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The Effects of Isopropyl 2-(1,3-dithioetane-2-ylidene) -2-[N-(4-methyl-thiazol-2-yl)carbamoyl]acetate (YH439) on Potentiated Carbon Tetrachloride Hepatotoxicity

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ABSTRACT

The reactive intermediates formed during the metabolism of therapeutic agents, toxicants and carcinogens by cytochromes P450 are frequently capable of covalently binding to tissue macromolecules and causing tissue damage. It has been shown that YH439, a congener of malotilate, is effective in suppressing hepatic P450 2E1 expression. The present study was designed to further establish the mechanistic basis of YH439 protection against toxicant by assessing its effects against chemical-mediated potentiated hepatotoxicity. Retinoyl palmitate (Vit-A) pretreatment of rats for 7 days substantially enhanced carbon tetrachloride hepatotoxicity, as supported by an ~ 5-fold increase in serum alanine aminotransferase (ALT) activity, as compared to CCl_4 treatment alone.

The elevation of ALT activity due to Vit-A was completely blocked by the treatment of $GdCl_3$, a selective inhibitor of Kupffer cell activity. Concomitant pretreatment of rats with both YH439 and Vit-A resulted in a 94% decrease in Vit-A-potentiated CCl_4 hepatotoxicity. YH439 was also effective against propyl sulfide-potentiated CCl_4-induced hepatotoxicity. Whereas propyl sulfide (50 mg/kg, 7d) enhanced CCl_4-induced hepatotoxicity by >5-fold, relative to CCl_4 treatment alone, concomitant treatment of animals with both propyl sulfide and YH439 at the doses of 100 and 200 mg/kg prevented propyl sulfide-potentiated CCl_4 hepatotoxicity by 35% and 90%, respectively. Allyl sulfide, a suppressant of hepatic P450 2E1 expression, completely blocked the propyl sulfide-enhanced hepatotoxicity, indicating that propyl sulfide potentiation of CCl_4 hepatotoxicity was highly associated with the expression of P450 2E1 and that YH439 blocked the propyl sulfide-enhanced hepatotoxicity through modulation of P450 2E1 levels. Propyl sulfide- and CCl_4-induced stimulation of lipid peroxidation was also suppressed by YH439 in a dose-related manner, as supported by decreases in malonedialdehyde production. The role of P450 2E1 induction in the potentiation of CCl_4 toxicity and the effects of YH439 were further evaluated using pyridine as a P450 2E1 inducer. Pyridine pretreatment substantially enhanced the CCl_4 hepatotoicity by 23-fold, relative to CCl_4 alone. YH439, however, failed to reduce the pyridine-potentiated toxicity, suggesting that the other form(s) of cytochrome P450 inducible by pyridine, but not suppressible by YH439 treatment, may play a role in potentiating CCl_4-induced hepatotoxicity. YH439 was capable of blocking cadmium chloride-induced liver toxicity in mice. These results demonstrated that YH439 efficiently blocks Vit-A-enhanced hepatotoxiciy through Kupffer cell inactivation and that the suppression of P450 2E1 expression by YH439 is highly associated with blocking of propyl sulfide-mediated hepatotoxicity.

Key Words: YH439, Hepatoprotective effect, Kupffer cell, Potentiated hepatotoxicity, Vitamin A

INTRODUCTION

Metabolism of toxicants such as carbon tetrachloride activates Kupffer cells to release toxic secretory products, and activation of Kupffer cells and increases in the number of Kupffer cells are highly associated with the death of the hepatocytes (Edwards *et al.*, 1993). Certain hepatoprotective agents may exert their effects through the modulation of Kupffer cell activity and the inhibition of Kupffer cell infiltration. The underlying mechanism of toxicant-induced potentiated hepatotoxicity in associtation with Kupffer cell activation as well as with hepatic P450 2E1 expression was previously studied (Kim *et al.*, 1996).

Studies have shown that pretreatment of animals with large doses of vitamin A dramatically enhances the hepatotoxicity of a number of chemicals including carbon tetrachloride and that the potentiation of hepatotoxicity is mediated by reactive oxygen species released from Kupffer cells, representing that vitamin A activates liver macrophages (ElSisi 1993a, 1993b and 1993c). The present research was designed to examine whether YH439 exerts protective effects against chemical-mediated potentiated hepatotoxicity through modulation of Kupffer cell activity, and whether the enhanced P450 2E1 expression in association with the Kupffer cell activation further potentiates hepatotoxicity.

The reactive intermediates formed during the metabolism of therapeutic agents, toxicants and carcinogens by cytochromes P450 are frequently capable of covalently binding to tissue macromolecules, such as proteins, and causing tissue damage (Seeff *et al.*, 1986; Johansson and Ingelman-Sundberg, 1988). In particular, hepatotoxicity or carcinogenicity is correlated with toxic reactive intermediates produced from a wide variety of small organic compounds through P450 2E1-catalyzed metabolism (English and Anders, 1985; Nelson *et al.*, 1976; Lewis *et al.*, 1988). Studies in this laboratory have shown that saturated alkyl sulfides potentiate carbon tetrachloride-induced hepatotoxicity whereas diallyl disulfide and diallyl sulfide inhibit P450 2E1-catalyzed metabolic activities as well as suppress the expression of P450 2E1 (Kim *et al.*, 1996). YH439, a congener of malotilate, was effective in suppressing hepatic constitutive or inducible P450 2E1 expression (Choi *et al.*, 1996). Although the implication that P450 2E1 suppression by YH439 may contribute to hepatoprotection, direct experimental correlation between the suppression in P450 2E1 expression and hepatoprotective effects, and its molecular basis have not been demonstrated yet.

