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EFFECTS OF PHORONE (DIISOPROPYLIDENE ACETONE), A GLUTATHIONE (GSH) DEPLETOR, ON HEPATIC ENZYMES INVOLVED IN DRUG AND HEME METABOLISM IN RATS: EVIDENCE THAT PHORONE IS A POTENT INDUCER OF HEME OXYGENASE

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SUMMARY: Concomitant with the depletion of glutathione content, phorone (250 mg/kg, ip.) produced a marked increase in heme oxygenase activity, biphasic effect on δ-aminolevulinic acid synthetase activity, and slight decreases in cytochrome P-450 content and aminopyrine demethylase activity in the liver of rats. The increase in heme oxygenase activity evoked by phorone was almost completely blocked by pretreatment of rats with actinomycin D and cycloheximide. Phorone was able to produce the changes in these parameters in a dose-dependent manner. Buthionine sulfoximine, a GSH depletor by inhibition of biosynthesis, failed to affect these hepatic parameters.

It has been shown that phorone (diisopropylidene acetone) is able to deplete glutathione (GSH) content from animal tissues and thus, may be useful as a GSH depleting agent (1). Of the various agents which could deplete GSH, diethyl maleate which was introduced by Boyland and Chausseud (2) has been widely used for toxicological studies in order to investigate the possible role of GSH-mediated reactions in the metabolism of various drugs and chemicals (3-5). However, diethyl maleate has also been shown to exert effects unrelated to GSH depletion, such as the induction of heme oxygenase (6,7), inhibition or stimulation of drug-metabolizing enzyme activities (8,9) and the increase in ornithine decarboxylase activity (10).

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Since phorone, like diethyl maleate, is one of the α,β-unsaturated carbonyl compounds, and may be useful as a GSH depleting agent, its other biochemical effects on animal tissues should be investigated in detail. From this viewpoint, we firstly examined the effects of phorone on hepatic enzymes involved in drug and heme metabolism because cytochrome P-450 plays an important role in drug action and toxicity, and because both drug and heme metabolism are likely to be related.

MATERIALS AND METHODS

Phorone was obtained from Wako Pure Chemical Co. Ltd. G-6-P, NADP, NADPH, G-6-P dehydrogenase, hemin and buthionine sulfoximine (BSO) were purchased from Sigma Chemical Co. Ltd. All other chemicals were of the highest grade available commercially.

Male Wistar rats, weighing 160-180 g, were injected ip. with phorone dissolved in corn oil at the doses indicated in Fig. and Tables. In some experiments, rats were injected ip. with BSO (4 mmol/kg) dissolved in distilled water (pH 8.5). The animals were killed at appropriate time periods as indicated in Fig. and Tables. The animals were fasted for 48 hr before being killed. The livers were perfused *in situ* with 0.9% NaCl solution, excised, and homogenized with 0.05M phosphate buffer (pH 7.2). The homogenate was centrifuged at 9,000 g for 20 min, and the resulting supernatant fraction was ultracentrifuged at 105,000 g for 60 min. The microsomal pellet was washed once, resuspended in a phosphate buffer and used for the determining cytochrome P-450 content, aminopyrine demethylase and heme oxygenase activities. The final supernatant fraction obtained from the control rats was saved and used as a source of biliverdin reductase for the assay of heme oxygenase activity. A portion of the liver was homogenized by 10 vol of 5% TCA containing 1mM EDTA. The homogenate was centrifuged at 2,000 g and the resulting supernatant was used for the determination of GSH content. Another portion of the liver was homogenized with 4 vol of 0.9% NaCl containing 10mM Tris-HCl buffer and 0.5mM EDTA for the assay of δ-aminolevulinic acid (ALA) synthetase activity.

Microsomal cytochrome P-450 content, aminopyrine demethylase and heme oxygenase activities were measured by the methods of Omura and Sato (11), Cochin and Axelrod (12) and Tenhunen *et al.* (13), respectively. Mitochondrial ALA synthetase activity was determined by the method of Marver *et al.* (14), by using the total homogenate as the enzyme source. Hepatic GSH content was measured by the method of Ellman (15) as described by Costa and Murphy (16). Microsomal protein concentration was assayed by the method of Lowry *et al.* (17).

