GST, which catalyzes detoxification of electrophilic/oxidizing drugs, and the activity of G6PDH, which plays an essential role in the regulation of cell redox level. In addition, we showed that the long-term ASA administration to diabetic rats partially reversed these alterations.

Nine weeks after the injection of streptozotocin to rats, we observed typical signs of diabetes mellitus, such as hyperglycemia, high glycated hemoglobin level and growth retardation. Severe long-term hyperglycemia resulted in hepatolysis reflected by the increased ALT and AST activities in blood plasma of the diabetic rats. Earlier, when investigating red blood cells in diabetic humans, we observed hyperpolarized membranes in diabetic erythrocytes, as well as an increased content of erythrocyte endogenous lipid peroxidation products (Augustyniak et al., 1996). In our present study, we did not observe enhanced liver TBARS accumulation in the course of long-term hyperglycemia in STZ-diabetic rats. One of the possible reasons for non-reduced or even lowered TBARS production in the livers from diabetic animals might be due to the reduced lipid content (lipolysis) in the course of long-term STZ diabetes in rats. The duration of experimental diabetes in our present approach was relatively long (9 weeks) compared to the duration of experimental diabetes studied by other
investigators (2 or 4 weeks). Earlier, it was found that hepatocyte mitochondria from diabetic rats showed a 20% reduction in lipid peroxidation, while a 10–20% increase in TBARS production was demonstrated in the microsomal fraction (Raza et al., 2004b). Hence, we argue that the TBARS accumulation, reflecting the extent of oxidative stress and antioxidative defense, may be tissue-specific and certainly depends also upon diabetes duration.

GSH, a co-substrate of GSHPx and GST and a major endogenous antioxidative agent involved in the conjugation of several types of compounds, protects cells from toxic effects and maintains the cellular redox status (Meister, 1988). In our experiments, the GSH level in the livers of diabetic rats did not change. To interpret this finding, we recall the recent report by Raza et al. (2004b), who showed that increased oxidative stress under the conditions of diabetes might differentially affect mitochondrial and cytosolic GSH metabolism. The mitochondrial GSH level in pancreas, kidney and liver was reported to be moderately increased (20–35%) in the diabetic animals, while the GSH level in the cytosolic fractions was raised only in the liver (~10%). The authors suggested that either the mitochondrial GSH biosynthesis increased in these tissues or the cytosolic GSH was transported to mitochondria in the course of diabetes (Raza et al., 2004b).

In experimental diabetes, we noted markedly reduced activities of the GSH-metabolizing enzymes, GSHPx and GST. These observations cannot be easily reconciled with the above-mentioned finding of unchanged GSH, and even more so, because of a considerable variability of the data in the literature concerning the effects of diabetes on these enzymes in different tissues (Anwar and Meki, 2003; Petievski et al., 2003; Ulusu et al., 2003; Yavuz et al., 2003; Latha and Pari, 2004; Raza et al., 2004b). It seems that the observed fluctuations in the enzyme activities may not be a physiological response to altered substrate concentrations, but rather result from not yet defined molecular distortions occurring in diabetes. Also, we have previously shown that GSHPx activity in red blood cells from diabetic patients slightly decreased in comparison to healthy donors (Zavodnik et al., 1998), and our present outcomes are in line with these earlier findings.

Numerous studies report a marked decrease in the activity of antioxidiant enzymes such as GSHPx and catalase, as well as GST activity, in liver, kidney and pancreatic tissues from diabetic rats (Anwar and Meki, 2003; Petievski et al., 2003; Yavuz et al., 2003; Latha and Pari, 2004). On the other hand, using immunoblotting, Raza et al. (2004b) revealed increased levels of cytosolic GST form A4-4 in pancreas, kidney and liver from diabetic rats; however, the cytosolic CDNB-metabolizing activity was marginally decreased in various tissues, except for the liver (where there was a 15–20% increase). Alloxan-induced diabetes in rats resulted in a marked increase in hepatic activities of cytochrome P450, NADPH-cytochrome C reductase, GST, and other phases I and II drug-metabolizing enzymes (Shewerita et al., 2002). McClung et al. (2004), in turn, reported that overexpression of the antioxidiant enzyme glutathione peroxidase in mice resulted in hyperglycemia, hyperinsulinemia and mild insulin resistance, which might contradict the general belief in the beneficial effects of antioxidiant enzymes on insulin function.

