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ENALAPRIL HEPATOTOXICITY IN THE RAT

EFFECTS OF MODULATORS OF CYTOCHROME P450 AND GLUTATHIONE

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Abstract—The effects of modulators of cytochrome P450 and reduced glutathione (GSH) on the hepatotoxicity of enalapril maleate (EN) were investigated in Fischer 344 rats. Twenty-four hours following the administration of EN (1.5 to 1.8 g/kg), increased serum transaminases (ALT and AST) and hepatic necrosis were observed. Pretreatment of the animals with pregnenolone-16α-carbonitrile, a selective inducer of the cytochrome P450IIIA gene subfamily, enhanced EN-induced hepatotoxicity, whereas pretreatment with the cytochrome P450 inhibitor, cobalt protoporphyrin, reduced the liver injury. Depletion of hepatic non-protein sulfhydryls (NPSHs), an indicator of GSH, by combined treatment with buthionine sulfoximine (BSO) and diethyl maleate (DEM) produced marked elevations in serum transaminases by 6 hr after EN treatment. Administered on its own, EN decreased hepatic NPSH content and when combined with the BSO/DEM pretreatment, the liver was nearly completely devoid of NPSHs. Protection from EN-induced hepatotoxicity was observed in animals administered L-2-oxothiazolidine-4-carboxylic acid, a cysteine precursor. Together, these observations suggest the involvement of cytochrome P450 in EN bioactivation and GSH in detoxification. The results corroborate previous *in vitro* observations pertaining to the mechanism of EN-induced cytotoxicity towards primary cultures of rat hepatocytes. Although the doses of EN used in this study were far in excess of therapeutic doses, under certain circumstances, this metabolism-mediated toxicologic mechanism could form the basis for idiosyncratic liver injury in patients receiving EN therapy.

The efficacy of enalapril maleate (EN)†, an angiotensin-converting enzyme inhibitor, in the treatment of hypertension and congestive heart disease has been well established [1]. EN can be considered a safe drug with relatively few side effects compared to other therapeutic agents indicated for these conditions. Dry cough is the most common adverse effect from chronic use, with an incidence as high as 10% [2]. Other chronic toxicities, such as neutropenia, taste disturbance, rash, proteinuria, bilateral renal artery stenosis and angioedema are rare [1]. We have focused our interest towards EN-associated hepatotoxicity which has been described in several case reports [3–8] and is also a rare but potentially serious adverse effect.

In previous studies using primary cultures of rat hepatocytes, we observed cytotoxicity at EN concentrations of 0.5 mM or higher [9]. Inhibitors of cytochrome P450, SKF 525-A, α-naphthoflavone and metyrapone, added *in vitro*, diminished the cytotoxic response. *In vivo* induction with pregnenolone-16α-carbonitrile (PCN) dramatically enhanced the cytotoxicity whereas phenobarbital and β-naphthoflavone had minimal effects. Depletion of hepatocellular glutathione (GSH) by *in vitro* pretreatment with buthionine sulfoximine (BSO) or diethyl maleate (DEM) augmented the EN-induced cytotoxicity while addition of *N*-acetylcysteine provided protection [10]. Together these results suggested that EN in rat hepatocytes may form a reactive metabolite by a PCN-inducible cytochrome P450 (CYP3A gene subfamily) [11] and that detoxification is by a GSH-dependent pathway. To date, the metabolism of EN is known to involve ester hydrolysis to the active compound enalaprilat which is excreted into the urine (Fig. 1) [12, 13]. No additional metabolites have been reported. In our previous investigations, enalaprilat was not cytotoxic towards rat hepatocytes [9]. In the present studies, we undertook to investigate in rats the hepatotoxicity of EN *in vivo*, to determine whether the *in vitro* results can be corroborated based on a hypothesis of cytochrome P450-mediated bioactivation and GSH-dependent detoxification.

MATERIALS AND METHODS

Animals. Male Fischer rats (175–200 g, Taconic Farms Inc., Germantown, NY) were cared for in accordance with the principles contained in the *Guide to the Care and Use of Experimental Animals*

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† Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSO, buthionine sulfoximine; CPP, cobalt protoporphyrin; DEM, diethyl maleate; DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid); EN, enalapril maleate; GSH, reduced glutathione; LDH, lactate dehydrogenase; NPSH, non-protein sulfhydryl; OTC, oxothiazolidine-4-carboxylate; PCN, pregnenolone-16α-carbonitrile; and SKF525-A, 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride.