RESULTS AND DISCUSSION

Time course studies on the effects of phorone (250 mg/kg) on hepatic GSH content, heme oxygenase and ALA synthetase

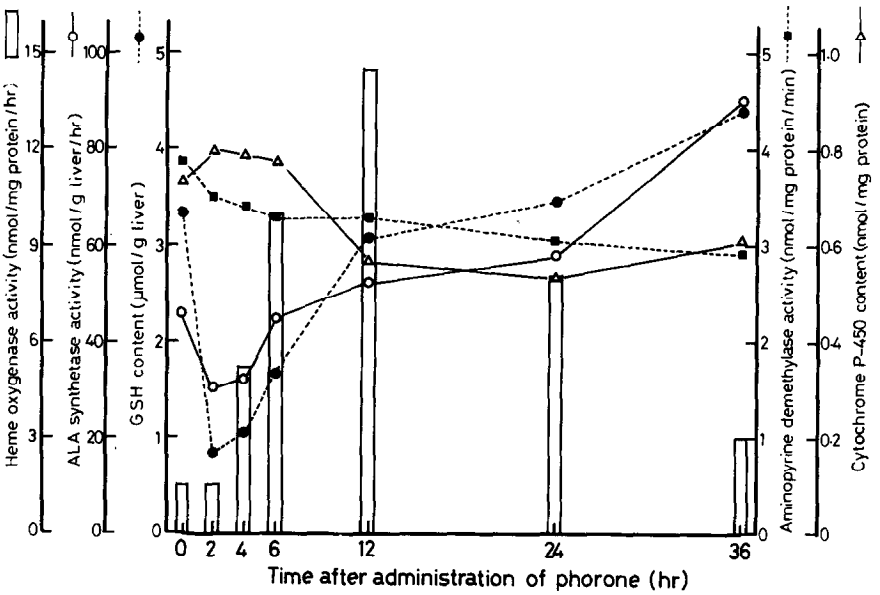

Fig. 1. Time Course of the Effects of Phorone on hepatic GSH Content, Heme Oxygenase and ALA Synthetase Activities, Cytochrome P-450 Content and Aminopyrine Demethylase Activity in Rats: Rats were injected ip. with Phorone (250 mg/kg) and killed at the times indicated. Values are the mean of three to five rats.

activities, cytochrome P-450 content and aminopyrine demethylase activity are shown in Fig. 1. The dosage of phorone used in this experiment was chosen according to the published data as described by Van Doorn et al. (1). As shown in Fig. 1, phorone produced a significant decrease in hepatic GSH content soon after its administration as has already been reported by Van Doorn et al. (1). Of interest was a remarkable increase in microsomal heme oxygenase activity due to the administration of phorone. An increase in heme oxygenase activity began within 4 hr after the administration of phorone, reached its peak (10-fold of the controls) at 12 hr and returned nearly to the control levels by 36 hr. In contrast, mitochondrial ALA synthetase activity declined somewhat at early time periods, remained unchanged within 24 hr and increased at 36 hr after the administration of phorone. Additionally, microsomal cytochrome P-450 content and aminopyrine demethylase activity reduced to lesser extents under

Table I. Effects of Actinomycin D and Cycloheximide on Phorone-induced Increase of Heme Oxygenase Activities

Treatment	Heme oxygenase (nmol/mg protein/hr)
Control	1.28 ± 0.09
Phorone (250 mg/kg, ip.)	13.2 ± 0.99
Actinomycin D + Phorone	2.32 ± 0.54
Cycloheximide + Phorone	2.61 ± 0.79

Rats were pretreated with either actinomycin D (2 mg/kg, ip.) cycloheximide (5 mg/kg, ip.) 1 hr prior to the administration of phorone (250 mg/kg, ip.) and killed 12 hr later.
Each value is the mean ± S.E.M. for three rats.

the experimental conditions. The changing patterns of these enzyme activities evoked by phorone were almost similar to those of diethyl maleate (18 and our unpublished results). The inverse relationship between the increase in heme oxygenase activity and the decrease in cytochrome P-450 content, as well as drug-metabolizing enzyme activities, has been well documented by the administrations of various agents including metal ions, hepatotoxins and interferon inducers (for reviews, see 18-20). In this respect, the findings that phorone, like diethyl maleate (18 and our unpublished results), produced a marked increase in heme oxygenase activity without showing the profound decreases in drug-metabolizing enzymes, suggest that GSH depleting agents may bring about the changes in these enzyme activities in a somewhat different manner.

