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INCREASE IN LIVER MICROSOMAL GLUTATHIONE *S*-TRANSFERASE ACTIVITY BY PHENOBARBITAL TREATMENT OF RATS

POSSIBLE INVOLVEMENT OF OXIDATIVE ACTIVATION VIA CYTOCHROME P450

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Abstract—The possible involvement of oxidative activation of liver microsomal glutathione (GSH) *S*-transferase by the cytochrome P450 system was investigated. When rats were given phenobarbital (PB) intraperitoneally for 3 days, liver microsomal GSH *S*-transferase activity was stimulated 1.3–1.4-fold and the effect of PB on the transferase was potentiated by combination with a catalase inhibitor, 3-amino-1,2,4-triazole. Immunoblotting of microsomal proteins from PB-treated rats with anti-microsomal GSH *S*-transferase antibody after SDS–PAGE showed the presence of a dimer of the transferase. When microsomal suspensions prepared from PB-treated rats were placed on ice without GSH, the microsomal GSH *S*-transferase activity gradually increased with time and reached 200% of the initial level at 3 hr when activation of the transferase by *N*-ethylmaleimide was lost. The time-dependent increase in GSH *S*-transferase activity in PB-treated microsomes was prevented by addition of 0.1 mM GSH. The increase in microsomal GSH *S*-transferase activity by NADPH was depressed by cytochrome P450 inhibitors such as SKF 525-A (2-diethylaminoethyl-2,2-diphenylvalerate), metyrapone or isoniazid in agreement with the concomitant decrease in generation of hydrogen peroxide in microsomes. These results indicate that the increase in GSH *S*-transferase activity in liver microsomes by PB treatment of rats is due to the oxidative modification of the enzyme by reactive oxygen species which are concomitantly increased following induction of cytochrome P450.

Glutathione (GSH†) *S*-transferases (EC 2.5.1.18) detoxify xenobiotics or toxic metabolites by conjugation with GSH [1, 2]. Microsomal GSH *S*-transferase in rat liver, which contains one cysteine residue per subunit, is activated by modification of the sulfhydryl group with *N*-ethylmaleimide or by thiol/disulfide exchange [3–7]. We reported that microsomal GSH *S*-transferase was also activated by forming a protein dimer or a mixed-disulfide bond via the sulfhydryl group when the liver was under conditions of oxidative stress such as hydrogen peroxide perfusion or ischemia–reperfusion, or when the microsomes were incubated with oxygen radicals *in vitro* [8–11]. Since oxygen radicals are generated endogenously in liver microsomes via the cytochrome P450 system [12–15], it was expected that the cytochrome P450 system contributes to modulation of microsomal GSH *S*-transferase activity *in vivo*. Our preliminary study showed an increase in microsomal GSH *S*-transferase activity after treatment of rats with the cytochrome P450 inducer, phenobarbital (PB). Considering that microsomal GSH *S*-transferase was not induced by PB in spite of the induction of cytosolic GSH *S*-transferase activity [16, 17], it was suggested that the increase in microsomal GSH *S*-transferase activity caused by PB treatment may be due to oxidative modification of the transferase by reactive oxygen species which were increased by cytochrome P450 induction. In the present study, the possible involvement of oxidative activation of microsomal GSH *S*-transferase via the cytochrome P450 system was investigated using cytochrome P450 inducers or inhibitors.

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† Abbreviations: GSH, glutathione; SKF 525-A, 2-diethylaminoethyl-2,2-diphenylvalerate; isoniazid, isonicotinic acid hydrazide; PB, sodium phenobarbital; 3-MC, 3-methylcholanthrene; 3-AT, 3-amino-1,2,4-triazole.

MATERIALS AND METHODS

Chemicals. Reduced glutathione (GSH), 3-methylcholanthrene (3-MC), GSH reductase, glucose 6-phosphate, 3-amino-1,2,4-triazole (3-AT) and cumene hydroperoxide were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). 1-Chloro-2,4-dintrobenzene and sodium phenobarbital (PB) were obtained from Wako Pure Chemicals (Osaka, Japan). NADPH and glucose 6-phosphate dehydrogenase were obtained from the Oriental Yeast Co. (Tokyo, Japan). The Immuno-Blot Assay kits were purchased from Bio-rad Laboratories (Richmond, CA, U.S.A.). All other chemicals used were of analytical reagent grade.

