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Suppression of Rat Hepatic Cytochrome P450 2E1 Expression by Isopropyl 2-(1,3-dithioetane-2-ylidene)-2-[N-(4-methyl-thiazol-2-yl)carbamoyl]acetate (YH439), an Experimental Hepatoprotectant: Protective Role Against Hepatic Injury

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ABSTRACT. The expression of cytochromes P450 2E1, P450 2B and P450 1A was examined in rat hepatic tissue in response to YH439, an experimental hepatoprotective agent. P450 2E1 metabolic activities relatively specific for P450 2E1 were decreased up to 57% of control activities in the hepatic microsomes prepared from rats treated with YH439 for 3 days. Immunoblot analyses showed that P450 2E1 levels were decreased below the limit of detectability in hepatic microsomes prepared from YH439-treated rats. YH439 at doses from 25 to 100 mg/kg completely suppressed isoniazid-inducible P450 2E1 levels as monitored by both metabolic activities and immunoblot analysis. RNA hybridization analysis revealed that P450 2E1 mRNA levels failed to change after YH439 treatment. These results demonstrate the YH439 effectively suppresses P450 2E1 expression in the absence of transcriptional inactivation. YH439 failed to affect P450 2B1/2 expression, whereas this agent enhanced the hepatic P450 1A1/2 levels. The hepatoprotective effects of YH439 were also examined. Animals treated with CCl_4 and ethanol for 9 weeks showed hepatic injury as demonstrated by 2.5- and 2-fold increases in serum alanine aminotransferase and alkaline phosphatase activities, respectively. Concomitant YH439 treatment resulted in a significant protective effect against the experimental hepatic injury. The toxicant-induced elevation in hepatic hydroxyproline level was completely blocked by YH439 treatment. These data indicate that YH439 suppresses the expression of P450 2E1 and protects the liver against chemical-induced hepatic injury and that the selective modulation of detoxifying enzymes by YH439 may contribute to the protection of liver from xenobiotic-induced intoxication. BIOCHEM PHARMACOL 52;8:1219–1225, 1996.

KEY WORDS. cytochrome P450 2E1; P450 2B; P450 1A1; YH439; hepatoprotective agent; enzyme suppression

P450 2E1,¶ an ethanol-inducible form of cytochrome P450, is active in the metabolism of both endogenous substances and small organic molecules including alcohols, acetone, acetaminophen, carbon tetrachloride, N-nitrosodimethylamine and benzene [1]. P450 2E1 has been implicated in the intoxication of patients by ethanol and isoniazid [2–4], and the metabolism of carbon tetrachloride to a hepatotoxic intermediate appears to be associated in part with increased levels of P450 2E1 [5]. N-nitrosodimethylamine is a potent carcinogen and appears to require P450 2E1-catalyzed metabolic activation to exert its carcinogenic effect [6]. P450 2E1 has also been shown to be the high-affinity enzyme responsible for metabolic activation of benzene, a hematotoxic compound [7]. Induction of P450 2E1 would potentiate the toxicity of many xenobiotics via metabolic activation and/or accumulation of reactive metabolites. Because P450 2E1 is inducible by many structurally diverse compounds and certain pathophysiological conditions [1, 8], exposure to P450 2E1 inducers in combination with toxicants specifically metabolized by P450 2E1 would result in harmful effects to the organs, including the liver. Allylsulfide and a few other organosulfur compounds inhibit P450 2E1 catalytic activities and exert an inhibitory effect on the induction of colon and liver cancer initiated by

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¶ *Abbreviations:* ALT, alanine aminotransferase; ALP, alkaline phosphatase; 4-AP, 4-aminophenol; INH, isoniazid; EDTA, ethylenediamine tetraacetic acid; 3-MC, 3-methylcholanthrene; 4-NP, 4-nitrophenol; PB, phenobarbital; P450 2E1, cytochrome P450 2E1; P450 2B, cytochrome P450 2B; P450 1A1, cytochrome P450 1A1; SDS-PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis; SSPE, standard saline phosphate with EDTA; YH439, isopropyl 2-(1,3-dithioetane-2-ylidene)-2-[N-(4-methyl-thiazol-2-yl)carbamoyl]acetate.

