

The role of glutathione in the toxicity of styrene

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PARKKI, M. G. The role of glutathione in the toxicity of styrene. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 53—59. The role of glutathione in the toxicity of styrene has been proposed based on the observations that glutathione inhibits the covalent binding of styrene and styrene oxide in vitro and that the administration of these compounds to animals decreases the glutathione content in the liver in vivo. In this study methionine (a precursor of reduced glutathione) or diethylmaleate (a depletor of reduced glutathione) was administered to hamsters concomitantly with styrene. Methionine protected the liver against the hepatotoxicity of styrene as indicated by the serum alanine aminotransferase activity. Diethylmaleate potentiated the hepatotoxic effect of styrene. A threshold value for hepatic glutathione of about 1 mmol/l was found for styrene toxicity. Cell damage occurred when the concentration of reduced glutathione decreased below this level. The results indicate that the reduced glutathione in the liver has a central role in the development of cell damage caused by styrene.

Key words: diethylmaleate, glutathione, hepatotoxicity, methionine, styrene, toxicity.

Glutathione has been shown to decrease the covalent binding of styrene oxide (8) and styrene (12) in liver tissue in vitro. Styrene oxide (8) and styrene (13, 18) has also been shown to decrease the liver content of reduced glutathione (GSH) after in vivo administration to animals. These findings suggest that GSH could play a role in the hepatotoxicity of styrene. When the liver GSH stores are depleted in reaction with the reactive intermediate(s) in the metabolism of styrene, these intermediates begin to attack the cell structures and cause damage. The object of the present study was to determine whether hepatic lesion caused by styrene can be opposed by the administration of a GSH pre-

cursor to animals and whether depletion of liver GSH stores by diethylmaleate can potentiate the hepatotoxicity of styrene.

METHODS

Male Syrian hamsters weighing 60—120 g were used in all the studies.

Single dose exposure

Styrene (6 g/kg, 600 mg/ml in corn oil; to one group 4.5 g/kg, 600 mg/ml) was administered to hamsters as a single intragastric dose 24 h before they were killed. Control groups received corn oil only.

In one series the animals were pretreated with 3-methylcholanthrene (20 mg/kg, 10

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mg/ml in corn oil ip.) 96 and 74 h before the styrene dose. In addition DL-methionine was given to a styrene and a control group in three doses of 200 mg/kg (40 mg/ml in saline) intraperitoneally 1 h before and 7 and 15 h after the styrene (oil) dose. Diethylmaleate was administered to a styrene and a control group as two doses of 500 mg/kg (250 mg/ml in corn oil) intraperitoneally 1 h before and 15 h after the styrene dose.

In the second series the 3-methylcholanthrene pretreatment was omitted. L-methionine was administered as four intraperitoneal doses of 200 mg/kg 1 h before and 5, 11 and 17 h after the styrene dose.

Repeated dose exposure

Styrene (1 g/kg 0.5 g/ml) was intragastrically administered daily for five or seven consecutive days. L-methionine (200 mg/kg, 7-d exposure) and diethylmaleate (300 mg/kg, 5-d exposure) were administered

intraperitoneally twice a day 1 h before and 11 h after the styrene doses. The animals were killed 24 h after the last styrene dose.

The liver nonprotein sulfhydryl (mainly GSH) content was measured with the Ellman method (4). The serum alanine aminotransferase (EC 2.6.1.2) (ALAT) (17) and alkaline phosphatase (orthophosphoricmonoester phosphohydrolase, EC 3.1.3.1) (Afos) (2) activities were measured with an Olli 3000-Analyzer, Finland. Because all the control groups showed a close range in the activities of ALAT and Afos and because the enzyme measurements were standardized with clinical control sera, all the control values were unified as "normal" values for these enzymes in the strain of hamsters used. The GSH control value was obtained in a similar manner (a GSH standard was always run with the measurement). Epoxide hydratase activity was measured with the method of Oesch (10); styrene oxide was used as the substrate. 7-Ethoxycoumarin O-deethylation and

Table 1. Effects of methionine and diethylmaleate on serum alanine aminotransferase (ALAT) and alkaline phosphatase (Afos) activities after styrene administration (6 g/kg, 24 h before sacrifice) and the effect of 3-methylcholanthrene pretreatment of the hamsters. Means $\pm$ standard errors are given. The statistical treatment of the results in connection with reduced glutathione (GSH) was carried out with Student's *t*-test (significances: a: $2P < 0.001$ in comparison to the control — a' represents the same level of significance but in comparison with styrene) and, in connection with ALAT and Afos, with the Wilcoxon test for two samples (significances: a: $2a < 0.001$, b: $2a < 0.005$, c: $2a = 0.03$, d: $2a = 0.06$, e: not significant in comparison with the control — the letters with accent marks represent the same levels of significance but in comparison to other substances, i.e., a' to styrene; b' to styrene, 3-methylcholanthrene; c' to styrene; d' to styrene; e' to styrene). The "pathological" values in the enzyme activities were considered to be those above the limit of the control level plus two standard deviations (outside the 97.7 % confidence limit) (for ALAT 122 mol/min l, for Afos 306 mol/min l).