Animal treatment. Male Sprague–Dawley rats (180–250 g) were used. Rats were given PB (75 mg/kg), 3-MC (40 mg/kg) or 3-AT (700 mg/kg)

Table 1. Effects of cytochrome P450 inducers on liver GSH *S*-transferase (GST) and GSH peroxidase (GSHpx) activities

Treatment	N	Microsomes		Cytosol	
		GST	GSHpx	GST	GSHpx
		(μmol/mg/min)			
Control	3	0.101 ± 0.015	0.088 ± 0.002	1.838 ± 0.033	ND
PB 1 hr	3	0.091 ± 0.025	0.058 ± 0.016	1.891 ± 0.326	ND
3.5 hr	3	0.064 ± 0.014	0.076 ± 0.012	1.902 ± 0.269	ND
24 hr	3	0.131 ± 0.017	0.137 ± 0.003*	2.282 ± 0.296	ND
Control	6	0.092 ± 0.004	0.076 ± 0.007	1.412 ± 0.110	0.282 ± 0.013
PB 3 days	6	0.135 ± 0.018†	0.142 ± 0.002†	2.613 ± 0.319†	0.345 ± 0.018†
Control	3	0.080 ± 0.018	0.055 ± 0.004	1.644 ± 0.215	ND
3-MC 3 days	4	0.097 ± 0.020	0.060 ± 0.005	2.531 ± 0.306†	ND

Rats were given PB (75 mg/kg) intraperitoneally and killed at indicated times. With repeated injection, PB (75 mg/kg) or 3-MC (40 mg/kg) was administered intraperitoneally to rats once a day for 3 days and the animals were killed 24 hr after the last injection. The activity of GST and GSHpx in liver cytosol and microsomes was measured as described in Materials and Methods.

* $P < 0.05$, † $P < 0.01$ control vs treated. ND, not determined.

intraperitoneally and were killed after overnight starvation by decapitation at the indicated times. Control rats received the same volume of saline or olive oil. Liver cytosol and microsomes were prepared as described previously [9].

In vitro *treatment of microsomes with various reagents*. Microsomes prepared from control or PB-treated (75 mg/kg, i.p., 3 days) rats were suspended in 0.05 M potassium phosphate buffer (pH 7.4) containing 0.3 mM EDTA and 0.25 M sucrose and were incubated with isonicotinic acid hydrazide (isoniazid, 1 mM) or metyrapone (1.5 mM) in the presence or absence of a NADPH generating system [9]. After removal of residual reagents from the reaction mixture by centrifugation at 105,000 *g* for 60 min, GSH *S*-transferase activity in the resultant pellets was measured. If necessary, microsomes were preincubated with anti-cytochrome P450 (IIB1) antibody in 0.05 M potassium phosphate buffer (pH 7.4) at room temperature for 60 min and aliquots were reincubated for the assay of GSH *S*-transferase activity in the presence or absence of the NADPH generating system at 37° for 10 min.

Measurement of hydrogen peroxide and enzyme activity. The activities of GSH *S*-transferase for 1-chloro-2,4-dinitrobenzene and GSH peroxidase for cumene hydroperoxide were measured by the methods of Habig *et al.* [18] and Reddy *et al.* [19], respectively. Catalase activity in liver homogenates and microsomes was measured by the method of Beers and Sizer [20]. Hydrogen peroxide content in microsomes was measured photometrically using ferrithiocyanate [13]. Protein concentration was determined by the method of Lowry *et al.* [21].

Gel electrophoresis and immunoblotting. SDS–PAGE was carried out in 15% polyacrylamide gels by the method of Laemmli [22] under non-reducing conditions and was followed by immunoblotting as described previously [10].

Statistical analysis. Data were expressed as means ± SD. Significance was calculated using Student's *t*-test, where $P < 0.05$ was taken as significant.

Table 2. Effect of 3-AT and PB on liver catalase activity

Treatment	N	Catalase (H_2O_2 μmol/mg/min)	
		Homogenates	Microsomes
Control	8	123.81 ± 13.79	4.66 ± 0.81
PB	2	123.42	2.78
3-AT	6	54.08 ± 6.90*	2.92 ± 0.42
3-AT + PB	6	39.05 ± 5.0*†	2.48 ± 0.70*

Rats were treated by the same method as used in Table 1.