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chemical carcinogens [9, 10]. In this regard, the identification and exploitation of agents that suppress the expression of P450 2E1 would substantially contribute to the protection of body organs from toxicants and, potentially, to the prevention of cancer in humans. In addition, it is generally accepted that induction of phase II detoxifying enzymes is associated with the inactivation of electrophiles or nucleophiles, thus representing potential chemoprotection. Induction of phase II enzymes is a critical and sufficient mechanism to engender protection against the toxic and carcinogenic actions of reactive intermediates [11–13].

YH439, which is an analogue of malotilate [14], is being investigated as a potential hepatoprotective agent for the treatment of patients with hepatitis, compensated liver cirrhosis and chemical-induced liver injury. This compound is currently being evaluated in phase I clinical trials. It has also been shown that pretreatment of animals with YH439 enhances the excretion of acetaminophen through glucuronide conjugation [15], which may in part represent the role of detoxification by YH439.

A previous study in our laboratories showed that malotilate, an anticirrhotic agent, suppresses hepatic P450 2E1 expression in rats [14]. In this research, we were interested in studying the effects of this agent on the expression of major drug metabolizing enzymes, which potentially modulate oxidative and conjugative metabolism and the interaction with electrophiles. The protein and mRNA levels of P450 2E1 were assessed by metabolic activities, immunoblot and RNA blot analyses. This study was designed to characterize the expression of major metabolizing enzymes in response to YH439 in association with hepatoprotective effects against toxicant-induced liver injury. YH439 turned out to be an efficacious agent in suppressing hepatic P450 2E1 expression. The present study aims to elucidate the mechanism of action and potential application of YH439 as a hepatoprotective agent.

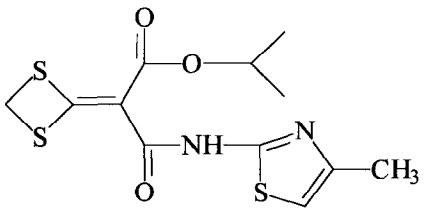
MATERIALS AND METHODS

Materials

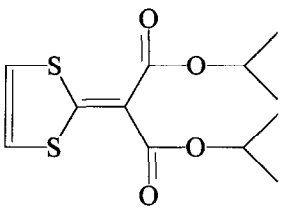
p-Nitrophenol, 4-nitrocatechol and other reagents used in these studies were obtained from Sigma Chemical Co. (St. Louis, MO, USA). γ-[^{32}P]ATP (>110 TBq/mmol) was obtained from Amersham (Arlington Heights, IL, USA). YH439 was synthesized at Yuhan Research Center Yuhan Corporation (Kunpo, Korea). The chemical structures of YH439 and malotilate are shown in Fig. 1.

Preparation of Microsomes

Male Sprague-Dawley rats were treated with either YH439 (25–100 mg/kg/day, p.o., 2–3 days), isoniazid (150 mg/kg/day, p.o., 3 days), or PB (100 mg/kg/day, i.p., 3 days). 3-MC-induced hepatic microsomes were prepared at 3 days after a single injection (25 mg/kg, i.p.). Animals were killed 24 hr after the last dose after having been fasted for 16 hr before killing. Microsomes were prepared by differential



YH439



Malotilate

FIG. 1. Chemical structures of YH439 and malotilate.

centrifugation and washed in pyrophosphate buffer and stored at −70°C until use. Protein was assayed by the method of Lowry *et al.* [16].

p-Nitrophenol Hydroxylase Assay

p-Nitrophenol hydroxylase activity was assayed as previously described [17].

Aniline Hydroxylase

Aniline hydroxylase was determined by measuring *p*-aminophenol formation as described by Brodie and Axelrod [18].