Treatment of animals	GSH (μ mol/g liver wet wt.)	N	ALAT (μ mol/ min l)	"Patho- logical" values/N	Afos (mmol/ min l)	"Patho- logical" values/N
Control	6.50 $\pm$ 0.22	25	74.4 $\pm$ 4.7	1/25	201 $\pm$ 17	0/10
Styrene	0.98 $\pm$ 0.10 a	10	781 $\pm$ 370 a	8/10	316 $\pm$ 226	7/10
Styrene + L-methionine	1.82 $\pm$ 0.18 a'	8	130 $\pm$ 19 c'	4/8	269 $\pm$ 22 e'	3/8
Styrene, 3-methylcholanthrene	0.62 $\pm$ 0.05 a	10	162 $\pm$ 21 a, d'	6/9	412 $\pm$ 61 b	7/10
Styrene, 3-methylcholanthrene + DL-methionine	1.10 $\pm$ 0.3 a	7	162 $\pm$ 36 e	2/4	183 $\pm$ 15 b'	0/7
Styrene, 3-methylcholanthrene + diethylmaleate	0.42 $\pm$ 0.06 a	5	370 $\pm$ 190 b	4/4	424 $\pm$ 63 a	4/4

UDPGlucuronosyltransferase (p-nitrophenol) were measured according to the methods of Aitio (1) and Hänninen (6), respectively.

RESULTS

In a preliminary study cysteamine, one 200 mg/kg dose 1 h before styrene (3 g/kg, intraperitoneal administration), was found to potentiate strongly the acute lethal effect of styrene (death occurring in a couple of hours after styrene administration). Methionine also potentiated this acute effect of styrene. Of the 23 animals dosed with 6 g/kg of styrene (the animals pretreated with 3-methylcholanthrene and the untreated ones combined) 20 survived after 24 h. When the methionine treatment was applied, only 11 of 23 animals survived. The groups differed with a chi-square value below 0.05 in the Mantel-Haenszel test (7).

The liver GSH contents, as well as the serum ALAT and Afos activities, 24 h after the single intragastric dose of styrene are listed in table 1. Styrene (6 g/kg) decreased the hepatic GSH level to 15 % of the control level and caused a strong elevation in the serum ALAT activity. The methionine treatment (4×200 mg L-methionine) was able to oppose partially the GSH depletion caused by styrene (GSH level was 30 % of the control value). In this group the serum ALAT was near the control level. Only minor changes in the serum Afos activity were seen. When the animals were treated in advance with 3-methylcholanthrene, the elevation in the serum ALAT activity was less pronounced after styrene administration. The GSH content in the liver was, however, strongly decreased (to 10 % of the control value). The lower methionine dosage (3×200 mg DL-methionine), used with the animals pretreated with 3-methylcholanthrene caused only a minor reversal in the GSH level. However, Afos, which was somewhat elevated in the group administered styrene only, was stored at the control level in the methionine-treated group. In