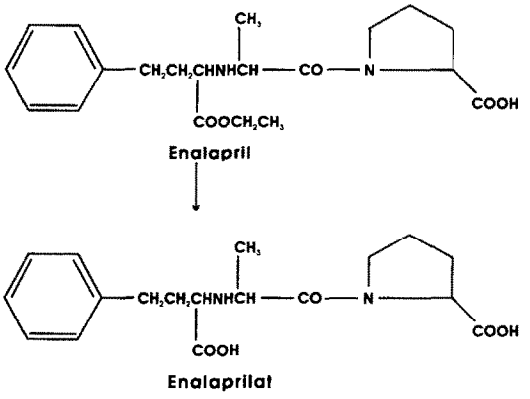

Fig. 1. Structural formulae of enalapril and its known metabolite, enalaprilat.

as prepared by the Canadian Council on Animal Care. Standard laboratory chow and tap water were provided *ad lib*.

Chemicals. EN was a gift from Merck Frosst Canada Inc. (Pointe-Claire, Quebec). GSH, DEM (98%), DL-buthionine-*S,R*-sulfoximine (BSO), L-2-oxothiazolidine-4-carboxylic acid (OTC), PCN, cobaltic protoporphyrin IX chloride (CPP), and 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) were obtained from the Sigma Chemical Co. (St. Louis, MO). Reagents for the determination of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were purchased from Abbott Laboratories (Irving, TX).

Pregnenolone-16α-carbonitrile study. PCN was prepared as an aqueous suspension containing 0.3% gum tragacanth. Groups of six rats were treated with PCN (100 mg/kg, p.o.) or an equivalent volume of vehicle (0.5 mL/100 g body weight) on 4 consecutive days. Twenty-four hours after the last dose, EN (1.5 g/kg of a 150 mg/mL solution prepared by dissolution in a small volume of 0.1 M NaOH, adjusting the pH to 7.0 and bringing the solution to final volume with normal saline) or an equivalent volume of normal saline was administered i.p. Twenty-four hours after the administration of EN or saline, rats were anaesthetized with sodium pentobarbital, blood was collected from the abdominal aorta, and the livers were excised, rinsed in saline and weighed. A section of the liver was fixed in 5% Formalin in 0.15 M phosphate-buffered saline (pH 7.2), processed by standard histological techniques, and examined by light microscopy by a pathologist who was unaware of the drug treatment.

Cobalt protoporphyrin study. CPP solution was prepared and administered to rats as described by Spaethe and Jollow [14]: CPP was dissolved in a small volume of 0.1 M NaOH and normal saline used to bring the solution to final volume. To groups of six rats, the CPP solution was administered (0.5 mL/100 g body weight) immediately after preparation at a dose of 59 mg/kg, s.c. Control animals received an equivalent volume of saline. Four days later, EN (1.8 g/kg of a 180 mg/mL solution) or an equivalent volume of normal saline was administered i.p. Twenty-four hours later, samples of blood and liver were collected as described for the PCN study.

L-2-Oxothiazolidine-4-carboxylate study. OTC solution was prepared as described for EN, and administered to groups of six rats (1 mL/100 g body weight) at a dose of 400 mg/kg, i.p. Control rats received an equivalent volume of normal saline. One hour later, EN (1.8 g/kg of a 180 mg/mL solution) or an equivalent volume of saline was injected i.p. Two hours after the EN injection, a second dose of OTC (400 mg/kg, i.p.) or saline was administered. Twenty-four hours after the administration of EN, samples of blood and liver were collected as described for the PCN study. In another study, groups of four to six rats received either OTC and EN or OTC and saline, as described above, and were killed at 6 hr after receiving EN or saline. A sample of liver was obtained for determination of non-protein sulfhydryl (NPSH) concentration.

NPSH depletion study. Groups of six to seven rats were treated with BSO (890 mg/kg, i.p.) coadministered with DEM (537 mg/kg, i.p.) in corn oil at 1 mL/100 g body weight. Control rats received an equivalent volume of corn oil. One hour later, EN (1.5 g/kg of a 150 mg/mL solution) or an equivalent volume of normal saline was administered i.p. Rats were killed 6 hr after receiving EN or saline. Blood and liver samples were collected as described for the PCN study. An additional sample of liver was collected for determination of NPSH content.

Determination of serum enzymes. AST and ALT were measured spectrophotometrically by the methods of Karmen [15], and Wróblewski and LaDue [16], respectively, using a biochromatic autoanalyzer (Abbott Laboratories, Irving, TX).