Western Blot Analysis

Microsomal proteins were separated by 7.5% SDS-PAGE according to Laemmli [19]. Western blot analysis was performed as previously described [20]. Microsomal proteins were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose paper, which was immunoblotted with antibodies specifically detecting P450 2E1 (provided by Dr. R. F. Novak, Wayne State University, MI, USA), P450 2B1/2 or P450 1A (provided by Dr. S. S. Park, NCI-Frederick Cancer Research, MD, USA).

Isolation of Total and Poly(A)$^+$ RNA

Total RNA was isolated by using the improved single-step method of thiocyanate-phenol-chloroform RNA extraction [21]. Poly(A)$^+$ RNA was isolated from the total RNA by using an oligo(dT)-cellulose column [22].

Oligonucleotides Probe Preparation

Antisense oligonucleotides for P450 2E1 (19-mer) were synthesized based on the published corresponding rat cDNA nucleotide sequences. The oligonucleotide sequence was P450 2E1, 5′-(CAAAGCCAACTGTGACAGG)-3′. The specificity of the probe used in this study was confirmed by Northern blot analysis, also as reported previously [13, 23].

RNA Slot Blot Hybridization

Poly(A)$^+$ RNA was serially diluted in 15 × SSC (1 × SSC: 150 mM NaCl and 15 mM sodium citrate) and applied to slots according to the manufacturer's protocols of the Schleicher & Schuell slot blot system (Minifold II). The nitrocellulose paper was baked in a vacuum oven at 80°C for 2 hr. The filter was prehybridized in a solution of 6 × SSPE (1 × SSPE: 0.15 M NaCl, 10 mM NaH_2PO_4 and 1 mM Na_2EDTA, pH 7.4) containing 0.1% sodium dodecylsulfate and 5 × Denhardt's solution without probe for 2 hr in a heat-sealable bag. Hybridization for P450 2E1 mRNA was carried out with a ^{32}P-end-labeled 19-mer oligonucleotide or cDNA probe, as described previously [23, 24]. Filters were washed at the hybridization temperature for 1 hr with three changes of a solution of 6 × SSPE and 0.1% SDS. Membranes were autoradiographed with Kodak X-Omat AR film at −80°C. Both oligonucleotide and a 600-bp P450 2E1 cDNA fragment were utilized as probes to assess P450 2E1 mRNA levels.

Scanning Densitometry

Scanning densitometry was performed with a Soft Laser Scanning Densitometer (Model SLR-1D/2D, Bio-Med Instrument Incorporation, Fullerton, CA, USA). The quantitation of the mRNA loaded on the slot blot was accomplished by hybridization of stripped membranes with ^{32}P-end-labeled poly(dT)$_{16}$, and the relative change in mRNA was determined from normalization of hybridization signal to the mRNA loaded onto the slots.

Hydroxyproline, ALT, ALP, Triglyceride and Cholesterol Contents

The amount of hydroxyproline in hepatic tissue was determined as previously described [25]. Serum ALT and ALP were measured with an automatic blood chemistry analyzer (Gilford SBA300). The levels of triglyceride and cholesterol in hepatic tissue were analyzed with commercially available kits.

RESULTS

Effects of YH439 on P450 2E1 Protein Expression

p-Nitrophenol hydroxylase activity, known to be relatively specific for P450 2E1, was monitored in the hepatic microsomes prepared from rats after YH439 treatment. Microsomal *p*-nitrophenol hydroxylase activity in the rats treated with YH439 at doses from 25 to 100 mg/kg was decreased by 5–23%, relative to control at 3 days posttreatment (Fig. 2A, left panel). The effects of YH439 treatment and INH (150 mg/kg/day, p.o., 3 days), an inducer of P450 2E1, were also examined. Although the rates of microsomal 4-NP hydroxylase activity were elevated to 4.48 nmol/min/mg protein in INH-induced hepatic microsomes, this INH-inducible P450 2E1 catalytic activity was completely suppressed by a concomitant treatment of animals with YH439 at doses from 25 to 100 mg/kg (Fig. 2A, right panel).