* $P < 0.01$ control vs treated, † $P < 0.01$ 3-AT alone vs 3-AT plus PB.

RESULTS

PB and 3-MC treatment

Table 1 represents the effect of PB and 3-MC treatment on the liver GSH *S*-transferase and peroxidase activities of rats. Both activities in microsomes had slightly decreased at 1 hr and 3.5 hr after PB treatment. However, a 30% increase in GSH *S*-transferase and a 55% increase in GSH peroxidase activity in microsomes were seen 24 hr after PB injection. When PB or 3-MC was given to rats for 3 days, cytosolic GSH *S*-transferase activity was significantly increased in both cases, whereas microsomal GSH *S*-transferase was stimulated only in PB-treated rats. GSH peroxidase activity in cytosol and in microsomes was also increased to 120% and 180% of the control, respectively, by PB treatment.

3-AT and PB treatment

As shown in Table 2, catalase activity in liver

Table 3. Effect of 3-AT and PB on liver GSH S-transferase (GST) and GSH peroxidase (GSHpx) activities

Treatment	N	Microsomes		Cytosol	
		GST	GSHpx	GST	GSHpx
		(μmol/mg/min)			
Control	6–8	0.098 ± 0.022	0.087 ± 0.008	1.459 ± 0.055	0.381 ± 0.027
PB	2	0.132	0.112	2.220	0.456
3-AT	6	0.129 ± 0.029	0.077 ± 0.006*	1.845 ± 0.275*	0.386 ± 0.042
3-AT + PB	5–6	0.155 ± 0.027†	0.095 ± 0.006‡	2.744 ± 0.186†‡	0.404 ± 0.043

Rats were given PB (75 mg/kg), 3-AT (700 mg/kg) or both intraperitoneally once a day for 3 days and were killed 24 hr after the last injection. GST and GSHpx activities in liver cytosol and microsomes were measured as described in Materials and Methods.

*$P < 0.05$, †$P < 0.01$ control vs treated, ‡$P < 0.01$ 3-AT alone vs 3-AT plus PB.

homogenates was decreased to 43% of the control by 3-AT alone and to 31% by combination of 3-AT with PB. Under the same conditions microsomal catalase activity was also decreased by either 3-AT alone or 3-AT plus PB treatment. Microsomal GSH S-transferase activity was increased about 30% by either PB or 3-AT treatment and was potentiated by the combination of 3-AT and PB, as well as there being an increase in cytosolic GSH transferase (Table 3). GSH peroxidase activity in microsomes was significantly decreased by 3-AT compared to a 28% increase in PB-treated rats. No increase in cytosolic GSH peroxidase activity was observed in 3-AT and 3-AT plus PB-treated rats.

Gel electrophoresis and immunoblotting

Immunoblotting with anti-microsomal GSH S-transferase antibodies after electrophoretic separation of the liver microsomal proteins of PB-treated rats showed the presence of greater amounts of the protein with a M_r of 34,000 than in control microsomes in addition to the native microsomal GSH S-transferase (M_r 17,000). The protein with a M_r of 34,000 was abolished by addition of dithiothreitol, showing a dimer protein of the transferase. In control microsomes, a small amount of the protein with a M_r of 51,000 was also observed (Fig. 1).