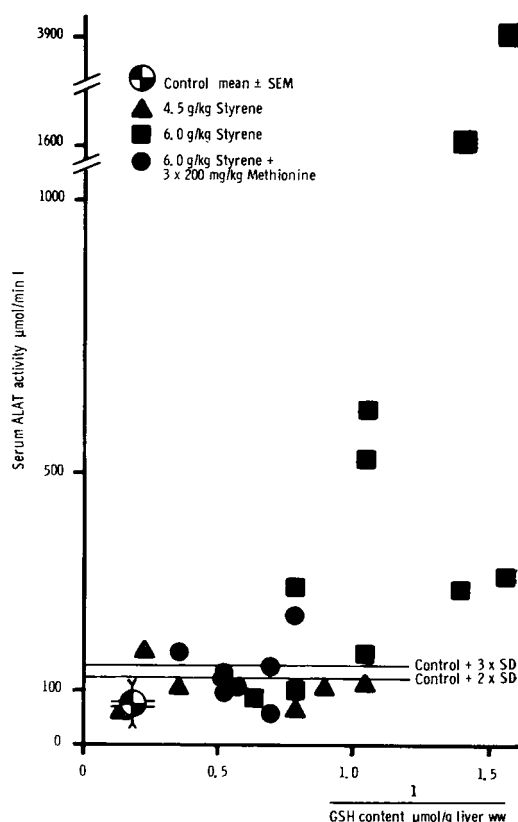


Fig. 1. Correlation between the hepatic reduced glutathione (GSH) content (reverse number) and the serum alanine aminotransferase (ALAT) activity 24 h after styrene administration. The dots indicate discrete cases.

the ALAT activity this protecting effect was not seen. On the other hand, diethylmaleate potentiated the elevating effect of styrene on the serum ALAT activity. Administrations of 3-methylcholanthrene, methionine, or diethylmaleate alone, or styrene as an intragastric dose of 4.5 g/kg did not elevate the serum activities of ALAT or Afos (data not shown).

Fig. 1 shows the relationship between the concentration of GSH in the liver and the serum ALAT activity 24 h after a single dose (6 g/kg) of styrene. Above a GSH concentration of about $1 \mu\text{mol/g}$ of liver (wet weight) there was no change in the activity of ALAT. Below this threshold a strong elevation could be seen. In the animals pretreated with 3-methylcholanthrene a correlation between elevated

Table 2. Effects of methionine and diethylmaleate on the liver glutathione content and serum alanine aminotransferase (ALAT) activity after intragastric administration of styrene (1 g/kg) for seven and five successive days, respectively. Means $\pm$ standard errors are given. The statistical treatment of the results in connection with reduced glutathione (GSH) has been carried out with Students t-test (significances: a: $2P < 0.001$, b: $2P < 0.005$, c: $2P < 0.05$), and in connection with ALAT with the Wilcoxon test for two samples, (significances: b: $2a < 0.005$, d: not significant). The "pathological" values in the ALAT activity were considered to be those above the limit of the control level plus two standard deviations (outside the 97.7 % confidence limit) ($122 \mu\text{mol/min l}$).

Treatment of animals	GSH ($\mu\text{mol/g}$ liver wet wt.)	N	ALAT ($\mu\text{mol/min l}$)	"Pathological" values/N
Control	6.50 ± 0.22	25	74.4 ± 4.7	1/25
Methionine	5.01 ± 0.16	7	70.6 ± 8.4	0/4
Diethylmaleate	3.82 ± 0.14^b	7	83.0 ± 12	1/6
Styrene	4.77 ± 0.27^c	12	121 ± 26^d	6/21
Styrene + methionine	5.43 ± 0.27	13	80.0 ± 11	1/13
Styrene + diethylmaleate	2.38 ± 0.25^a	7	345 ± 220^b	5/9

Table 3. The effects of methionine, diethylmaleate, and styrene (1 g/kg) administrations (alone and in combination) for seven, or in the case of diethylmaleate five, successive days on drug metabolizing enzymes. The statistical treatment of the results has been carried out with Student's t-test; significances are given as follows: a: $2P < 0.001$, b: $2P < 0.01$, c: $2P < 0.05$.

Treatment of animals	Ethoxycoumarin deethylase (nmol/ min g liver wet wt.)		Epoxide hydratase (nmol/min g wet wt.)		UDPGlucuronosyl- transferase (nmol/ min g liver wet wt.) (Digitonin-treated microsomes)	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE
Control	7	19.0 ± 3.8	8	361 ± 24	6	66.2 ± 15
Methionine	7	25.3 ± 6.5	7	327 ± 9.2	5	81.8 ± 12
Diethylmaleate	5	22.6 ± 1.5	4	498 ± 32^b	4	145 ± 59
Styrene	12	33.3 ± 5.6^c	12	596 ± 43^a	8	163 ± 23^b
Styrene + methionine	13	44.9 ± 7.9^b	12	596 ± 21^a	8	131 ± 9.0^b
Styrene + diethylmaleate	2	30.0 ± 10	2	959 ± 215		—

ALAT values and low GSH level was also seen, in spite of the lesser elevation in ALAT activity (not shown in the figure).

The effects of methionine and diethylmaleate on the liver GSH content and the serum ALAT activity in connection with the repeated administration of styrene are given in table 2. Styrene alone caused no significant elevation in the activity of ALAT. The GSH content of the liver was only slightly depressed 24 h after the last styrene dose. On the contrary, when diethylmaleate was administered concomitantly with styrene, an elevation in the activity of ALAT was detected. In this

group a stronger decrease in the GSH level was also seen. In the activity of Afos no significant changes were seen (data not shown).

