Glutathione-Related Detoxication Functions in Streptozotocin-Induced Diabetic Rats

Noriko SAITO-YAMANAKA, Harumichi YAMANAKA*, and Shigeyoshi NAGASAWA

National Institute of Animal Health, 3–1–1 Kannondai, Tsukuba, Ibaraki 305, Japan

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ABSTRACT. In order to analyze the detoxication functions in rats with diabetes induced by streptozotocin, the authors administered to the diabetic animals two drugs, ethionine and benzo(*a*)pyrene, which affect mainly the liver and are metabolized through a glutathione conjugation process, and examined the changes in the content of glutathione and activities of related enzymes in the liver. In the liver of the rats with streptozotocin-induced diabetes, the total glutathione content, glutathione *S*-transferase activity and glutathione-insulin transhydrogenase activity were lower than those of normal rat livers, while the glutathione peroxidase activity showed high values. Although specific changes in the glutathione-related detoxication functions were observed in the rats to which ethionine or benzo(*a*)pyrene had been administered, these changes were not revealed under diabetic conditions. It is suggested that in diabetic rats responses to toxic stimuli are suppressed.—KEY WORDS: detoxication, diabetes, glutathione, rat, streptozotocin.

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Glutathione (GSH) plays an important role mainly in the detoxication and metabolism as a cofactor or a substrate of some enzymes. Glutathione *S*-transferases (GST) are involved in the conjugation of various xenobiotics in phase II biotransformation, while glutathione peroxidase (GSH.Px) is one of the radical scavengers preventing oxygen toxicity and glutathione-insulin transhydrogenase (GIT) catalyzes the first reaction in the insulin degradation process [12, 15]. It is generally recognized that the detoxication function is impaired in diabetic animals. Hanasono *et al.* [6, 7] reported that the serum glutamyl-pyruvic transaminase activity and triglyceride levels were higher and glucose-6-phosphatase activity was lower in the rats with alloxan- or streptozotocin (STZ)-induced diabetes than in normal rats after carbon tetrachloride administration, and that the toxicity of various drugs including chloroform, trichloroethane and galactosamine, etc. was enhanced in rats with alloxan-induced diabetes. It was reported that the GST activities decreased in experimental diabetic rats [1, 14]. These findings indicate that the diabetic condition may modify the detoxication metabolism.

This study aimed at analyzing the glutathione and glutathione-related enzymes, GST and GSH.Px, which are associated with the detoxication functions. In addition, the activities of GIT, a putative marker of the levels of insulin and glutathione, as suggested previously [12], were estimated. At first, we analyzed the changes in the glutathione-related detoxication functions in rats with STZ-induced diabetes. Thereafter, we studied the effects of the administration of ethionine (ET) and benzo(*a*)pyrene (B(a)P), which are both of metabolized by the glutathione conjugation process [2], in diabetic rats.

* PRESENT ADDRESS: YAMANAKA, H., National Grassland Research Institute, 768 Senbonmatsu, Nishinasuno-machi, Nasu-gun, Tochigi 329–27, Japan.

MATERIALS AND METHODS

Reagents: Streptozotocin, *dl*-ethionine, 5,5'-dithio-bis-(2-nitrobenzoic acid) and 1-chloro-2,4-dinitrobenzene were purchased from Wako pure chemical industries Ltd. Benzo(*a*)pyrene, reduced glutathione and glutathione reductase were purchased from Sigma Chemical Co. Dimethylsulfoxide was purchased from E. Merk and cumene hydroperoxide was from Nacalai tesque. Inc.

Animals and treatments: Male F344 rats aged 7 weeks (180–200 g B.W.) were divided into two groups of 18 rats each. After overnight fasting, one group received streptozotocin (40 mg/kg B.W. i.v.) dissolved in physiological saline solution, and the other the saline solution only. Every six rats of both groups were kept under observation for 10 days and killed by bleeding under ether anesthesia to obtain tissue samples. The other rats of both groups were observed for a period of one week, and then they were subjected to the intra-peritoneal administration of two drugs, ethionine (8.7 mg/kg B.W.) and benzo(*a*)pyrene (20 mg/kg B.W.). The drugs were dissolved in dimethylsulfoxide. On the third day after the administration of the drugs, tissue samples were collected.

Preparation of tissue samples: Animals which fasted over night were bled under ether anesthesia. Serum and liver were collected for analysis. Liver was perfused *in situ* to avoid blood contamination. Serum was used for the estimation of the blood glucose and insulin contents, and liver for the determination of the glutathione content and for enzyme assays.