Determination of hepatic NPSH. A sample (approximately 1 g) of liver was homogenized in 4 vol. of ice-cold trichloroacetic acid containing 5 mM EDTA. The homogenate was centrifuged at 12,000 *g* for 20 min at 4°. Using the autoanalyzer, the supernatant was assayed for NPSH by the method of Ellman [17] which monitors the colorimetric reaction of sulfhydryl groups with DTNB. NPSH concentration was calculated from a standard curve of GSH since this is the predominant NPSH in the liver.

Table 1. Effect of EN dose on serum markers of liver injury

EN dose (g/kg, i.p.)	N*	ALT (U/mL)	AST (U/mL)
0	4	40.4 ± 1.3†	88.2 ± 7.7
0.3	4	41.9 ± 1.6	74.7 ± 6.3
1.2	2	105, 100	112, 134
1.5	2	269, 349	430, 418
1.8	3	365 ± 97	630 ± 100
2.0	4	299 ± 119	834 ± 172

* Number of rats.
† SEM is reported where N = 3–4.

Table 2. Time-course of elevation of serum markers of liver injury after EN

Time after EN* (hr)	N†	ALT (U/mL)	AST (U/mL)
0	4	45.1 ± 1.5‡	80.7 ± 2.6
2	2	44.9, 45.4	82.3, 75.3
4	2	31.0, 36.6	70.7, 75.1
6	4	40.8 ± 4.7	82.2 ± 4.8
8	2	44.6, 37.7	86.9, 81.0
24	4	69.7 ± 10.9	348 ± 87
48	4	94.2 ± 40.7	227 ± 82

* Rats were administered 1.2 g/kg, i.p., of EN. Blood was collected from the tail vein.
† Number of rats.
‡ SEM is reported where N = 4.

Statistical analysis. Results are expressed as means ± SEM. Homogeneity of variance was tested using Bartlett's test. Log transformations were performed on data where the variance was heterogeneous. Analysis of variance (ANOVA) was applied using the least significant difference test for multiple comparisons between treatment groups. The criterion for significance was $P < 0.05$ for all comparisons.

RESULTS

A preliminary study was carried out to establish the dose-range for liver injury in rats caused by EN, as manifested by elevations in serum markers. There appeared to be a threshold of about 1.2 g/kg, i.p., below which no changes were observed in ALT and AST (Table 1) nor amongst a full battery of other biochemical parameters. A dose of 1.5 g/kg, i.p., was selected for the studies in which chemical modulators were expected to produce an enhancement of hepatotoxicity. For the studies in which chemical modulators were expected to have a protective effect, the EN dose was raised to 1.8 g/kg, i.p. At these doses, EN did not cause mortality of any animals during a 24-hr observation period. Results for a preliminary study with only a few animals suggested that ALT, AST and serum lactate dehydrogenase (LDH) activities increased in response to EN administration. Serum alkaline phosphatase and γ-glutamyltransferase activities were not affected. For the actual studies, total bilirubin concentration (although not identified in preliminary studies as possibly affected), and ALT, AST and LDH activities were measured in all animals. However, in the final data analyses, there were no significant effects on total bilirubin concentration and LDH activity by any of the drug

