Glutathione S-Transferase Activity in the Liver of Mice with Different Sensitivity to Hepatocarcinogenic Effects of *o*-Aminoazotoluene

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Glutathione S-transferase activity was detected in the liver of inbred mice sensitive (CBA) and resistant (CC57BR and C57Bl) to hepatocarcinogenic effects of *o*-aminoazotoluene. High liver glutathione S-transferase activity was found in CC57BR and C57Bl and low in CBA mice treated with this carcinogen. Thus, interstrain differences in glutathione S-transferase activity probably determine the resistance to *o*-aminoazotoluene-induced hepatocarcinogenesis.

Key Words: *o-aminoazotoluene; inbred mice; sensitivity to hepatocarcinogenesis; glutathione S-transferase*

o-Aminoazotoluene (OAT) is an azobenzene compound producing carcinogenic effects in animals and humans and characterized by high species and organ specificity [2]. OAT gains carcinogenic properties after metabolic activation with enzymes of the microsomal monooxygenase system (1a subfamily of cytochromes P-450, cyp1a) [4]. N-Hydroxylation leads to the formation of highly reactive metabolites attacking nucleophilic sites of macromolecules, including DNA [8]. Detoxification of carcinogenic metabolites in the body involves phase II enzymes, including glutathione S-transferase (GST) [7], catalyzing their conjugation with glutathione SH-groups and neutralizing electrophilic sites of the substrate. The formed conjugates are hydrophilic compounds easily eliminated from the body [6]. Thus, GST plays a role in the inactivation of carcinogenic metabolites, including N-hydroxy-OAT.

Various mouse strains display different resistance to hepatocarcinogenic effects of OAT [2]. The sensitivity or resistance to OAT-induced carcinogenesis is determined by OAT metabolism and depends on the prevalence of activating or detoxifying processes. However, there is no correlation between hepatocarcinogenic effects of OAT and inducibility of cyp1a at the initial stages of chemical carcinogenesis [1]. It can be expected that phase II enzymes contribute to the resistance to OAT-induced hepatocarcinogenesis.

Here we studied *in vitro* GST activity in mice of 3 cyp1a-inducible strains (Ah+ genotype) resistant (CC57BR and C57Bl) and sensitive (CBA) [2] to carcinogenic effects of OAT [1].

MATERIALS AND METHODS

Experiments were performed on CBA, CC57BR, and C57Bl mice obtained from the nursery of the Institute of Cytology and Genetics.

Induction was performed by intraperitoneal injection of 200 mg/kg OAT. The animals were kept under standard conditions with *ad libitum* water supply and were deprived of food 24 h before decapitation. Liver supernatants were obtained by differential centrifugation 3 days after the administration of OAT [10], which corresponded to maximum GST induction (data not shown). Protein concentration in the supernatant was measured by the method of Lowry [9]. The activity of GST was measured spectrophotometrically [6]

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using 1-chloro-2,4-dinitrobenzene as the substrate. The calibration curve was constructed using various concentrations of 2,4-dinitrophenyl-S-glutathione.

The results were analyzed using Statgraphics software.

RESULTS

C57Bl and CBA mice had various basal GST activities ($p<0.05$). OAT-induced GST activity differed between CC57BR and CBA and between C57Bl and CBA mice ($p<0.01$, Table 1).

The basal activity of liver GST was maximum in C57Bl mice, lower in CC57BR mice, and minimum in CBA mice ($p<0.05$). OAT increased GST activity, in particular, in CC57BR mice ($p<0.01$). The activity of liver GST in C57Bl and CC57BR mice treated with OAT was high, while in CBA mice sensitive to OAT-induced hepatocarcinogenesis this parameter increased only slightly and was lower than in resistant animals ($p<0.01$). Taking into account that studied mice have the genotype of inducibility, our findings suggest that considerable amounts of N-hydroxy-OAT are produced in the liver, and further detoxification of this reactive metabolite depends on the activity of cytosolic aminotransferases. The high content of N-hydroxy-OAT in the liver of CBA mice can be distributed to low activity of GST involved in detoxification, which contributed to their high sensitivity to hepatocarcinogenic effects of OAT. Expression of some GST isoforms is a marker of neoplasia [3]. Low GST activity correlates with high sensitivity to toxic effects of xenobiotics. Interstrain differences in GST activity can be associated with increased risk for colon cancer in mice treated with 1,2-dimethylhydrazine [5]. Thus, various sensitivity of studied mice to hepatocarcinogenic effects of OAT is probably related to interstrain differences in GST activity.

TABLE 1. Activity of GST in the Liver of Control and OAT-Treated Mice ($M\pm m$, $n=8$)

Strain	GST activity, nmol/min/mg protein	
	Control	OAT
C57Bl	1550±177	1779±146
CC57BR	1030±94	1950±232*
CBA	817±108	1117±69**

Note. *$p<0.01$ and **$p<0.05$ compared to the control.

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REFERENCES

1. L. F. Gulyaeva, L. Yu. Zakharova, V. V. Lyakhovich, *et al.*, *Byull. Eksp. Biol. Med.*, **126**, No. 7, 201-203 (1998).
2. V. I. Kaledin, I. A. Serova, Yu. G. Tsellarius, and L. A. Semenova, *Eksper. Onkol.*, **12**, 28-30 (1990).
3. G. J. Beckett and J. D. Hayes, *Adv. Clin. Chem.*, **30**, 318-323 (1993).
4. Y.-L. Cheung, S. M. Puddicombe, T. J. B. Gray, and C. Ioannides, *Carcinogenesis*, **15**, 1257-1263 (1994).
5. D. A. Delker, T. K. Bammler, and D. W. Rosenberg, *Drug Metab. Dispos.*, **24**, 408-413 (1996).
6. W. H. Habig, M. J. Pabst, and W. B. Jacoby, *J. Biol. Chem.*, **249**, 7130-7139 (1974).
7. J. A. Hinson and F. F. Kadlubar, *Glutathione Conjugation: Mechanisms and Biological Significance*, London (1988), pp. 235-280.
8. E. L. Loechler, *Carcinogenesis*, **17**, 895-902 (1996).
9. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).
10. T. Omura and R. Sato, *Ibid.*, **239**, 2379-2385 (1964).