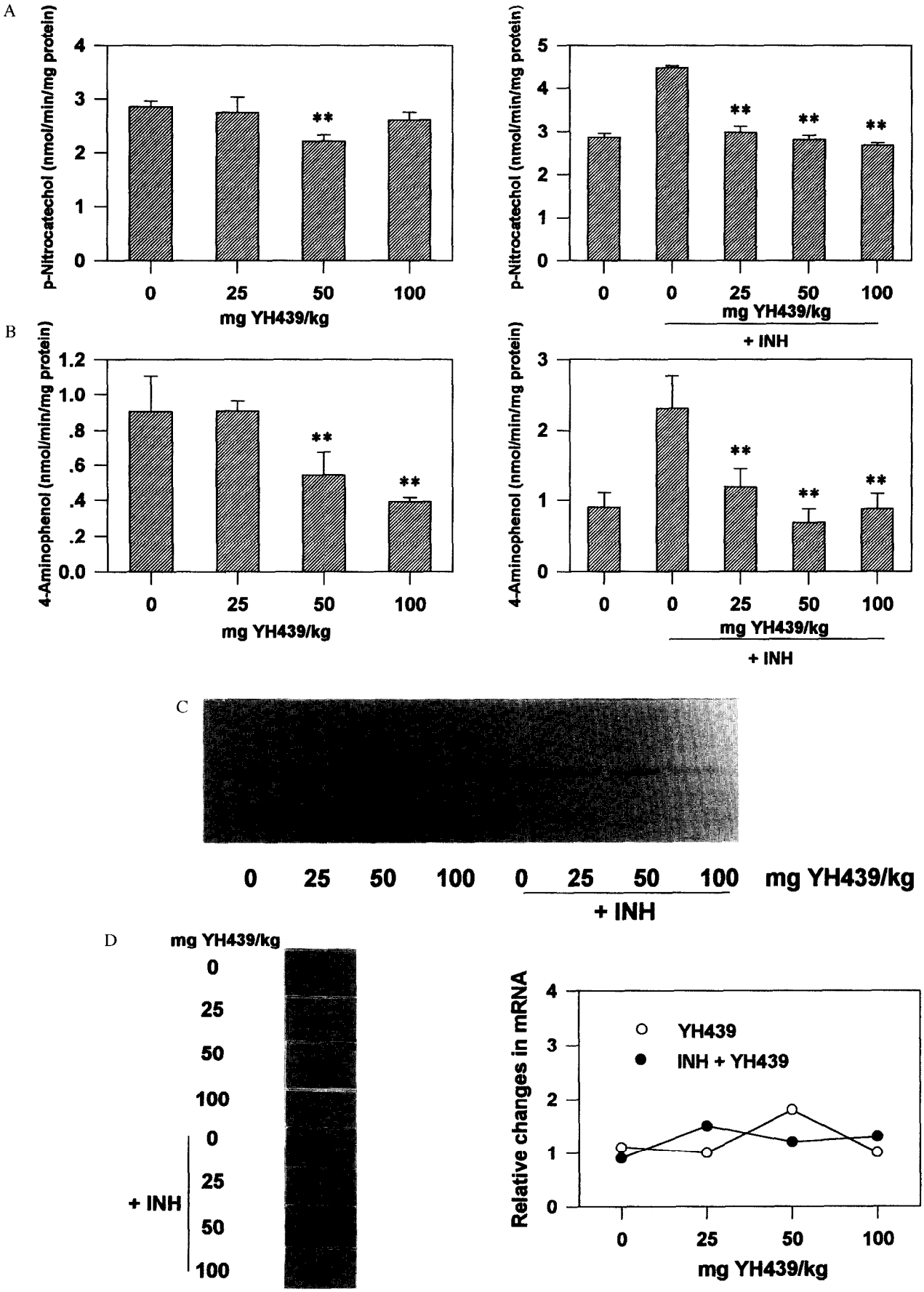
In addition, the hepatic microsomes obtained from YH439-exposed rats (100 mg/kg, p.o., 3 days) exhibited a maximal 57% decrease in the metabolism of aniline compared with the activity obtained from vehicle-treated animals (Fig. 2B, left panel). INH-induced hepatic microsomes isolated at 3 days posttreatment exhibited an enhanced rate of aniline hydroxylase activity with the value of 2.30 nmol 4-AP/min/mg protein as opposed to the control (0.91 nmol 4-AP/min/mg protein; Fig. 2B, right panel). Complete suppression of induced aniline hydroxylase activity (i.e. to 1.19–0.69 nmol 4-AP/min/mg protein) was also noted in hepatic microsomes isolated at 3 days after treatment with the same doses of YH439 (Fig. 2B, right panel).