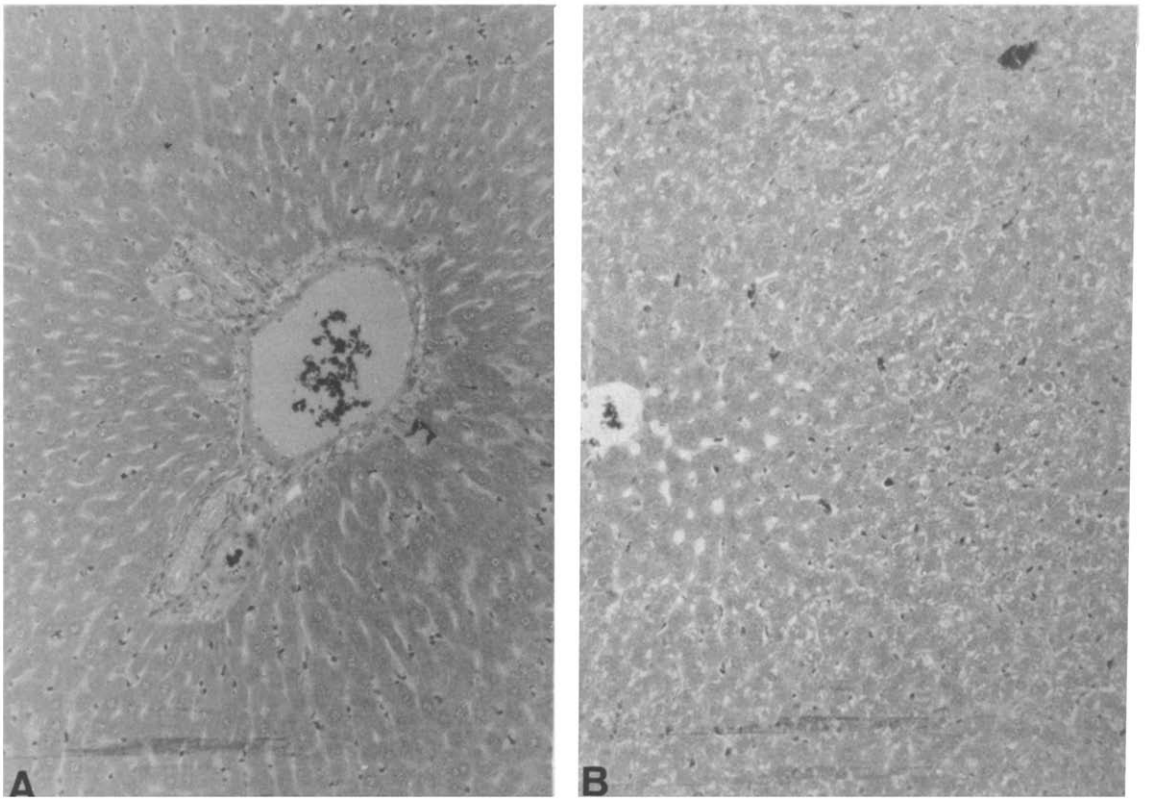

Fig. 2. EN-induced hepatic necrosis in rats. Liver tissue from animals treated with saline (A) or EN (1.5 g/kg, i.p.) (B). The animals were killed 24 hr after treatment. Stained with hemotoxylin and eosin, medium magnification.

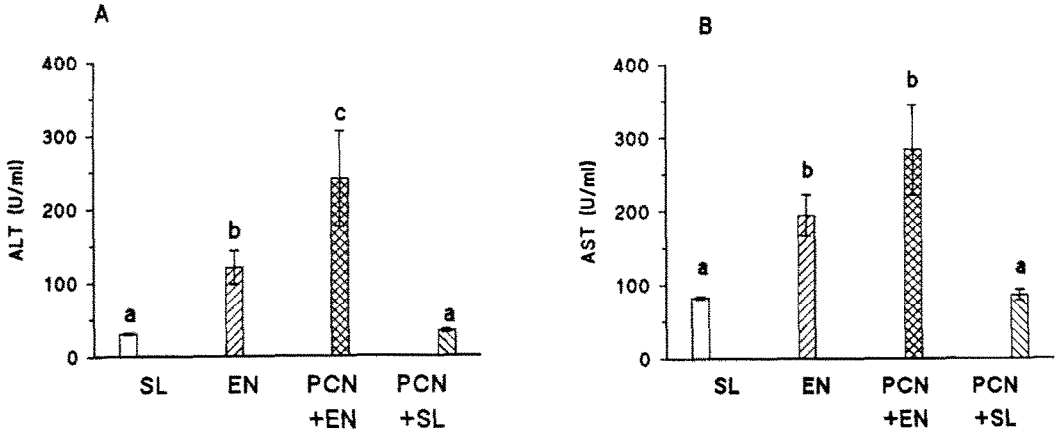

Fig. 3. Effect of PCN induction on ALT (A) and AST (B) activities 24 hr after EN treatment. Rats were pretreated with PCN (100 mg/kg, p.o., for 4 days) or vehicle, and received either EN (1.5 g/kg, i.p.) or saline (SL) 24 hr after the last dose of PCN. Values are means ± SEM, N = 6. Treatment groups that did not differ significantly ($P < 0.05$) in a multiple-comparison test using ANOVA have the same letter.

or chemical treatments and, therefore, these results are not shown.

Another preliminary study examined the time–course of elevation of serum liver enzymes after EN administration (Table 2). The data indicated that ALT and AST activities peaked at about 24 hr and remained elevated up to 48 hr. Therefore, 24 hr was selected as the endpoint for subsequent studies. Also, this duration was equivalent to the incubation time in previous *in vitro* investigations of EN cytotoxicity using primary cultures of rat hepatocytes [9, 10].

Figure 2 illustrates microscopically the effect of EN on liver morphology. Liver from saline-treated control rats (Fig. 2A) exhibited normal lobular architecture and cell morphology. At an EN dose of 1.5 g/kg, i.p., the liver demonstrated severe necrosis in Zone 3 (centrilobular region), and degeneration and vacuolization in Zone 1 (periportal region) (Fig. 2B).