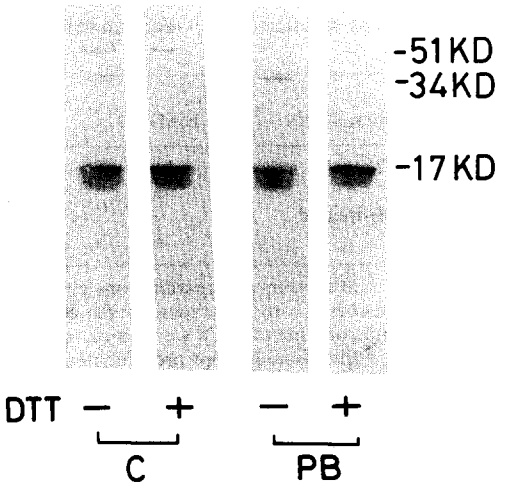

Fig. 1. Electrophoresis and immunoblotting of control and PB-treated microsomes. Microsomes freshly prepared from control and PB-treated (75 mg/kg, i.p., 3 days) rats were incubated with or without 10 mM dithiothreitol for 10 min at room temperature. The microsomal proteins (50 μg) were applied to 15% SDS–polyacrylamide gel and the electrophoresis was carried out by the method of Laemmli [22] under non-reducing conditions at 6°. Immunoblotting was performed by transferring proteins from gels to nitrocellulose paper as described in Materials and Methods.

Cytochrome P450 inhibitors

Tables 4 and 5 show the effect of isoniazid and metyrapone, which are inhibitors of cytochrome P450, on hydrogen peroxide formation and microsomal GSH S-transferase activity. The formation of hydrogen peroxide in PB-treated microsomes in the presence of NADPH was 2.6-fold of the control and was depressed significantly by the addition of isoniazid. GSH S-transferase activity, which was stimulated by NADPH 1.8-fold in control and 1.5-fold in PB-treated microsomes, was also decreased when the microsomes were incubated with isoniazid. In the case of metyrapone, the formation of hydrogen peroxide in control and PB-treated microsomes was decreased to 46% and 60%, respectively, and GSH S-transferase activity was also decreased to 89% and 73% (Table 5). The GSH S-transferase activity in control and PB-treated microsomes was decreased to 78% and 57%, respectively, by 2-diethylaminoethyl-2,2-diphenylvalerate (SKF 525-A) treatment. No increase in GSH S-transferase activity was observed by the addition of NADPH in microsomes prepared from isoniazid-treated rats (Table 6). When control microsomes were incubated with the anti-cytochrome P450 antibody in the presence of NADPH, GSH S-transferase activity was decreased to 86% (Table 7).

Table 4. Effect of isoniazid (INH) on H_2O_2 generation and microsomal GSH *S*-transferase (GST) activity

Microsomes	NADPH	INH (1 mM)	H_2O_2* (nmol/mg)	GST activity† (μmol/mg/min)
Control	−	−	7.1 ± 0.2	0.099 ± 0.010
	−	+	7.7 ± 1.8	0.100 ± 0.015
	+	−	97.7 ± 9.4	0.182 ± 0.020
	+	+	62.1 ± 4.3‡	0.140 ± 0.021‡
PB-treated	−	−	27.2 ± 2.4	0.220 ± 0.033
	−	+	24.9 ± 1.7	0.182 ± 0.025§
	+	−	259.1 ± 25.9	0.327 ± 0.012
	+	+	128.6 ± 20.5§	0.266 ± 0.037§

* Microsomes prepared from control or PB-treated (75 mg/kg, i.p., 3 days) rats were incubated with 1 mM INH in the presence or absence of a NADPH generating system at 37° for 30 min and contents of H_2O_2 were measured.

† Microsomes were preincubated with 1 mM INH at room temperature for 30 min and the GST activity was measured after removal of residual INH by centrifugation.

Each value shows the mean ± SD for triplicate incubations.

‡ $P < 0.05$, §$P < 0.01$ non-treated vs INH-treated.

Table 5. Effect of metyrapone on H_2O_2 generation and microsomal GSH *S*-transferase (GST) activity

Microsomes	NADPH	Metyrapone (1.5 mM)	H_2O_2* (nmol/mg)	GST activity† (μmol/mg/min)
Control	+	−	97.0 ± 10.7	0.228 ± 0.031
	+	+	44.3 ± 8.2‡	0.203 ± 0.030
PB-treated	+	−	230.9 ± 45.7	0.317 ± 0.041
	+	+	138.3 ± 5.3	0.230 ± 0.023§

* Microsomes prepared from control or PB-treated (75 mg/kg, i.p., 3 days) rats were incubated with metyrapone in the presence of a NADPH generating system at 37° for 10 min and contents of H_2O_2 were measured.

† Microsomes were preincubated with metyrapone at room temperature for 30 min and GST activity was measured after removal of residual metyrapone by centrifugation.

Each value shows the mean ± SD for triplicate incubations.

