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SUB-CHRONIC EFFECTS OF STYRENE AND STYRENE OXIDE ON LIPID PEROXIDATION AND THE METABOLISM OF GLUTATHIONE IN RAT LIVER AND BRAIN

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Abstract.....Sub-chronic effects of styrene and styrene oxide on lipid peroxidation, glutathione contents and glutathione reductase activities in the liver and brain were examined after intraperitoneal administration to rats 3 times a week for 7 weeks.

Styrene (300, 400 and 500 mg/kg) and styrene oxide (200 and 300 mg/kg) increased lipid peroxidation in the liver after 7 weeks of treatment. Hepatic lipid peroxidation in rats treated with a higher dose of styrene oxide (400 mg/kg) was significantly enhanced even after 2 weeks of treatment. On the other hand, no change in lipid peroxidation was observed in the brain under the above conditions.

Neither glutathione contents nor glutathione reductase activities in the liver and brain were altered at 40 h after the last of these sub-chronic treatments. To elucidate the cause of lipid peroxidation, the time courses of glutathione content after treatment with either styrene or styrene oxide (300 mg/kg) were studied in more detail. Significant decreases in both the GSH and GSSG contents were detected shortly after these treatments and the levels recovered to the control values at 40 h in these organs, although the changes were less significant in the brain of rats treated with styrene.

These results suggest that enhancement of lipid peroxidation in the liver after treatment with styrene or styrene oxide was a consequence of repeated

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depletions of glutathione to certain critical levels and delayed recovery of lipid peroxides.

Key words : Styrene, styrene oxide, lipid peroxidation, glutathione, glutathione reductase.

INTRODUCTION

Styrene is widely used in the plastics industry, and styrene oxide is also utilized largely as an intermediate in the production of styrene glycol and its derivatives. Styrene, as an environmental pollutant, poses a severe threat to humans (Götell et al., 1972 ; Stewart et al., 1968). Both hepatic and neuronal disorders caused by styrene have been reported. For example, styrene has a depressive effect on the central nervous system and also causes peripheral neuropathy (Spencer and Irish, 1942). The effects of styrene observed in animal experiments seem to depend on its biotransformation into reactive metabolites. Metabolic conversion of styrene has been extensively studied. The major metabolic pathway of styrene is its conversion to styrene oxide, which is considered to be one of the most reactive metabolites of styrene by many investigators (Vsinio et al., 1984 ; Bardodej, 1978). At least two major pathways are involved in the further metabolism of styrene oxide (Leibman, 1975). One is catalyzed by epoxide hydrolase, and the other is its conjugation with glutathione (GSH) by glutathione-S-transferase. Styrene oxide has also been shown to cause hepatotoxicity and neurotoxicity (Charkrabarti and Brodeur, 1981 ; Misumi et al., 1986), although the mechanism of toxicity of these chemicals is currently unknown.

Lipid peroxidation is a common toxic mechanism for a number of agents (Babson et al., 1981). While several studies have demonstrated that the toxic effects of these agents, such as lipid peroxidation, could be protected by glutathione (Reed, 1986). In an earlier study of acute exposure, styrene was reported to cause a decrease in glutathione content and an increase in lipid peroxidation in the liver of treated rats (Srivastava et al., 1983). However, the sub-chronic effects of both styrene and its metabolites, *i.e.*, styrene oxide, have not been investigated. In this study, to elucidate the mechanism of both neuronal and hepatic disorders caused by styrene and styrene oxide, we focused on the sub-chronic effects of these agents on lipid peroxidation, glutathione content and the metabolism of this compound in the liver and brain of rats.

MATERIALS AND METHODS

The chemicals used in this study were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo).

Male Wistar rats (Kyudo Animal Center, Kumamoto, Japan) weighing 180–210 g

were divided into 8 groups and treated as described below. The LD₅₀ of styrene oxide was considered to be 460 mg/kg body weight (Bardodej, 1978). For the present study, we used doses of 200, 300 and 400 mg/kg body weight of styrene oxide and 300, 400 and 500 mg/kg body weight of styrene for each injection. Styrene or styrene oxide dissolved in olive oil (1 g/ml) was given to individual rats by i.p. injection 3 times a week for 7 weeks. The control animals received an equivalent volume of the same vehicle. Unless otherwise stated, animals were killed by decapitation 40 h after the last injection. The liver and brain were immediately removed and weighed.