Dose-related decreases in hepatic P450 2E1 levels posttreatment with YH439 were confirmed by immunoblot analyses (Fig. 2C). Western blot analysis exhibited dose-dependent decreases in P450 2E1 protein levels at 3 days posttreatment with YH439, which were more distinct then those in catalytic activities. Hepatic microsomes prepared from rats treated daily with YH439 at doses from 25 to 50 mg/kg exhibited more than 50% decrease in the amount of P450 2E1 protein. The dose of 100 mg/kg was sufficient to decrease the constitutive P450 2E1 protein levels below the limit of detectability. INH-induced rat hepatic microsomes showed a time-dependent increase in immunodetectable P450 2E1 levels, whereas hepatic microsomes isolated at 72 hr after daily treatment of rats with both YH439 and INH exhibited P450 2E1 band intensities comparable to those in uninduced microsomes (Fig. 2C), indicating that YH439 is capable of efficiently blocking INH-inducible P450 2E1 expression.

The level of hepatic P450 2E1 mRNA at 3 days posttreatment with YH439 failed to be altered (i.e. 1.3-fold of control; Fig. 2D). Slot blot analysis of hepatic P450 2E1 mRNA levels in the rats treated with both YH439 and INH for 3 days also showed no significant change in P450 2E1 message (Fig. 2D).

Immunoblot Analyses of P450 2E1 and P450 1A Levels

P450 2E1 and P450 1A levels were monitored among the major forms of P450s by using form-specific monoclonal antibodies. In contrast to the suppression in P450 2E1 levels, expression of P450 2B1/2 was not modulated after 3 days of YH439 treatment (Fig. 3A). The levels in P450 1A,


A
p-Nitrocatechol (nmol/min/mg protein)
0 1 2 3 4
0 25 50 100
mg YH439/kg
**
p-Nitrocatechol (nmol/min/mg protein)
0 1 2 3 4 5
0 0 25 50 100
mg YH439/kg
+ INH
**
B
4-Aminophenol (nmol/min/mg protein)
0.0 .2 .4 .6 .8 1.0 1.2
0 25 50 100
mg YH439/kg
**
4-Aminophenol (nmol/min/mg protein)
0 1 2 3
0 0 25 50 100
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+ INH
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0 25 50 100 0 25 50 100 mg YH439/kg
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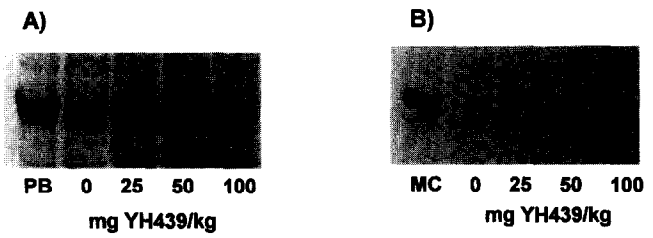

FIG. 3. Immunoblot analyses of P450 2B1/2 and P450 1A1/2. A: Immunoblot analysis for P450 2B1/2 levels after YH439 treatment with monoclonal antibody PB 2-66-3. Lanes represent hepatic microsomes isolated from rats treated with PB, vehicle or YH439 at doses of 25, 50 and 100 mg/kg for 3 days, respectively. B: A replicate membrane was immunoblotted with monoclonal antibody MC 1-7-1 to assess hepatic P450 1A/2 levels.

however, were slightly increased in YH439-treated hepatic microsomes, although the induction of P450 1A by YH439 was substantially less than by 3-MC (Fig. 3B).