We found that the activity of G6PDH, the rate-limiting enzyme of the pentose phosphate pathway oxidative reaction, was depressed in the liver postmitochondrial fraction from diabetic rats. This is in line with the report by Zhang et al. (2000), who showed that increased glucose (10–25 mM) caused inhibition of G6PDH, resulting in decreased NADPH levels in bovine aortic endothelial cells. On the other hand, the earlier work by Gunma et al. (1969) demonstrated that the activities of the pentose phosphate pathway enzymes did not change, in either the oxidative (with the key enzyme G6PDH) or the non-oxidative reactions, in rat adipose tissue under alloxan-induced diabetes.

Enzymatic activities in various hyperglycemic tissues could be changed due to either an altered enzyme gene expression (Bojunga et al., 2004) or a post-translational enzyme modification (glycation), or a combination thereof. It has been suggested that glycation of antioxidiant enzymes due to hyperglycemia may be responsible for the reduction in GSHPx activity (Adachi et al., 1991; Wolff et al., 1991). It was suggested that low-dose ASA might reduce glycoxidative damage in diabetes (Contreras et al., 1997), mainly due to inhibition of post-Amador Maillard reactions (a complex cascade of reactions initiated by glycation leading to production of "advanced glycation end-products") (Hadley et al., 2001). In our experiment, we did not observe any hypoglycemic effect of the long-term ASA treatment (Table 1), but the ASA administration resulted in a slight decrease in the levels of glycated hemoglobin, presumably due to a competition between the two processes, protein glycosylation by a high glucose level and acetylation by ASA (Watala et al., 2006).

In our study, we demonstrated that the effects of long-term hyperglycemia and ASA treatment were largely convergent in the reduction of enzymatic activities. In general, the present study provides evidence that both hyperglycemia in diabetic animals and treatment with ASA in nondiabetic rats considerably reduced the activities of GSHPx, catalase and GST, but not G6PDH. The long-term ASA administration affected the activities of different enzymes in the liver tissue of diabetic and control rats, reversing the decreased GSHPx and G6PDH activities in the diabetic rats, inhibiting GSHPx, catalase and GST, and enhancing G6PDH activity in the control rats. The reason for these changes in enzymatic activities has been presumed to be acetylation by ASA, a process which is apparently quite selective for different proteins (Rendell et al., 1986) and has been shown to effectively compete with glucose for free amino groups (Watala et al., 2006). It should be noted that both glycation due to hyperglycemia in diabetes and acetylation under ASA treatment of control animals had similar effects on the activities of all studied enzymes except G6PDH. A reasonable explanation for such a discrepancy might be found in the earlier study by Jeffery et al. (1985). The authors showed that ASA treatment of G6PDH from baker's yeast modified essentially one lysine residue per subunit of G6PDH, which resulted in enzyme inactivation. Our present study shows that
such an activation pathway may be less efficient in diabetic animals, likely due to chemical competition between glucose and ASA in the terms of chemical kinetics (higher glucose concentration retards acetylation) (Rendell et al., 1986). It has been also shown in another study that gentisic acid (an ASA biotransformation product) increased the percentage of glucose metabolized through the pentose phosphate pathway and hence contributed to G6PDH activation (Sturman and Smith, 1967). Thus, these earlier findings may reconcile the observed diverse alterations in various enzymes upon ASA treatment in nondiabetic and diabetic animals.

Overall, our present findings point out that diabetes per se may alter the activities of drug-metabolizing enzymes, but also that antiplatelet therapy with ASA may become modified in a diabetic state. This fact should be considered during the drug administration to diabetic patients.

Conclusions

Chemical competition between protein glycation by a high glucose and acetylation by ASA may lead to decreased glycated hemoglobin under long-term ASA administration to diabetic rats. The effects of protein glycation due to hyperglycemia in diabetic rats and protein acetylation due to ASA treatment were convergent in the reduced activities of all studied enzymes (catalase, GSHPx and GST) except G6PDH. Such a convergence is likely to result from a common mechanism of non-enzymatic modification of enzyme activities by either glucose or ASA.

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References


streptozotocin induced diabetic rats: modulation by \textit{Momordica charantia} (karela) extract. Life Sciences 74, 1503–1511.


