Tissue-specific induction of intestinal glutathione S-transferases by αβ-unsaturated carbonyl compounds

T. Masukawa[1], T. Nishimura and H. Iwata

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, 1-6, Yamadaoka, Suita-shi, Osaka 565 (Japan), 29 December 1983

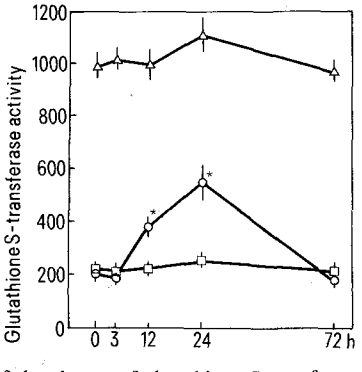
Summary. Glutathione S-transferase activity in rat intestinal mucosa was increased by the injection of αβ-unsaturated carbonyl compounds such as phorone and diethylmaleate, but that in the liver and kidney was not. Since the administration of cycloheximide completely blocked the increase of the enzyme activity by phorone, the increase of the activity may be due to de novo synthesis rather than enzyme activation.

Key words. Glutathione depleter; phorone; diethylmaleate; αβ-unsaturated carbonyl compound; glutathione S-transferases; intestinal mucosa; induction.

Glutathione S-transferases are a group of enzymes that catalyse the conjugation of reduced glutathione (GSH) with a large variety of compounds carrying an electrophilic site[2]. The enzymes, which are widely located in various tissues, are thought to participate in the detoxication of the xenobiotics ingested[3-5]. Like the induction by drugs of the microsomal drug-metabolizing enzymes, glutathione S-transferases are known to be induced by various compounds such as phenobarbital, polycyclic aromatic hydrocarbons and synthetic antioxidants[5-7]. While their inducibility is generally highest in the liver, induction also occurs in extrahepatic tissues, e.g. intestine, kidney and lung[5]. The intestine, especially, is an organ directly exposed to a number of drugs, food additives and xenobiotics, and therefore the induction of intestinal enzymes which participate in the detoxication is thought to be of importance in drug metabolism. However, there has been little information on this aspect of intestinal glutathione S-transferases. This study presents evidence that αβ-unsaturated carbonyl compounds such as phorone (diisopropylidene acetone) and diethylmaleate induce glutathione S-transferases in the intestinal mucosa, but not in the liver and kidney.

Materials and methods. 5-week-old male Wistar rats were used. The rats were decapitated at approximately the same time (9.00–10.00), and the tissues were removed. Intestinal mucosa was carefully removed with a razor blade. The liver was perfused with cold saline. The tissues obtained were homogenized in 4 vol. of 0.25 M sucrose. The homogenate was centrifuged at 10,000 × g for 20 min and the resulting supernatant fraction was used for the enzyme assay. In the case of the assay of soluble and microsomal enzyme activity, the above supernatant fraction was centrifuged at 105,000 × g for 60 min. The resultant supernatant was designated the cytosolic fraction. The microsomal pellet was washed with 0.15 M Tris-HCl (pH 8.0) equivalent to 10 vol. of original tissue, and recentrifuged at 105,000 × g for 60 min to remove cytosolic contamination. The resulting pellet was suspended in 0.15 M Tris-HCl (pH 8.0), and was used as the microsomal fraction. Glutathione S-transferase activity was measured with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate using the method of Habig et al.[8]. The assay was performed in 1 mM CDNB and 5 mM GSH in 0.1 M potassium phosphate buffer (pH 6.5) at 25°C. Protein was determined by the method of Lowry et al.[9].

Results and discussion. The time-course of the change of glutathione S-transferase activity after injection of phorone (250 mg/kg, i.p.) is shown in the figure. In the intestinal mucosa, the enzyme activity increased markedly to about twice the initial level 12 h after injection and reached a peak, 3 times as high as the inital activity, 24 h later. The activity was found to be restored to the normal level 72 h after administration of phorone. However, there was no alteration in glutathione S-transferase activity of the liver and kidney. The increase of the enzyme activity in the intestinal mucosa was found to be dose-dependent (table 1). Since phorone is thought to be useful as a GSH depeleting agent[10], the effect of diethylmaleate, the agent most widely used as a GSH depleter, was examined to assess whether the phenomena described above are specific for αβ-unsaturated carbonyl compounds as GSH depleting agents. Diethylmaleate had the same activity as phorone (table 1). Ad-



Time course of the change of glutathione S-transferase activity in the various tissues of rats treated with phorone (250 mg/kg, i.p., dissolved in sesame oil). Enzyme activity is expressed as nmol CDNB conjugated/min/mg protein. Points and bars represent the mean and SEM respectively of results from 4 rats. Significantly different from the control (* $p < 0.01$.). ○, Intestinal mucosa, △, liver, □, kidney.

Table 1. Effect of phorone and diethylmaleate on glutathione S-transferase activity in the various tissues of rats

Treatment	Enzyme activity		
	Liver	Kidney	Intestinal mucosa
Control (i.p.)	977 ± 47	230 ± 21	206 ± 21
Phorone 100 mg/kg (i.p.)	978 ± 40	228 ± 11	372 ± 4*
250 mg/kg (i.p.)	1125 ± 63	254 ± 27	546 ± 21*
Diethylmaleate 500 mg/kg (i.p.)	854 ± 49	197 ± 8	387 ± 18*
Control (s.c.)	987 ± 35	246 ± 5	212 ± 7
Phorone 250 mg/kg (s.c.)	1124 ± 56	260 ± 7	567 ± 15*

Rats were sacrificed 24 h after the injection of drug (dissolved in sesame oil). Enzyme activity is expressed as nmol CDNB conjugated/min/mg protein. Values represent the mean ± SEM of results for 4 rats. Significantly different from control (* $p < 0.01$).