Table 3 shows the activities of drug metabolizing enzymes after the repeated administration of methionine and diethylmaleate alone and in combination with styrene. Styrene caused elevation in the activities of ethoxycoumarin deethylase (1.8-fold), epoxide hydratase (1.7-fold) and UDPglucuronosyltransferase (2.5-fold measured in digitonin-activated microsomes and 1.8-fold measured in nontreated microsomes; the numbers of the nontreated

microsomes are not given in the table). Methionine had no effects on these enzymes whereas diethylmaleate increased the activity of epoxide hydratase (1.4-fold).

DISCUSSION

Glutathione has been found to be depleted in the liver *in vivo* after styrene (13, 18) and styrene oxide (8) administration. Glutathione also inhibited the covalent binding of styrene and styrene oxide in liver tissue (8, 12). Moreover, glutathione conjugate has been shown to be the major metabolite of styrene oxide in the liver (15). Now it was shown that reduced glutathione (GSH) plays a protecting role against the hepatotoxicity of styrene. Liver damage was avoided when the decrease in the GSH level caused by styrene was opposed with methionine, a precursor of GSH (11). Potentiation of GSH depletion with diethylmaleate also potentiated the hepatotoxicity of styrene. Diethylmaleate depletes GSH in the cell by being bound to it both enzymatically and non-enzymatically (3). A critical concentration of GSH (around 1 $\mu\text{mol/g}$ liver wet weight $\sim 1 \text{ mmol/l}$) was found; below this threshold cell damage occurred. This is the concentration reported also for other reactive intermediates (9).

Administration of styrene as a dose of 1 g/kg for 7 d was not sufficient as such to cause clear hepatotoxic effects. However, when diethylmaleate was administered with styrene, liver damage was seen indicating that GSH has a protecting role also during longer exposure to styrene. The GSH concentration was not, however, detected to be below the critical level. This finding was to be expected, because the maximal suppression of the GSH level caused by the smaller dose (1 g/kg) of styrene used in this experiment is not likely to last for 24 h.

That the effects of methionine and diethylmaleate were mediated via induction

or deterioration of the styrene-metabolizing enzymes seems unlikely because methionine administration had no effect on these enzyme activities. Rather, diethylmaleate administration increased the activity of epoxide hydratase, an enzyme regarded as an inactivator of reactive intermediates. Neither did these compounds change the induction of drug metabolism caused by styrene. Mediation of the effects of methionine and diethylmaleate via competition with styrene at the active sites of drug metabolizing enzymes *in vivo* cannot, however, be excluded. The induction seen in this experiment in the hamster has earlier been shown in the rat after intraperitoneal administration (14) and inhalation exposure (16).

The protecting effect of GSH against the hepatotoxicity of styrene does not prove that this toxicity is based only on the covalent binding. Other toxic properties of the epoxides, as well as the reactive oxygen species possibly generated during the oxidation of styrene at cytochrome P-450, could also be opposed by GSH (5).

The serum ALAT activity seemed to be more sensitive to styrene exposure than Afos. ALAT can, however, be regarded as a more specific indicator of liver damage than Afos.

Pretreatment of hamsters with 3-methylcholanthrene seemed to protect them against the hepatotoxicity of styrene (without elevating the GSH level) because the high ALAT values seen in the noninduced animals after styrene administration were absent in these animals. This result could have been caused by a change in the metabolism of styrene to a direction where less of the hepatotoxic molecular species is formed.

Methionine and cysteamine potentiated the acute lethal effect of styrene (mediated by the possible effects of styrene on the central nervous system, lungs or some other critical target organ). The pretreatment of hamsters with phenobarbital has been found to increase this acute toxicity of styrene also (13). The effect of phenobarbital, however, may be mediated by a different mechanism because it was seen 48 h after the administration of this compound (13).

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QUESTIONS AND ANSWERS

Question to Mr. PARKKI

Dr. VAINIO:

I was influenced by the high intragastric doses you are using. I wonder whether factors such as changes in gastrointestinal motility might explain your findings.

Mr. PARKKI:

In a macroscopic inspection at the moment of sacrifice it was found that a great part of the dose (6 g/kg) was still in the gastrointestinal channel, and slow absorption was indicated. With the use of the intragastric administration route, this high a dosage was needed to reach the threshold value for hepatic GSH concentration in 24 h.