The liver was homogenized with a 0.25 M sucrose solution. Homogenate was centrifuged at 9,000 ×g for 15 min to obtain the postmitochondrial fraction (PF). PF was centrifuged at 105,000 ×g for 60 min to obtain the cytosol. GIT activity was estimated with the PF. Total and oxidized glutathione contents while the activities of GST and GSH.Px were measured with cytosol.

Analysis: Blood glucose and insulin contents were determined using kits, Glucose C-test Wako (Wako Pure

Chemical Industries Ltd.) by enzymatic method and Insulin EIA kit "MITSUI" II (Kainosu Co., Ltd.) by the enzyme immunoassay, respectively.

Protein content of the tissue samples was measured by the method of Lowry *et al.* using bovine serum albumin as the standard [9].

The amounts of total and oxidized glutathione were assayed by the method of Grifith [5]. GST activities were determined by the method of Warholm *et al.* [16] with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. GSH.Px activity was measured by the method of Jensen and Clausen [8] with hydrogen peroxide and cumene hydroperoxide as substrates. The activity of hydrogen peroxide as a substrate expresses the selenium-dependent activity. The activity of cumene hydroperoxide expresses both the selenium-dependent and independent activities. The selenium-independent activity is one of the isozymes of GST. GIT activity was estimated by the HPLC method previously reported [12].

Statistics: Data were analyzed with Tukey's multiple-range test.

RESULTS

At first, the changes in the glutathione-related detoxication functions in the rats with STZ-induced diabetes were analyzed. Blood glucose level was significantly higher in the STZ-treated group than in the control group which received a physiological saline solution only (Table 1). In contrast with blood glucose, the serum insulin level was lower in the STZ-treated group (24.7±11.1 μIU/d*l*, Table 1). These results indicated that the STZ treatment induced diabetes mellitus in these rats which displayed typical symptoms consisting of polydipsia, polyuria and glycosuria.

In these diabetic rats, the content of total and oxidized glutathione in the cytosol, cytosolic activities of GST and GSH. Px, and GIT activity in PF were measured. In the diabetic rats, the total glutathione content remarkably decreased, while the content of oxidized glutathione (GSSG) did not change appreciably (Fig. 1, Cont). GST activity (Fig. 2, Cont) and GIT activity (Fig. 3, Cont) were significantly lower than those in the untreated group, while the GSH.Px activities with hydrogen peroxide and cumene hydroperoxide as substrates were higher (Fig. 4, Cont).

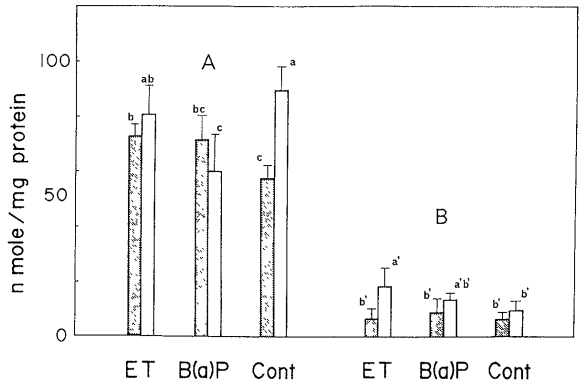

Fig. 1. Total and oxidized glutathione content in rat liver. A: total glutathione content. B: oxidized glutathione content. Dotted columns indicate the diabetic groups. Open columns indicate the nondiabetic groups. ET: ethionine-treated groups. B(a)P:benzo(*a*)pyrene-treated groups. Cont: groups which did not receive ET or B(a)P. The same superscripts indicated the absence of significant difference among the groups.

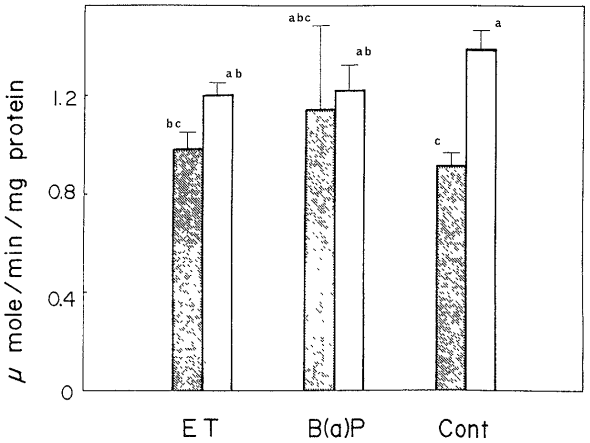

Fig. 2. Glutathione *S*-transferase activity in rat liver. Activity was estimated with 1-chloro-2,4-dinitrobenzene as a substrate. Dotted columns indicate the diabetic groups. Open columns indicate the nondiabetic groups. ET: ethionine-treated groups. B(a)P: benzo(*a*)pyrene-treated groups. Cont: groups which did not receive ET or B(a)P. The same superscripts indicate the absence of significant difference among the groups.