‡ $P < 0.05$, §$P < 0.01$ non-treated vs metyrapone-treated.

Table 6. Effect of SKF 525-A on GSH *S*-transferase (GST) activity in microsomes from PB- or isoniazid (INH)-treated rats

Microsomes	NADPH	SKF 525-A (50 μM)	GST activity (μmol/mg/min)
Control	−	−	0.070 ± 0.003
	−	+	0.066 ± 0.005
	+	−	0.090 ± 0.003*
	+	+	0.070 ± 0.005†
PB-treated	−	−	0.106 ± 0.022*
	−	+	0.079 ± 0.021
	+	−	0.130 ± 0.007*
	+	+	0.074 ± 0.009†
INH-treated	−	−	0.080 ± 0.006
	+	−	0.070 ± 0.004

Microsomes prepared from control, PB (75 mg/kg, 3 days)- or INH (75 mg/kg, 30 min)-treated rats were incubated with SKF 525-A at 37° for 30 min in the presence or absence of a NADPH generating system and the GST activity was measured. Values show the means ± SD for triplicate incubations.

*$P < 0.05$ control vs treated, †$P < 0.05$ non-treated vs SKF 525-A-treated.

Table 7. Effect of cytochrome P450 (IIB1) antibody on microsomal GSH S-transferase (GST) activity

Addition	Antibody	GST activity (μmol/mg/min)
None	−	0.081
	+	0.075
NADPH	−	0.151
	+	0.130

Microsomes (3.2 mg/mL) prepared from control rats were incubated with or without cytochrome P450 (IIB1) antibody (80 μL) for 60 min at room temperature and the GSH S-transferase activity was measured after incubation at 37° for 10 min in the presence or absence of a NADPH generating system.

Values show the means of duplicate incubations as the representative experiment.

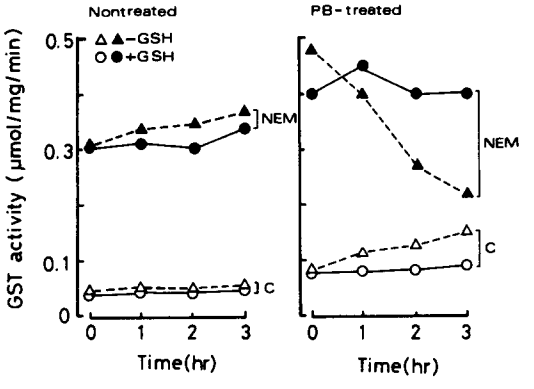

Fig. 2. Effect of N-ethylmaleimide (NEM) on GSH S-transferase activity in control or PB-treated microsomes. Microsomal pellets prepared from control or PB-treated (75 mg/kg, i.p., 3 days) rats were suspended in 0.05 M potassium phosphate buffer (pH 7.4) containing 0.3 mM EDTA and 0.25 M sucrose and placed on ice in the presence or absence of 0.1 mM GSH. Aliquots were taken at indicated times and were treated with 1 mM NEM for 2 min at room temperature. GSH S-transferase activity was measured as described in Materials and Methods. Typical data from several experiments are shown. Each point shows the mean of duplicate incubations. (△) Microsomes without GSH and NEM, (▲) microsomes treated with NEM in the absence of GSH, (○) microsomes with GSH, (●) microsomes treated with NEM in the presence of GSH.

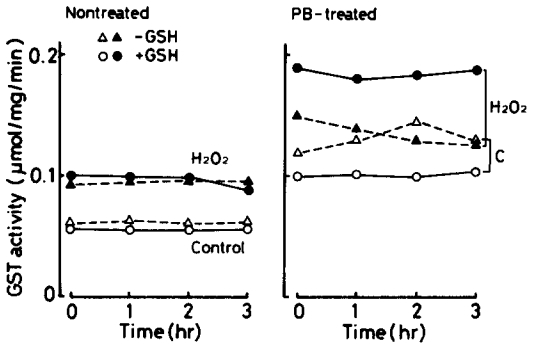

Fig. 3. Effect of hydrogen peroxide (H_2O_2) on GSH S-transferase activity in control or PB-treated microsomes. Control or PB-treated microsomes, which were suspended in the same buffer as used in Fig. 1, were incubated with 0.75 mM H_2O_2 for 30 min at room temperature and GSH S-transferase was measured as described in Materials and Methods. Typical data from several experiments are shown. Each point shows the mean of duplicate incubations. (△) Microsomes without GSH and H_2O_2, (▲) microsomes treated with H_2O_2 in the absence of GSH, (○) microsomes with GSH, (●) microsomes treated with H_2O_2 in the presence of GSH.