EN administration produced statistically significant, moderate increases in serum transaminases: 3- to 4-fold increases in ALT and greater than 2-fold increases in AST (Figs. 3–6). EN treatment also produced a significant 10—17% reduction in liver weight (expressed as a percentage of body weight prior to EN administration) by 24 hr (data not shown). The various chemical modulators, except for PCN (see below), did not affect this EN-induced reduction in liver weight.

Pregnenolone-16α-carbonitrile study. Figure 3 illustrates the enhancement of EN hepatotoxicity by induction with PCN. Given alone, PCN did not alter ALT and AST levels significantly. After PCN induction, however, ALT activity in response to EN was increased a further 2-fold compared to EN administration alone. AST activity was further elevated by approximately 1.5-fold but, because of the large standard deviation, the average value was not significantly different from the average value for EN treatment alone.

PCN, administered on its own, caused a significant (21 ± 2%) increase in liver weight which was associated with its well-known enzyme-inducing effect. When EN was administered to PCN-pretreated rats, the 24-hr liver weight was not significantly different from controls (data not shown), since the weight increase produced by PCN compensated for the weight decrease produced by EN.

Cobalt protoporphyrin study. When administered alone, CPP did not alter serum aminotransferase activities significantly. However, it provided protection against EN-induced liver injury as manifested by a 50% reduction in the degree of ALT elevation by EN alone, and a 39% reduction in AST elevation (Fig. 4). These serum parameters in CPP-pretreated animals were not significantly different after EN administration from values in saline-treated controls.

L-2-Oxothiazolidine-4-carboxylate study. OTC treatment on its own did not have any significant effects on ALT and AST activities. In a preliminary experiment, a single dose (400 mg/kg, i.p.) of OTC was found to be insufficient to significantly protect against elevations of serum transaminases by EN. However, when administered at a dose of 400 mg/kg 1 hr prior to, and 400 mg/kg again 2 hr after EN treatment, it reduced the EN-induced elevation of ALT significantly by 44% and the elevation of AST by 34%, although the latter parameter did not attain statistical significance (Fig. 5). Liver NPSH content, measured 7 hr after the first dose of OTC, was elevated significantly compared to control levels. OTC diminished the reduction in liver NPSHs observed at 6 hr after EN administration although the differences between the OTC plus EN group and the saline control group or the EN group were not statistically significant (Table 3).

NPSH depletion study. In a preliminary experiment, the hepatic NPSH content 1 hr after the administration of BSO alone, DEM alone, and BSO in combination with DEM was determined to be

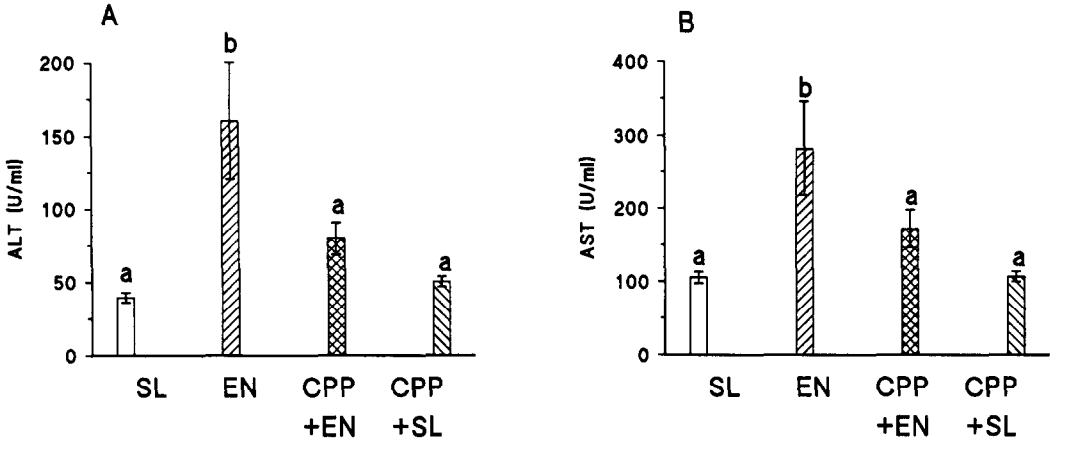

Fig. 4. Effect of CPP on ALT (A) and AST (B) activities 24 hr after EN treatment. Rats were pretreated with CPP (59 mg/kg, s.c.) or saline (SL) 4 days before receiving either EN (1.8 g/kg, i.p.) or SL. Values are means ± SEM, N = 6. Treatment groups that did not differ significantly ($P < 0.05$) in a multiple-comparison test using ANOVA have the same letter.