Hepatoprotective Effects of YH439

To establish whether YH439 has protective effects against hepatic injury caused by hepatotoxicants, another study was designed with malotilate as a comparative agent. When animals were simultaneously treated with carbon tetrachloride and ethanol, liver injury was observed, as confirmed by ~2.5- and 2-fold increases in ALT and ALP activities, respectively (Table 1). The serum ALT and ALP activities in rats treated with YH439 at the daily doses of 50 or 100 mg/kg with carbon tetrachloride and ethanol insult, however, were comparable to those of untreated animals (Table 1). Biochemical changes such as alterations in the levels of triglyceride and cholesterol are related to histopathological changes in rat liver such as cell necrosis and fat infiltration. The toxicant-induced elevated liver triglyceride and cholesterol levels were also decreased to the levels of untreated animals after either YH439 or malotilate treatment (Table 2). These data support the conclusion that YH439 effectively protects the liver against experimental hepatic injury.

When a chronic inflammatory process is induced by toxicants, collagen synthesis overcomes its degradation in liver tissue. The effect of YH439 and malotilate on liver fibrosis was assessed by measuring hydroxyproline contents. Insult by CCl_4 and ethanol for 9 weeks caused a 13-fold increase in hepatic hydroxyproline content (Table 3). Concomitant treatment of rats with YH439 and carbon tetrachloride and ethanol resulted in protection against the formation of collagen, as supported by the decreases in hydroxyproline contents (Table 3).

TABLE 1. Effects of YH439 or malotilate on serum ALT and ALP activities in rats coadministered both carbon tetrachloride and ethanol

Treatment	ALT (unit/L)	ALP (unit/L)
Untreated	38.6 ± 1.7	54.0 ± 4.3
CCl_4/ethanol	100.2 ± 9.4	110.8 ± 16.0
YH439 50 mg/kg + CCl_4/ethanol	34.0 ± 1.3***	60.4 ± 2.8*
YH439 100 mg/kg + CCl_4/ethanol	34.4 ± 1.8***	59.2 ± 4.2**
Malotilate 50 mg/kg + CCl_4/ethanol	36.5 ± 1.4***	60.4 ± 4.3*
Malotilate 100 mg/kg + CCl_4/ethanol	35.2 ± 1.0***	59.5 ± 2.5**

All data represent mean ± SE of 10 animals.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, statistically different from CCl_4-treated animal as assessed by one-way analysis of variance. Rats were gavaged with 0.4 mL/kg of CCl_4 twice a week for 9 weeks and supplied with 5% aqueous ethanol instead of drinking water during the experimental period. YH439 or malotilate was administered for 9 weeks (6 times/week, p.o.).

DISCUSSION

Because P450 2E1 induction is associated with hepatotoxicity and nephrotoxicity and with carcinogenesis in response to certain organic toxicants, the selective suppression in P450 2E1 level by chemical agents may contribute to the protection of organs, particularly the liver, from xenobiotic-induced intoxication and, potentially, to the chemoprevention of cancer. Studies have demonstrated that diallylsulfide blocks the induction of carcinogenic responses in liver and colon, which develops following dimethylhydrazine treatment in animals [26–28]. A plausible mechanism for this suppression in cancer development is the selective inhibition of P450 2E1 [9, 10].