Table 1. Levels of blood glucose and insulin

Treatment	Blood glucose (mg/d*l*)	Insulin (μ IU/m*l*)
Ethionine, Diabetic	264±102$^{c)}$	25.5± 5.2$^{b'c')}$
Ethionine, Nondiabetic	149± 11$^{c)}$	137.3±97.3$^{a')}$
Benzo(*a*)pyrene, Diabetic	382±119$^{b)}$	15.9± 8.9$^{c')}$
Benzo(*a*)pyrene, Nondiabetic	150± 9$^{c)}$	34.5±30.3$^{b'c')}$
Diabetic	508± 46$^{a)}$	24.7±11.1$^{b'c')}$
Control	177± 12$^{c)}$	97.1±41.9$^{a'b')}$

a), b), c), a'), b'), c'') The same superscripts indicate that there is no significant difference among the groups. Data are expressed as mean±SD.

Subsequently, we investigated the changes in the glutathione-related detoxication functions in nondiabetic rats subjected to administration of the two drugs, ET and B(a)P. In the drug-treated nondiabetic rats, the blood glucose levels did not show significant changes compared with the control (Table 1). Although the serum insulin level in the ET-treated rats was higher, and level in B(a)P-treated rats was lower than in the control rats, the changes were not significant (Table 1). The total glutathione content decreased in the B(a)P-treated rats and the oxidized glutathione content increased in the ET-

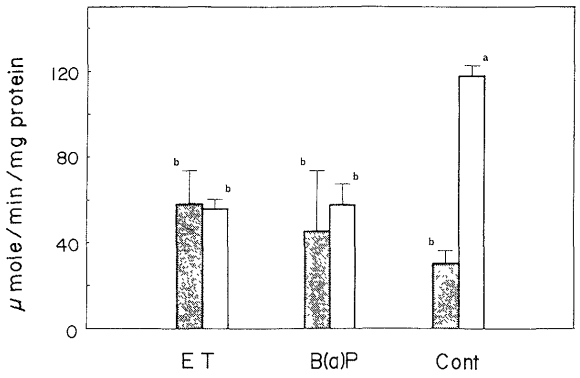

Fig. 3. Glutathione-insulin transhydrogenase activity in rat liver. Dotted columns indicate the diabetics groups. Open columns indicate the nondiabetic groups. ET: ethionine-treated groups. B(a)P:benzo(*a*)pyrene-treated groups. Cont: groups which did not receive ET or B(a)P. The same superscripts indicate the absence of significant difference among the groups.

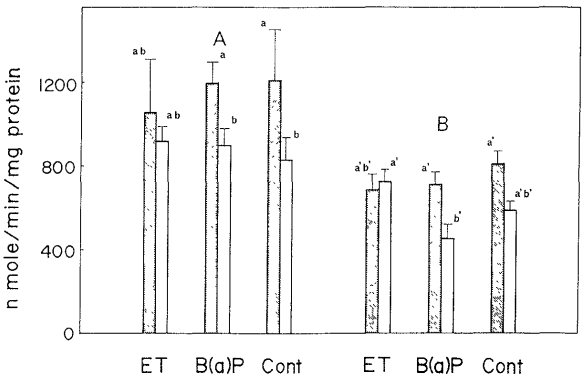

Fig. 4. Glutathione peroxidase activity in rat liver. A: activity with cumene hydroperoxide as a substrate. B: activity with hydrogen peroxide as a substrate. Dotted columns indicate the diabetic groups. Open columns indicate the nondiabetic groups. ET: ethionine-treated groups. B(a)P: benzo(*a*)pyrene-treated groups. Cont: groups which did not receive ET or B(a)P. The same superscripts indicate the absence of significant difference among the groups.

treated rats (Fig. 1). The GST activity decreased but the changes were not significant (Fig. 2). The GIT activity decreased remarkably (Fig. 3). Although the GSH.Px activity with hydrogen peroxide as a substrate did not exhibit any significant changes, that with cumene hydroperoxide as a substrate increased in the ET-treated rats and decreased in the B(a)P-treated rats (Fig. 4).