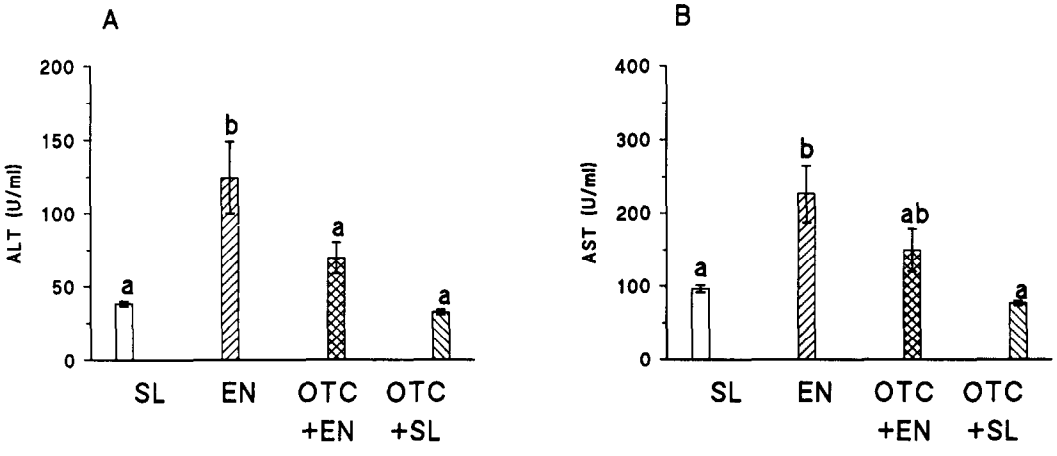

Fig. 5. Effect of OTC on ALT (A) and AST (B) activities 24 hr after EN treatment. Rats were administered OTC (400 mg/kg, i.p.) or saline (SL) 1 hr before and again 2 hr after receiving either EN (1.8 g/kg, i.p.) or SL. Values are means ± SEM, N = 6. Treatment groups that did not differ significantly ($P < 0.05$) in a multiple-comparison test using ANOVA have the same letter.

approximately 80, 15 and 10%, respectively, of normal values. Since the time–course of hepatic NPSH status in relation to EN hepatotoxicity was unknown, it was decided that the combined BSO/DEM treatment would ensure the most extensive and longest duration of NPSH depletion [18]. In preliminary experiments, a 1.5 g/kg, i.p., dose of EN, which is not lethal on its own, caused mortality within 24 hr to more than 50% of the rats that had been pretreated with BSO (890 mg/kg, i.p.) combined with DEM (537 mg/kg, i.p.). Therefore, to avoid mortality and to reduce the suffering of the animals, the effect of EN on the liver in NPSH-depleted rats was examined at 6 hr. The BSO/DEM pretreatment on its own did not affect ALT and AST activities. However, it enhanced liver toxicity induced by EN, as manifested by a greater than 6-fold elevation in ALT and a greater than 5-fold elevation in AST compared to EN treatment alone (Fig. 6). Six hours after administration, EN produced a slight (23%) but statistically significant decline in hepatic NPSH concentration (Table 3). When EN was administered after BSO/DEM pretreatment, the liver was nearly completely depleted of NPSHs.

DISCUSSION

In the present study, EN caused liver damage as assessed by elevation in serum transaminase (ALT and AST) activities. Histopathological examination

Table 3. Effects of BSO/DEM and OTC treatments on hepatic NPSH concentration 6 hr after EN administration

Treatment*	Hepatic NPSH (Mm)	% SL control
SL	5.33 ± 0.30	100
EN	4.10 ± 0.37†	77
BSO/DEM + SL	0.82 ± 0.01‡	15
BSO/DEM + EN	0.39 ± 0.05§	7
OTC + SL	10.82 ± 0.71‖	203
OTC + EN	4.69 ± 0.23	88

* Rats were treated with BSO (890 mg/kg, i.p.) plus DEM (537 mg/kg, i.p.) or OTC (400 mg/kg, i.p.) or corn oil vehicle 1 hr prior to either EN (1.5 g/kg, i.p.) or SL vehicle. A second dose of OTC (400 mg/kg i.p.) was administered 2 hr after EN treatment. Hepatic NPSH concentration was measured 6 hr after EN treatment. Values are means ± SEM, N = 4–7.

† Significantly different from SL, BSO/DEM + SL, and BSO/DEM + EN groups, $P < 0.05$.

‡ Significantly different from SL, EN, and BSO/DEM + EN groups, $P < 0.05$.

§ Significantly different from SL, EN, and BSO/DEM + SL groups, $P < 0.05$.