Table 2. Effect of cycloheximide treatment on the induction of glutathione S-transferase activity in the intestinal mucosa by phorone

Treatment	Enzyme activity
Control (4)	215 ± 10
Phorone (4)	305 ± 18*
Cycloheximide (4)	194 ± 11
Cycloheximide + phorone (3)	154 ± 12**

Rats were injected with cycloheximide (2 mg/kg, i.p., dissolved in saline) 30 min prior to and 2 h after phorone (250 mg/kg, s.c., dissolved in sesame oil), and the rats were sacrificed 12 h after phorone injection. Enzyme activity is expressed as nmol CDNB conjugated/min/mg protein. Values represent the mean ± SEM. The number of rats used is in parentheses. Significantly different from control (* $p < 0.01$, ** $p < 0.05$).

ditionally, the effect of phorone was also observed after dorsal s.c. injection in rats. The findings indicate that the tissue-specific-increase of glutathione S-transferase activity in the intestine is specific to the $\alpha\beta$-unsaturated carbonyl compound as a GSH depleter, and is not an artifact caused by the method of drug administration.

Recently, glutathione S-transferases were demonstrated to be located in microsomes as well as in the cytosol[11,12]. Thus, subcellular localization of the increased activity of intestinal glutathione S-transferases was investigated. The increase of the enzyme activity was found to be in the cytosol, but not in the microsomes (data not shown).

The addition of phorone (10^{-6}–10^{-4} M) into the enyzme assay medium described in the method did not increase the intestinal glutathione S-transferase activity. Moreover, as shown in the table 2, the treatment of cyclohexamide, an inhibitor of protein synthesis, completely blocked the increase in activity of intestinal glutathione S-transferases by phorone. Thus, the increase of intestinal enzyme activity by phorone is thought to be due to de novo synthesis rather than enzyme activation.

Both diethylmaleate and phorone were reported to result in a dramatic decrease of tissue GSH level, but GSH synthesis was increased when tissue GSH was depleted with these compound[10,13]. Under our conditions intestinal GSH was decreased by the injection of phorone (250 mg/kg, i.p.), to about 50% of the control at 3 h, but at 12–24 h after phorone, GSH levels rose to about twice those of the control rats, and then gradually returned to the control level (data not shown).

A similar time course pattern of GSH levels was found in the liver and kidney. Thus, the period of the induction of intestinal glutathione S-transferases corresponded to that of the increased synthesis of GSH. However, it is not clear whether the modification of GSH metabolism is related to the induction of intestinal glutathione S-transferases. Further studies using various types of compounds modifying GSH metabolism will be necessary to assess the relationship between the enzyme induction and the alternation of GSH metabolism. Most of the inducers used in previous studies produced the induction of glutathione S-transferase in the extrahepatic organs as well as in the liver[5,6]. In this regard, the present finding that the induction of the enzymes by phorone is restricted to the intestine is interesting. While a possible cause of organ specificity of the enzyme induction is not clear, the induction of intestinal glutathione S-transferases may be specific to $\alpha\beta$-unsaturated carbonyl compounds. Thus, $\alpha\beta$-unsaturated carbonyl compounds as GSH depleters may be useful tools to evaluate the role and detoxication capacity of intestinal glutathione S-transferases in drug metabolism.

1 Present address: Department of Pharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, Nagaotoge-cho, Hirakata, Osaka, 573-01, Japan.
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Tetracyclines inhibit parathyroid hormone-induced bone resorption in organ culture[1]

B.C. Gomes, L.M., Golub and N.S. Ramamurthy

School of Dental Medicine, State University of New York at Stony Brook, Stony Brook (New York 11794, USA), 8 November 1983

Summary. Several tetracyclines (minocycline, doxycycline, tetracycline), in levels approximating physiologic concentrations, were found to inhibit parathyroid hormone-induced bone resorption in organ culture; the specificity of this effect was demonstrated by comparison with other (non-tetracycline) types of antibiotics. The ability of tetracyclines to inhibit bone resorption is consistent with the recent proposal by Golub et al.[2] that these antibiotics can inhibit mammalian collagenolytic enzymes by a mechanism unrelated to the drug's antibacterial efficacy, a property which could be therapeutically useful in diseases characterized by excessive collagen breakdown.

Key words. Bone resorption; bone culture; tetracyclines; collagenolytic enzymes.

Recently, Golub et al.[2] reported that a tetracycline (minocycline) (1) inhibited both abnormally enhanced collagenolytic enzyme activity in gingiva, and excessive collagen resorption in the skin of rats, even under germ-free conditions, and (2) suppressed leukocyte collagenase activity in vitro and gingival fluid collagenase activity in vivo. These effects did not appear to be produced by non-tetracycline antibiotics. They concluded that tetracycline therapy can inhibit the activity of mammalian collagenolytic enzymes by a mechanism(s) unrelated to the drug's antibacterial efficacy, and suggested that this property could inhibit collagen degradation including that which occurs during bone resorption. Preliminary evidence indicated that minocycline therapy also reduced pathologically excessive alveolar (periodontal) bone loss in rats[2]. However, this effect could have resulted from the known ability of the antibiotic to suppress oral Gram-negative micro-organisms responsible for inflammatory periodontal disease[3], which includes alveolar bone loss, rather than a direct inhibitory effect of the drug on bone resorption. In the present study, we report that various tetracyclines, but not other antibiotics, directly inhibit bone resorp-