As shown in Table I, pretreatment of rats with either actinomycin D or cycloheximide resulted in an almost complete inhibition of a phorone-mediated increase in heme oxygenase activity. The results suggest that phorone is able to produce an increased synthesis of mRNA for heme oxygenase and its subsequent translation into the enzyme protein. Thus, it would be reasonable

Table II. Dose-related Effects of Phorone on Hepatic GSH Contents and Enzymes involved in Drug and Heme Metabolism

Treatment	GSH content (μmol/g liver)	Heme oxygenase (nmol/mg protein/hr)	ALA synthetase (nmol/g liver/hr)	P-450 content (nmol/mg protein)	Aminopyrine demethylase (nmol/mg protein/min)
Control	3.86 ± 0.76	1.66 ± 0.31	33.21 ± 6.14	0.68 ± 0.07	5.12 ± 0.71
Phorone (mg/kg)					
62.5	4.56 ± 0.65	3.06 ± 0.53	34.34 ± 2.72	0.67 ± 0.06	4.69 ± 0.36
125	4.05 ± 0.41	6.88 ± 0.28	38.62 ± 3.6	0.62 ± 0.04	4.69 ± 0.49
250	2.48 ± 0.19	15.51 ± 2.49	35.1 ± 2.08	0.47 ± 0.01	3.93 ± 0.25
500	1.72 ± 0.18	18.47 ± 3.73	26.79 ± 6.15	0.57 ± 0.01	3.55 ± 0.73

Rats were injected ip. with phorone at the doses indicated and killed 12 hr later. The values are the mean ± S.E.M. for three rats.

to state that phorone is an inducer of heme oxygenase of rat liver.

The dose-related effects of phorone on hepatic GSH content, heme oxygenase, ALA synthetase and drug-metabolizing enzymes are represented in Table II. Phorone produced the changes in these parameters in a dose dependent manner. Phorone was able to induce heme oxygenase at a dose of 62.5 mg/kg but failed to reduce GSH content. The highest dose of phorone (500 mg/kg) markedly reduced GSH content to 44 % of the controls and increased heme oxygenase activity to 13-fold of the controls. Even at the highest dose of phorone, the magnitudes of the decreases in cytochrome P-450 content and aminopyrine demethylase activity were less extensive. Therefore, phorone, when selected as a GSH depleting agent, may interact weakly with concomitantly administered drugs or chemicals with respect to their oxidative metabolism by cytochrome P-450 containing drug-metabolizing enzymes. However, the potent inducing effect of phorone on heme oxygenase may bring about a possible perturbation of heme metabolism in the liver; therefore the evaluation of the experimental results should be made with considerable care.

Table III. Effects of Phorone and Buthionine Sulfoximine on Hepatic GSH Contents and Enzymes Involved in Drug and Heme Metabolism

Treatment	GSH content (μmol/g liver)	Heme oxygenase (nmol/mg protein/hr)	P-450 content (nmol/mg protein)	Aminopyrine demethylase (nmol/mg protein/min)
Control	3.35 ± 0.45	1.66 ± 0.28	0.73 ± 0.08	3.87 ± 0.58
Phorone	1.68 ± 0.23	14.52 ± 1.06	0.80 ± 0.06	3.34 ± 0.31
Buthionine Sulfoximine	0.79 ± 0.06	1.79 ± 0.05	0.81 ± 0.04	3.69 ± 0.14

Rats were injected with phorone (250 mg/kg, ip.) or buthionine sulfoximine (4 mmol/kg, ip.) and killed 6 hr later.

Finally, to examine whether the observed effects of phorone are related to its GSH depleting effect, comparisons were made between the effects of phorone and BSO, a depletor of GSH by inhibition of biosynthesis at the step of γ-glutamylcysteine synthetase (21-23), on these hepatic enzymes. As shown in Table III, though BSO profoundly reduced hepatic GSH content, the agent did not produce any effects on other parameters examined, such as heme oxygenase activity and cytochrome P-450 content. The lack of the effects of BSO on drug-metabolizing enzymes in the liver of mice has been already reported by Drew and Miners (23). The present study, therefore, adds a lack of effect of BSO on hepatic heme metabolism as well as drug metabolism in rats. The results shown in Table III also suggest that the inducing effect of phorone on heme oxygenase is not simply due to a consequence of the depletion of GSH. Rather, phorone could produce a specific effect on the machinery of heme oxygenase synthesis.

The present findings with phorone, together with diethyl maleate (18 and our unpublished results), suggest that the inducibility of hepatic heme oxygenase is extremely sensitive to α, β-unsaturated carbonyl compounds.

In conclusion, this study would add new insight into the effect of phorone on hepatic heme metabolism.

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