Finally, we studied the additional effects of toxic stimulation through the administration of ET or B(a)P on the glutathione-related detoxication functions in the rats with STZ-induced diabetes.

The levels of blood glucose and serum insulin are shown in Table 1. Although similar changes were observed in the diabetic rats and in the drug-treated diabetic rats, the blood glucose level in the drug-treated diabetic group was lower than that in untreated diabetic group. These results suggested that the two drugs did not affect the severity of diabetes.

Figure 1 shows the contents of total and oxidative glutathione. Although the B(a)P-treated diabetic group showed a slight decrease in the total glutathione content, the decrease was not as pronounced as that recorded in the B(a)P-treated nondiabetic group. The GSSG content in the ET-treated diabetic group did not change, whereas a significant increase was observed in the ET-treated nondiabetic group.

The GST activity did not show significant alterations in both groups of diabetic and nondiabetic rats in the case of B(a)P and ET administration, while a significant decrease was observed in the diabetic rats without drug administration (Fig. 2).

Figure 3 shows the GIT activity. In the diabetic rats which received the drugs, the GIT activities were remarkably decreased, compared with the normal control rats. However, no additional changes were observed in the diabetic groups which received the drugs compared to nondiabetic groups which received the drugs.

Figure 4 shows the GSH.Px activities. The activity determined with cumene hydroperoxide as a substrate increased in the B(a)P-treated diabetic group, although no change was observed in the B(a)P-treated diabetic group. The activity determined with hydrogen peroxide as a substrate tended to increased in the B(a)P-treated diabetic group, while it decreased in the B(a)P-treated nondiabetic group. These changes were similar to those in diabetic rats. The ET-treated diabetic group did not show appreciable changes nor did the nondiabetic group.

DISCUSSION

STZ-induced diabetes mellitus affected the levels of glutathione and activities of related enzymes. The total glutathione content and the GST activity decreased, whereas the GSH.Px activity increased. Various abnormalities of the metabolism, e.g. hyperglycemia, disorders of lipid metabolism or inhibition of protein synthesis have been reported under STZ-induced diabetic conditions [10, 11]. Liver, the major organ of metabolism, is directly affected by these dysfunctions, especially in terms of inhibition of protein synthesis. The decrease in the total glutathione content and GST activity in the liver may be attributed to such liver disorders. On the other hand, it is known that oxidative stress increases under diabetic conditions. Sato *et al.* [13] reported that the thiobarbiturate value increased in diabetic patients. The increase in the activity of GSH.Px, which is conspicuous in selenium-dependent enzymes (compare Fig. 4A and 4B), could be one of the protective responses against oxidative stress.

The effect of the administration of ET and B(a)P in addition to the STZ-treatment on the responses of the diabetic rats is difficult to interpret. It was anticipated that the response in the drug-treated diabetic rats would be enhanced compared with the response of the drug-treated

nondiabetic rats. The three diabetic groups showed similar changes in the levels of glutathione and related enzymes. It appeared that the effects of drug administration were not obvious when the STZ treatment was carried out, although the two drugs used in this study exhibited different effects. For example, although the administration of ET resulted increase of the GSSG content in nondiabetic rats, such an increase was not observed in the ET-treated diabetic rats. The total glutathione content decreased in the B(a)P-treated nondiabetic group, while in the B(a)P-treated diabetic group, these changes were alternated.

The results described above suggest that standard responses against drugs could not be elicited in the animals with STZ-induced diabetes and that the biotransformation process through GST and protective action against oxygen toxicity through GSH.Px, glutathione related detoxication functions, were impaired.

The levels of cytochrome P-450, a series of enzymes in the phase I biotransformation, are reduced or the isozyme composition are altered in diabetic animals [3, 4]. These changes could partially account for the different responses to drugs between the diabetic animals and the nondiabetic ones. Similarly, a change in the composition of GST, corresponding to the phase II enzymes of biotransformation may occur and be related to these responses. However, these assumptions remains to be verified.

We measured GIT activity as a marker of both the glutathione-related detoxication functions and insulin metabolic state. There was no significant difference between the diabetic and nondiabetic groups in the presence of toxic stimulus, although a decrease of the activities was conspicuous after treatment with each drug. These results suggested that the factor which controls the GIT activity is not confined to the insulin level, although the GIT activity decreased in the diabetic rats.

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