One gram of each individual organ was homogenized in a 9 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.4, containing 1.15% KCl. An aliquot of this homogenate was used directly for the determination of malondialdehyde (MDA) content (Ohkawa et al., 1979), and the rest was centrifuged at 27,000 g for 20 min at 4°C. The resulting supernate was recentrifuged at 100,000 g for 60 min. Glutathione reductase activity in the 100,000 g supernate was determined by the method of Carlberg and Mannervik (1975).

Another aliquot (1 g) of tissue was homogenized separately in 15 ml of 50 mM sodium phosphate buffer, pH 8.0, containing 1 mM EDTA, mixed with 25% metaphosphoric acid (1/4 volumes of homogenate (v/v)) and centrifuged at 100,000 g for 30 min. The reduced and oxidized glutathione contents of the supernate obtained were determined by the fluorometric method of Hissin and Hilf (1976). Protein was determined by the procedure of Lowry et al. (1951).

For the time-course study, the animals were treated with either styrene or styrene oxide (300 mg/kg body wt.) for 1 week according to the same protocol as the sub-chronic experiments, and were sacrificed at a given time after the last i.p. injection. The contents of GSH and GSSG in individual livers and brains were determined as described above.

The results were analyzed by one-way analysis of variance. If the F-value was significant, the difference was clarified using Scheffé's method for comparison of means between groups. Probability values of 5% or less were considered significant.

RESULTS

Half of the styrene oxide 400 mg/kg-treated rats died within 2 weeks and so the remaining half of rats in this group were sacrificed after 2 weeks of treatment. On the other hand, all of the styrene 400 mg/kg-treated rats survived for 7 weeks. These results suggest that styrene oxide was more toxic for the rats than styrene.

In the early period of sub-chronic administration of styrene or styrene oxide, narcotic effects of these compounds were observed for several hours after i.p. injection. Narcotic effects in the treated groups, however, became less prominent 2 weeks after the start of the experiment, probably due to an increased capacity to

detoxify these compounds in the liver.

There was no significant difference in the relative weights of the liver and brain per 100 g body weight between the control and treated animals after 7 weeks of treatment (data not shown). The effects of styrene or styrene oxide administration on the levels of lipid peroxidation, the contents of both the reduced and oxidized forms of glutathione and glutathione reductase activity in the liver are shown in Table 1. In the styrene-treated rat livers, the levels of lipid peroxides increased roughly in proportion to the dose injected. Comparable increases in lipid peroxides were also detected in the rats treated with lower doses of styrene oxide (200–300 mg/kg). As described above, only half of the animals treated with styrene oxide 400 mg/kg survived after 2 weeks. The results obtained from these surviving rats are tentatively listed in Table 1. Marked enhancement of lipid peroxidation was observed in the livers of these rats, *i.e.*, 225% of that in control animals.

In contrast to the liver, no significant increase of lipid peroxide in the brains of styrene- or styrene oxide-treated rats was evident at any dose level of these chemicals.

Both glutathione contents and glutathione reductase activities in the liver (Table 1) and brain were determined usually 40 h after the last treatment. No significant changes were found in these results, although number of studies have shown a relationship between depletion of GSH and enhancement of lipid peroxidation (Younes and Siegers, 1980, 1981). To elucidate the cause of lipid peroxidation, changes in glutathione contents after treatment with either styrene or styrene oxide were studied in more detail. The time courses of depletion of GSH and GSSG in the liver at a dose of 300 mg/kg of styrene and styrene oxide are shown in Fig. 1. This dose is determined by the results of Table 1. It is likely that there is the critical dose of both styrene and styrene oxide in rats between 300 mg/kg and 400 mg/kg. Styrene caused a 55% depletion of GSH 2 h after the last treatment, whereas styrene oxide caused a 77% depletion of GSH after 2 h (Fig. 1A). A rise in the glutathione content of the liver occurred 6 h after the last treatment, increasing above the normal level and returning to normal at 40 h. The time course of GSSG was similar to that of GSH (Fig. 1B). The extent of depletion of GSH and GSSG was always greater in the styrene oxide-treated rats than in the styrene-treated animals. The time courses of the contents of GSH and GSSG in the brain of rats treated with 300 mg/kg of styrene and styrene oxide are shown in Fig. 2. In contrast to the liver, styrene did not cause any significant change in GSH and GSSG. However, styrene oxide caused an approximately 50% depletion of both GSH and GSSG at 2 h after the last treatment. These results indicate that at least transient depletion of glutathione content occurred repeatedly, that is, 3 times a weeks for 7 weeks under the conditions of the sub-chronic experiment.