About a 10% decrease in hydrogen peroxide generation was observed after treatment of microsomes with the cytochrome P450 antibody.

N-*Ethylmaleimide and hydrogen peroxide treatment* in vitro

Control and PB-treated microsomes suspended in 0.05 M potassium phosphate buffer (pH 7.4) containing 0.3 mM EDTA and 0.25 M sucrose were put on ice in the presence or absence of 0.1 mM GSH and the effect of N-ethylmaleimide on GSH S-transferase activity in the microsomes was evaluated at the indicated times (Fig. 2). In control microsomes GSH S-transferase activity was at almost the same level until 3 hr in the presence or absence of GSH, and the transferase activity was stimulated by N-ethylmaleimide 5- to 7-fold under either condition. On the other hand, GSH S-transferase activity in PB-treated microsomes gradually increased with time in the absence of GSH and reached 200% of the initial level at 3 hr, when the increase in transferase activity caused by N-ethylmaleimide was only 1.5-fold compared with 4.4-fold in the presence of GSH. As shown in Fig. 3, a 60% increase in GSH S-transferase activity in control microsomes was caused by hydrogen peroxide regardless of the presence or absence of GSH. However, in PB-treated microsomes without GSH the increase in GSH S-transferase activity by the agent was observed only when the microsomes were used within 30 min and was lost after 3 hr. A 90% increase in the transferase activity caused by hydrogen peroxide was obtained during 3 hr in PB-treated microsomes in the presence of 0.1 mM GSH.

DISCUSSION

In the present study, an increase in microsomal GSH S-transferase and GSH peroxidase activities as well as cytosolic GSH S-transferase was observed in the liver of the rat treated with PB for 3 days. In 3-MC-treated rats, microsomal GSH S-transferase activity was slightly increased in spite of a significant increase in the cytosolic activity. Although PB is an inducer of drug-metabolizing enzymes, it has been shown that microsomal GSH S-transferase is not induced by PB as there is no increase in expression at the RNA level [17]. In the present study, an

increase in the microsomal GSH *S*-transferase protein in PB-treated microsomes was not observed when the same amount of microsomal proteins from control or PB-treated liver was analysed by western blotting, supporting no induction of GSH *S*-transferase in PB-treated microsomes (Fig. 1). Thus it was assumed that microsomal GSH *S*-transferase is increased by PB via a different mechanism from that of enzyme induction.

PB is well known as an inducer of cytochrome P450 and NADPH cytochrome P450 reductase [23, 24], and both enzymes can generate reactive oxygen species [12–15]. We showed previously that liver microsomal GSH *S*-transferase of rats was activated by reactive oxygen species via oxidative modification of the sulfhydryl group in the enzyme [8–11]. It is therefore suggested that microsomal GSH *S*-transferase is activated by oxygen radicals which are generated via the cytochrome P450 system. In our study the amount of hydrogen peroxide in PB-treated microsomes was 2.6-fold compared with non-treated microsomes (Table 4). When the PB-treated microsomes were analysed by SDS–PAGE followed by immunoblotting, the protein with a M_r of 34,000, which was abolished by dithiothreitol, was detected in addition to the native GSH *S*-transferase with a M_r of 17,000, indicating formation of the dimer of the transferase. Since microsomal GSH *S*-transferase is activated by reactive oxygen species by forming a dimer protein or a mixed-disulfide bond [10, 11], it was clarified that microsomal GSH *S*-transferase was activated after PB treatment by reactive oxygen species which were increased with increasing cytochrome P450 content. Considering generation of more hydrogen peroxide in PB-treated microsomes than in 3-MC-treated microsomes [12], it is likely that the activity of GSH *S*-transferase in PB-treated microsomes is higher than that in 3-MC-treated microsomes. It is reasonable to assume that microsomal GSH peroxidase activity was also increased by PB treatment (Fig. 2) because microsomal GSH *S*-transferase can act as selenium-independent GSH peroxidase [25]. The increase in both activities in microsomes was also observed after ischemia–reperfusion or diethylmaleate perfusion of isolated rat liver [8, 26].