‖ Significantly different from SL, EN, and OTC + EN groups, $P < 0.05$.

confirmed the liver injury and indicated that it was primarily a tissue necrosis with predominantly Zone 3 (centrilobular region) affected. The predominantly Zone 3 necrosis which is seen with bromobenzene hepatotoxicity has been attributed to a greater concentration of cytochrome P450 and a lower concentration of GSH in this region compared to Zone 1 (periportal region) [19]. Therefore, it was postulated that in Zone 3 there is more bioactivation and less detoxification by GSH. An analogous scenario can be envisioned to account for the pattern of EN-induced necrosis within the rat liver. The decrease in liver weight after EN administration was likely related to necrosis.

The doses of EN used in this study were obviously very high. However, at these doses liver injury appeared to be a primary toxic event. Gross pathological examination of the animals did not reveal any other apparent organ or system toxicities other than severe hypotension (observational) before sacrifice, which was associated with a dose-related pharmacological effect of the drug. Functional changes may have occurred in other organs, such as the kidneys, as a result of the altered hemodynamics, but we did not investigate this possibility further beyond determining from preliminary experiments that standard clinical biochemical parameters, including creatinine and blood urea nitrogen (BUN) were unaltered. This acute single-dose study was designed to facilitate investigation of the mechanism of EN hepatotoxicity using compounds to induce or inhibit cytochrome P450, and deplete or enhance hepatic GSH. Whether hepatotoxicity can be demonstrated in rats in a chronic dosing study using lower doses of EN remains to be determined.

PCN is a selective inducer of the cytochrome P450IIIA gene subfamily [11]. The increased hepatotoxicity of EN in rats pretreated with PCN (Fig. 3) suggests that a cytochrome P450IIIA isozyme(s) may be involved in the metabolic activation of EN to a toxic metabolite(s). These results are in agreement with our previous *in vitro* findings of enhanced EN cytotoxicity towards hepatocytes isolated from PCN-induced rats compared to control rats [9]. The *in vitro* investigations demonstrated minor effects of phenobarbital and β-naphthoflavone pretreatments, which induce the

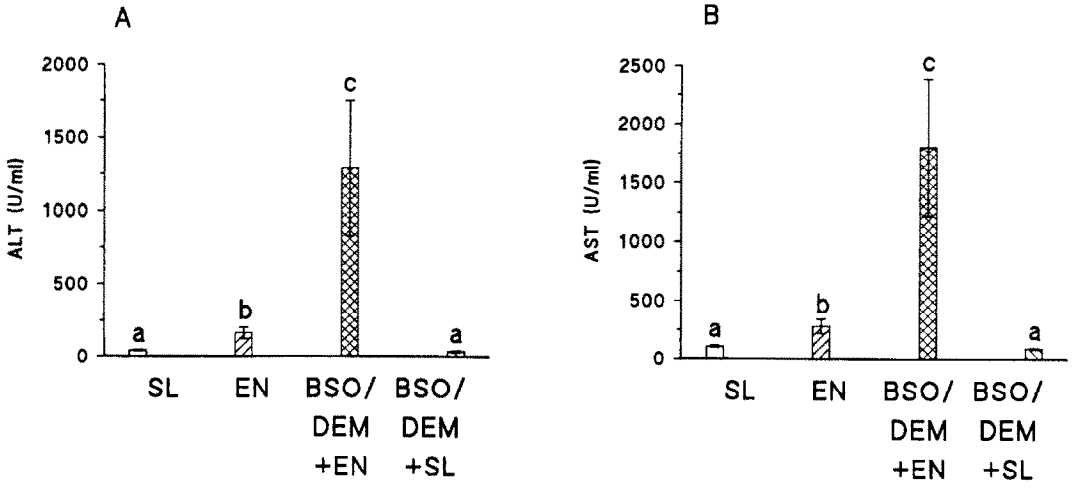

Fig. 6. Effect of combined BSO/DEM pretreatment on ALT (A) and AST (B) activities 6 hr after EN treatment. Rats were pretreated with BSO (890 mg/kg, i.p.) combined with DEM (537 mg/kg, i.p.) or corn oil vehicle 1 hr before receiving either EN (1.5 g/kg, i.p.) or saline (SL). Values are means ± SEM, N = 6–7. Treatment groups that did not differ significantly ($P < 0.05$) in a multiple-comparison test using ANOVA have the same letter.

P450IIB and P450IA gene subfamilies, respectively [20], on EN cytotoxicity. The possible contribution of these and other P450 subfamilies towards EN hepatotoxicity in rats *in vivo* remains to be determined.