In this study, the effects of YH439 were also compared with those of malotilate at protein and mRNA levels. Here, YH439 was capable of blocking both constitutive and inducible P450 2E1 expression in response to isoniazid, a P450 2E1 inducer, as had been observed in malotilate-

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FIG. 2. Dose-related effects of YH439 on hepatic constitutive and INH-inducible P450 2E1 expression. A: *p*-Nitrophenol hydroxylase activity was monitored in the hepatic microsomes isolated at 3 days after treatment with either vehicle or 25 mg/kg, 50 mg/kg or 100 mg/kg of YH439 (left panel), INH, INH + YH439 25 mg/kg, INH + YH439 50 mg/kg or INH YH439 100 mg/kg (right panel). Data represent the mean ± standard deviations of four determinations. B: Microsomal aniline hydroxylase activity was assayed in the hepatic microsomes shown in A. Data represent the mean ± SD of four determinations. **$P < 0.01$, significantly different from control (Student's *t*-test). C: The same microsomal preparations were subject to P450 2E1 immunoblot analysis. Each lane contained 12 μg of microsomal proteins. Multiple determinations of the samples produced from different groups of animals resulted in variations of less than 15%. D: RNA blot analyses of P450 2E1 mRNA levels in the rats treated as above. The relative changes in P450 2E1 mRNA as assessed by scanning densitometry are shown on the right. Multiple determinations of the samples produced from different groups of animals resulted in variations of less than 10%.

TABLE 2. Effects of YH439 and malotilate on liver lipid in rats coadministered both carbon tetrachloride and ethanol

Treatment	Triglycerides (mg/g liver)	Cholesterol (mg/g liver)
Untreated	15.4 ± 1.3	3.8 ± 0.1
CCl_4/ethanol	40.2 ± 8.4	5.5 ± 0.3
YH439 50 mg/kg + CCl_4/ethanol	24.7 ± 8.6*	4.1 ± 0.2*
YH439 100 mg/kg + CCl_4/ethanol	18.1 ± 4.2*	3.9 ± 0.2*
Malotilate 50 mg/kg + CCl_4/ethanol	17.1 ± 5.3*	3.4 ± 0.2*
Malotilate 100 mg/kg + CCl_4/ethanol	14.3 ± 3.1*	3.2 ± 0.1*

All data represent mean ± SE of 10 animals.

* $P < 0.05$, statistically different from CCl_4-treated animal as assessed by one-way analysis of variance. Rats were gavaged with 0.4 mL/kg of CCl_4 twice a week for 9 weeks and supplied with 5% aqueous ethanol instead of drinking water during the experimental period. YH439 or malotilate was administered for 9 weeks (6 times/week, p.o.).

TABLE 3. Effects of YH439 and malotilate on hepatic protein and hydroxyproline contents in rats coadministered both carbon tetrachloride and ethanol

Treatment	Protein (mg/g liver)	Hydroxyproline (μg/g liver)
Untreated	284.7 ± 19.5	103.9 ± 8.0
CCl_4/ethanol	246.0 ± 16.5	1381.3 ± 120.2
YH439 50 mg/kg + CCl_4/ethanol	284.5 ± 26.9*	131.4 ± 14.9*
YH439 100 mg/kg + CCl_4/ethanol	284.2 ± 14.9*	95.7 ± 7.3*
Malotilate 50 mg/kg + CCl_4/ethanol	285.6 ± 9.2*	180.5 ± 15.4*
Malotilate 100 mg/kg + CCl_4/ethanol	287.5 ± 17.9*	92.3 ± 3.8*

All data represent mean ± SE of 10 animals.

* $P < 0.05$, statistically different from CCl_4-treated animal as assessed by one-way analysis of variance. Rats were gavaged with 0.4 mL/kg of CCl_4 twice a week for 9 weeks and supplied with 5% aqueous ethanol instead of drinking water during the experimental period. YH439 or malotilate was administered for 9 weeks (6 times/week, p.o.).

treated animals [14]. In blocking P450 2E1 expression, the agent was effective even at the daily dose of 25 mg/kg body weight. The rates of *p*-nitrophenol and aniline hydroxylase activities in the hepatic microsomes produced from rats treated with both YH439 and isoniazid were comparable to those of untreated animals. This blocking effect on P450 2E1 induction was not dose-related at the time point of day 3. The effect on catalytic activities was much less pronounced than that on the immunochemically detectable protein level, indicating that the measured metabolic activities were not strictly specific for P450 2E1 [29]. This result would explain the lack of YH439 dose–response effect on the constitutive expression of P450 2E1 as monitored by *p*-nitrophenol hydroxylase activity.