Since superoxide anion and hydrogen peroxide are generated via the cytochrome P450 system, it was expected that inhibition of enzyme scavenging of such reactive oxygen species would enhance the PB-mediated increase in GSH *S*-transferase activity. To test this hypothesis, a catalase inhibitor 3-AT was used. As shown in Table 3, the stimulation of microsomal GSH *S*-transferase activity by PB treatment was potentiated in combination with 3-AT (a 30% increase with PB treatment alone vs a 60% increase in combination with 3-AT). This confirmed that the increase in microsomal GSH *S*-transferase activity after PB treatment of rats is due to oxidative activation of the enzyme, but not to induction, by reactive oxygen species which are stimulatively formed following cytochrome P450 induction. The depression of oxidative activation of microsomal GSH *S*-transferase by catalase or superoxide dismutase has also been shown *in vitro* [9].

The relationship between hydrogen peroxide formation and microsomal GSH *S*-transferase activity was further investigated using cytochrome P450 inhibitors such as isoniazid, metyrapone and SKF 525A. Isoniazid and metyrapone decreased the generation of hydrogen peroxide concomitant with a decrease in GSH *S*-transferase activity in either control or PB-treated microsomes (Tables 4 and 5). SKF 525-A also depressed microsomal GSH *S*-transferase activity in both microsomes in the presence of NADPH. In addition, the transferase activity was not enhanced by NADPH in microsomes prepared from isoniazid-treated rats, showing inhibition of hydrogen peroxide formation via cytochrome P450 *in vivo*. Furthermore, microsomal GSH *S*-transferase activity was decreased by treatment of microsomes with the anti-cytochrome P450 antibody (Table 7). Thus it was again established that microsomal GSH *S*-transferase activity is increased corresponding to the amounts of reactive oxygen species which are formed by the cytochrome P450 system.

We also noticed that GSH *S*-transferase activity in PB-treated microsomes gradually increased when the microsomal suspension was placed on ice, reaching 1.5-fold of the initial level after 1 hr. In addition, activation of the transferase by *N*-ethylmaleimide or hydrogen peroxide in PB-treated microsomes was depressed corresponding to an increase in the basal level of the transferase activity. The decrease in the effect of *N*-ethylmaleimide or hydrogen peroxide on microsomal GSH *S*-transferase in PB-treated microsomes was prevented when 0.1 mM GSH was added to the microsomes. This means that more reactive oxygen species are generated spontaneously in PB-treated microsomes, and the sulfhydryl group of the transferase is easily oxidized during storage of the microsomal suspension on ice resulting in an increase in the transferase activity. Therefore, we usually assayed the GSH *S*-transferase activity in PB-treated microsomes within 30 min of preparation from rat liver unless GSH was added.

All these results show that the increase in microsomal GSH *S*-transferase activity seen after PB treatment is due to an oxidative activation of the enzyme by reactive oxygen species which are increased following induction of the cytochrome P450 system.

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REFERENCES

1. Jakoby WB and Habig WH, Glutathione transferase. In: *Enzymatic Basis of Detoxication* (Ed. Jakoby WB), Vol. 2, pp. 63–94. Academic Press, New York, 1980.
2. Habig WH, Glutathione *S*-transferase: versatile enzymes of detoxication. In: *Radioprotectors and*