The nearly complete inhibition of the effect of EN on serum transaminases by CPP (Fig. 4) indicated that oxidative metabolism by cytochrome P450 was involved in the hepatotoxicity of EN. A single dose of CPP produces a rapid, dramatic (>80% depletion) and long-lasting (10 days or more) fall in hepatic cytochrome P450 [14]. Unlike cobalt chloride which enhances hepatic GSH [21] or SKF 525-A which depletes GSH [22], CPP does not affect hepatic GSH levels or phase II metabolizing enzymes [14]. CPP also appears to be less toxic than either of these other cytochrome P450 inhibitors [14, 22].

In this study, OTC reduced the hepatotoxicity of EN (Fig. 5) which supports our previous finding of a protective effect of *N*-acetylcysteine towards EN-induced cytotoxicity in primary cultures of rat hepatocytes [10]. Both OTC and *N*-acetylcysteine increase the hepatic pool of GSH by supplying L-cysteine, the limiting amino acid required for GSH biosynthesis [23]. OTC is converted by the enzyme 5-oxoprolinase to L-cysteine [24]. The BSO/DEM study confirmed the importance of GSH in preventing the development of EN-induced liver toxicity. BSO is an inhibitor of γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH biosynthesis, while DEM depletes hepatic GSH by conjugation to the sulfhydryl moiety [23]. EN produced a decrease in hepatic NPSH content (Table 3) which in livers drastically depleted of NPSHs by the combined treatment with BSO and DEM, resulted in severe injury (Fig. 6). OTC administration enhanced liver NPSH content and lessened the degree of EN-induced reduction in NPSHs (Table 3). Together these results indicate a role for GSH in the detoxification of EN, possibly by conjugating with a reactive metabolite formed by cytochrome P450. Alternatively, it is possible that reactive oxygen species may be formed during cytochrome P450-mediated metabolism of EN, and GSH is utilized for their reduction.

The first angiotensin-converting enzyme inhibitor to be marketed, captopril, has also been associated with rare incidence of hepatotoxicity [25, 26]. Helliwell *et al.* [27] demonstrated in mice, moderate increases in ALT, decreases in hepatic GSH and histological evidence of hepatic necrosis 24 hr after i.p. administration of 200–300 mg/kg of captopril. These doses also exceeded by far the usual therapeutic doses (1–6 mg/kg). The formation of GSH conjugates of captopril does not involve intermediary metabolism by cytochrome P450. Rather, the parent compound forms mixed disulfide conjugates with GSH, cysteine or proteins [28]. Captopril–plasma protein conjugates were found to have immunogenic potential [29], and captopril-specific antibodies have been detected in serum for some patients receiving the drug [30]. Unlike captopril, EN and its deesterified metabolite, enalaprilat, did not directly form GSH conjugates (unpublished observation). Based on the results of this study and our previous *in vitro* investigations, we postulate that a reactive metabolite(s) of EN is detoxified by conjugation with GSH. The detection and identification of such a metabolite(s) remain to be determined.

Although it is extremely unlikely that EN at therapeutic doses (10–40 mg/day) would lower hepatic GSH sufficiently to cause liver damage, the concurrent administration of other drugs which reduce GSH levels [31–33] may render the liver more susceptible to EN-induced injury. Patients with GSH synthetase deficiency [23] may be at greater risk of developing hepatotoxicity in a situation in which the detoxification capacity of the liver is challenged by drugs which are conjugated with GSH or which form metabolites which are conjugated with GSH. Additionally, if such patients have induced levels of cytochrome P450, for example, from therapy with macrolide antibiotics which selectively induce P450IIIA isozymes [20], the enhancement of the postulated EN bioactivation pathway may increase the risk of hepatotoxicity. Considering that EN concentrations in the liver may approach 1 mM (0.5 g/L) in patients with liver dysfunction [34], one can envision a scenario in which idiosyncratic hepatotoxicity can occur at therapeutic doses of EN by the mechanisms implicated in this study. Furthermore, it is conceivable that EN through bioactivation to a reactive metabolite, could act as a hapten and produce immunogenic effects, as have been described for captopril. Since, as of 1989, EN ranked amongst the top twelve most prescribed drugs in the United States [35], the number of accidental or deliberate overdoses can be expected to increase. Acute toxicity, in addition to the exaggerated pharmacological effects of the drug, may include hepatotoxicity.

In summary, EN administration to rats caused liver injury by a mechanism which appeared to require cytochrome P450 for bioactivation and which depended on GSH for detoxification. On a mechanistic basis, these results support our previous *in vitro* findings with primary cultures of rat hepatocytes.

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