The lack of P450 2B induction following YH439 treatment also shows the selective effects of the agent in modulating the levels of enzymes involved in metabolic activation. A minimal increase in P450 1A level was noted after treatment with a relatively large dose of YH439. Nonetheless, this dose would far exceed the dose range for clinical use.

Malotilate-mediated suppression of P450 2E1 expression may be associated with its hepatoprotective and anticirrhotic effects, given that P450 2E1 is associated with toxicant-mediated hepatic injury [14]. YH439 appeared to be equipotent (if not superior) to malotilate in suppressing P450 2E1 levels at 3 days posttreatment, as shown by the results from metabolic and immunochemical characterization of P450 2E1 levels. The selective effect of YH439 on hepatic P450 2E1 expression may also be beneficial against toxicant-induced hepatic injury. The difference between the effects of YH439 and malotilate on *p*-nitrophenol hydroxylase activity might be due to the pharmacokinetic differences of the compounds (e.g. absorption) or differences in metabolic pathway(s) (e.g. production of metabolites that might have interacted with the active site of the enzyme).

Suppression in P450 2E1 levels may occur through transcriptional inactivation, translational inhibition or inactivation of P450 2E1 protein. Previous studies from these laboratories have shown that malotilate, a hepatoprotective agent, suppresses P450 2E1 expression. P450 2E1 suppression by malotilate failed to accompany P450 2E1 mRNA suppression [14], indicating that the decrease in P450 2E1 protein levels by the agent may not be associated with transcriptional inactivation. P450 2E1 suppression by the agent might result from changes in P450 2E1 translational efficiency and/or protein inactivation. The possible transcriptional regulatory mechanism was also examined with YH439. The present work also showed no change in P450 2E1 message levels with concomitant suppression of P450 2E1 protein expression after YH439 treatment. The level of P450 2E1 mRNA failed to decrease after hybridization with either a labeled synthetic oligonucleotide probe or a cDNA probe for P450 2E1. Thus, the suppression of P450 2E1 by this agent occurs with no transcriptional inactivation, although a slight transient increase in poly(A)$^+$ P450 2E1 mRNA was monitored at day 2 following 200 mg/kg of YH439 treatment (data not shown).

We also examined the expression of other cytochromes P450, including P450 1A1/2 and P450 2B1/2, among the major cytochromes P450. Although YH439 affected P450 1A expression at a high dose of 200 mg/kg, this agent failed to modulate P450 2B expression and thus seems to be relatively specific for the expression of P450 2E1. The effects of YH439 on the protein and mRNA levels of glutathione S-transferases (GST) and microsomal epoxide hydrolase were also examined (unpublished data). YH439 possessed the capability to induce major GST subunits including Ya, Yb1/2 and Yc, which would be supportive in blocking the initiation of chemical carcinogenesis, indicating that YH439 might serve as a chemoprotective agent partly through modulation of phase II enzyme expression.

Additional studies were carried out to establish whether YH439 is indeed effective in preventing or treating toxicant-induced hepatotoxicity. YH439, an experimental synthetic hepatoprotectant, was effective in protecting the liver against 9-week CCl_4-induced hepatic injury, as shown by serum ALT and ALP activities and by hepatic lipid contents. Moreover, the agent was efficacious in blocking liver fibrosis. These results provide evidence that YH439 suppresses hepatic constitutive and inducible P450 2E1 expression and that the suppression of P450 2E1 along with enhanced expression of GSTs may be associated with the hepatoprotective effects of this agent.

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