Anticarcinogens (Eds. Nygaard OF and Simic MG), pp. 169–190. Academic Press, New York, 1983.
3. Morgenstern R and DePierre JW, Microsomal glutathione transferase: purification in unactivated form and further characterization of activation process, substrate specificity and amino acid composition. *Eur J Biochem* **134**: 591–597, 1983.
4. Morgenstern R, DePierre JW and Jornvall H, Microsomal glutathione transferase; primary structure. *J Biol Chem* **260**: 13976–13983, 1985.
5. Morgenstern R, DePierre JW and Ernster L, Activation of microsomal glutathione *S*-transferase activity by sulfhydryl reagents. *Biochem Biophys Res Commun* **87**: 657–663, 1979.
6. Morgenstern R, DePierre JW and Ernster L, Reversible activation of rat liver microsomal glutathione *S*-transferase activity by 5,5′-dithiobis (2-nitrobenzoic acid) and 2,2′-dipyridyl disulfide. *Acta Chem Scand* **B34**: 229–230, 1980.
7. Aniya Y and Anders MW, Regulation of rat liver microsomal glutathione *S*-transferase activity by thiol/disulfide exchange. *Arch Biochem Biophys* **270**: 330–334, 1989.
8. Aniya Y and Naito A, Oxidative stress-induced activation of microsomal glutathione *S*-transferase in isolated rat liver. *Biochem Pharmacol* **45**: 37–42, 1993.
9. Aniya Y and Anders MW, Activation of rat liver microsomal glutathione *S*-transferase by reduced oxygen species. *J Biol Chem* **264**: 1998–2002, 1989.
10. Aniya Y and Anders MW, Activation of rat liver microsomal glutathione *S*-transferase by hydrogen peroxide: role for protein-dimer formation. *Arch Biochem Biophys* **296**: 611–616, 1992.
11. Amiya Y and Anders MW, Activation of hepatic microsomal glutathione *S*-transferase by hydrogen peroxide and diamide. In: *Oxygen Radicals* (Eds. Yagi K, Kondo M, Niki E and Yoshikawa T), pp. 737–740. Elsevier, Amsterdam, 1992.
12. Hildebrandt A, Speck M and Roots I, Possible control of hydrogen peroxide formation and degradation in microsomes during mixed function oxidation reaction. *Biochem Biophys Res Commun* **54**: 968–975, 1973.
13. Thurman RG, Ley HG and Scholz R, Hepatic microsomal ethanol oxidation. Hydrogen peroxide formation and the role of catalase. *Eur J Biochem* **25**: 420–430, 1972.
14. Oprian DD, Gorsky LD and Coon MJ, Properties of the oxygenated form of liver microsomal cytochrome P-450. *J Biol Chem* **258**: 8684–8691, 1983.
15. Scholz W, Schutze K, Kunz W and Schwarz M, Phenobarbital enhances the formation of reactive oxygen in neoplastic rat liver nodules. *Cancer Res* **50**: 7015–7022, 1990.
16. Morgenstern R, Meijer J, DePierre JW and Ernster L, Characterization of rat-liver microsomal glutathione *S*-transferase activity. *Eur J Biochem* **104**: 167–174, 1980.
17. DeJong JL, Morgenstern R, Jornvall H, DePierre JW and Tu C-PD, Gene expression of rat and human microsomal glutathione *S*-transferases. *J Biol Chem* **263**: 8430–8436, 1988.
18. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferase. *J Biol Chem* **249**: 7130–7139, 1974.
19. Reddy CC, Tu C-PD, Burgess JR, Ho C-Y, Scholz RW and Massaro EJ, Evidence for the occurrence of selenium-independent glutathione peroxidase activity in rat liver microsomes. *Biochem Biophys Res Commun* **101**: 970–978, 1981.
20. Beers RF Jr and Sizer IW, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* **195**: 133–140, 1952.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
22. Laemmli UK, Cleavage of structural protein during the assembly of the head of bacteriophage. *Nature* **227**: 680–685, 1970.
23. Mannering GJ, Significance of stimulation and inhibition of drug metabolism in pharmacological testing. In: *Selected Pharmacological Testing Methods* (Ed. Burger A), Vol. 3, pp. 51–111. Marcel Dekker, New York, 1968.
24. Parke DV, Induction of the drug-metabolizing enzymes. In: *Enzyme Induction* (Ed. Parke DV), p. 207. Plenum Press, London, 1975.
25. Prohaska JR and Ganther HE, Glutathione peroxidase activity of glutathione *S*-transferase purified from rat liver. *Biochem Biophys Res Commun* **76**: 437–445, 1977.
26. Aniya Y and Teruya M, Activation of hepatic microsomal glutathione *S*-transferase of rats by a glutathione depletor, diethylmaleate. *J Pharmacobiodyn* **15**: 473–479, 1992.