Table 1. Effects of styrene and styrene oxide on the levels of lipid peroxide, glutathione contents and glutathione reductase in liver.

Treatment	Dose (mg/kg)	Lipid peroxide (nmol MDA/100mg protein)	Reduced glutathione (mg/Liver)	Oxidized glutathione (mg/Liver)	Glutathione reductase (nmol/min/mg protein)
Experiment 1					
Control	—	117.3 ± 9.9	19.11 ± 1.80	4.72 ± 0.57	76.4 ± 6.8
Styrene	300	129.2 ± 22.0	21.48 ± 1.50	4.82 ± 0.40	82.8 ± 7.2
Styrene	400	142.3 ± 15.2*	20.36 ± 2.10	4.64 ± 0.44	83.4 ± 6.5
Styrene	500	165.5 ± 17.5*	19.15 ± 2.27	4.54 ± 0.56	87.0 ± 5.3
Experiment 2					
Styrene oxide	200	131.5 ± 10.3	18.06 ± 2.31	4.15 ± 0.46	86.3 ± 11.7
Styrene oxide	300	127.3 ± 13.0	16.75 ± 2.31	3.72 ± 0.50	86.8 ± 8.9
Experiment 2					
Control	—	130.6 ± 8.0	18.03 ± 2.21	4.60 ± 0.45	77.3 ± 7.6
Styrene oxide	400	293.8 ± 10.2*	16.60 ± 2.36	3.65 ± 0.57	85.1 ± 8.5

Exp. 1. Samples from 6 animals in each group were assayed after 7 weeks with or without treatments.

Exp. 2. Samples from 3 animals were assayed at 2 weeks in both groups.

All values are mean ± S.D.

* Statistically significant ($P < 0.01$)

Fig. 1. Time course of hepatic GSH and GSSG levels in rats after administration of either styrene or styrene oxide three times a week. Rats were sacrificed at a given time after the third injection, as indicated on the abscissa. Three styrene- or styrene oxide-treated rats and three control rats were sacrificed at each time-point. All values are mean $\pm$ S. D. *Statistically significant ($P < 0.05$)

DISCUSSION

The present results show that sub-chronic administration of styrene and styrene oxide to rats enhances the rate of lipid peroxidation in the liver homogenate. In an acute experiment, Srivastava et al. (1983) found that styrene treatment (50, 100, 200 and 600 mg/kg) caused a dose-dependent decrease in glutathione and that the rate of hepatic lipid peroxidation was significantly increased in rats treated with 100, 200 and 600 mg/kg of styrene. In our sub-chronic experiment, we confirmed that hepatic glutathione contents were depleted by more than 50% shortly after

Styrene oxide on lipid peroxidation

Fig. 2. Time course of brain GSH and GSSG levels in rats treated as for Fig. 1.

treatment. However the levels recovered to the control values at 20 h (Fig. 1). This rather rapid recovery of glutathione is not due to the regeneration of GSH from GSSG by glutathione reductase, but *de novo* synthesis (Sies, 1983). These results suggest that in the case of sub-chronic treatment, the enhancement of lipid peroxidation was also related to the transient but severe depletion of glutathione content. This explanation is consistent with the observation of Younes and Siegers (1980) and the assumption that depletion of glutathione *per se* could cause enhanced lipid peroxidation possibly due to lack of a cellular defence system against the endogenous toxic intermediates following treatment with many -SH-depleting agents (Younes and Siegers, 1981).

The absence of any effect of styrene and styrene oxide on lipid peroxidation in the brain at least under the present conditions may be due to the fact that levels of

glutathione were not depleted severely to the critical level which may be necessary to enhance lipid peroxidation rates (Casini et al., 1985 ; Younes and Siegers, 1981). The toxic compounds from the portal vein are initially taken up into the liver and conjugated with GSH, whereas the brain may not receive the toxic compounds in amounts sufficient to deplete glutathione. It is likely that at least part of the depletion of GSH in the brain is due to insufficient supply of GSH from the liver.

Our results show that sub-chronic treatment with relatively high doses of styrene or styrene oxide enhances the rate of lipid peroxidation in the liver homogenate. The enhancement of lipid peroxidation appears to be a consequence of repeated depletions of glutathione to certain critical levels and delayed recovery of lipid peroxides. Lipid peroxidation can cause deleterious effects on cellular function both directly, by impairment of the membrane structure, or indirectly, by causing the evolution of toxic products. When we consider the chronic toxicity of styrene or styrene oxide, it is important to note the lipid peroxidation was induced by these chemicals.

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