Vitamin A, propyl sulfide and pyridine were used in this research to study YH439 effect on their potentiated carbon tetrachloride hepatotoxicity. In the present study, we showed the role of P450 2E1 in the potentiated hepatotoxicity and the related hepatoprotective role of YH439. As part of complete characterization of YH439 effects against a variety of toxicants, cadmium chloride was used as a toxicant to establish the possible protective effects of YH439 against metal-induced toxicity. To the end of this study, the effect of YH439 against thioacetamide-induced liver fibrosis was examined.

MATERIALS AND METHODS

Materials

Allyl sulfide and propyl sulfide were purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Vitamin A palmitate (USP type 500, 542,000 I.U./g) was provided from Hoffmann-La Roche (Basel, Switzerland). YH439 was a gift from Yuhan Research Center Yuhan Corporation (Kunpo, Korea). Other reagents were purchased from Sigma Chemical Co (St. Louis, MO, U.S.A.).

Animal treatment

Male Sprague-Dawley and female Wistar rats (200~250 g) and ICR mice were obtained from Daehan Laboratory animals (Seoul, Korea) and maintained at 20 to 23℃ with a relative humidity of 50%. Animals were caged under the supply of filtered pathogen-free air and given food (Samyang rodent chow, Korea) and water *ad libitum* unless specified. Corn oil was used as an administration vehicle for oily agents.

For the preparation of microsomes, animals were fasted 16 h prior to killing.

Collection of blood and tissue samples

Male Sprague-Dawley rats (200~250 g) were treated with either vitamin-A (Vit-A) or propyl sulfide followed by administration with a subtoxic dose of carbon tetrachloride (0.15 ml/kg, i.p., 10% solution mixed with corn oil) to induce liver injury. To assess the efficacy of YH439, animals were daily treated with YH439 +Vit-A or YH439+propyl sulfide for 7 days before CCl_4 administration. Animals were sacrificed at 24 h following the last treatment.

Effects on $CdCl_2$-induced hepatotoxicity

ICR mice were used to assess YH439 effects against $CdCl_2$ hepatotoxicity. $CdCl_2$ was injected through tail vein at the dose of 10 mg/kg. Animals were sacrificed at 6 h or 12 h after $CdCl_2$ insult and blood was collected to determine plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities.

Evaluation of hepatic toxicity

Liver injury was assessed by monitoring plasma ALT activity using a commercially available kit (Youngdong Pharmaceutical, Korea).

Assay for lipid peroxidation

Malonedialdehyde and other degradation products of peroxidized lipids were determined as described previously (Gutteridge 1982; Braughler *et al.*, 1986; Rajakumar and Rao, 1993). Briefly, incubation mixture for assaying lipid peroxidation contained (10 w/v%) liver homogenate, 0.15 M KCl, 0.2 mM ferric chloride and 0.2 mM ferrous sulfate in a total volume of 500 μl. Reaction was initiated by adding ferric and ferrous ions and continued for 20 min at 37℃. Reaction was stopped by addition of 0.5 ml of 15% trichloroacetic acid. After centrifugation at 1000xg for 10 min, 0.8 ml of the supernatant was combined with the same volume of 0.25 N HCl containing 0.67% thiobarbituric acid. Following heating at 80℃ for 30 min, samples were cooled and centrifuged at 1000xg for 10 min. The absorbance of the supernatant was determined at 532 nm using the molar extinction coefficient of $1.56\times105/M\times cm$.

Assays of hepatic triglycerides and total cholesterol contents

The levels of triglycerides and total cholesterol in the liver homogenates (1:10, w/v) were determined using the commercially available kits (Youngdong Pharmaceutical, Korea).

The effects on Kupffer cell activation

In order to examine the uptake of colloidal carbon by Kupffer cells, livers were perfused at 37℃ with Krebs-Henseleit bicarbonate buffer as described by Cowper *et al* (1990). Liver perfusate was pumped via a cannula placed in the portal vein and effluent was collected from the vena cava. Samples were collected at 15 seconds interval for 20 minutes and absorbance at 623 nm was monitored.

RESULTS

Effect on Kupffer cell-activated hepatotoxicity

The hypothesis that the protective effect of YH439 against chemical-induced liver injury is associated with the suppression of Kupffer cells was assessed. It has been shown that pretreatment of rats with large doses of vitamin-A (retinoyl palmitate) dramatically enhances the hepatotoxicity of a number of chemicals through activation of Kupffer cells. Vitamin-A pretreatment of rats for 7 days potentiated carbon tetrachloride-induced hepatotoxicity, as supported by an ~4-fold increase in plasma ALT activity (i.e. 1515±733 KA unit/L) (Fig. 1). The elevation of ALT activity due to vitamin-A was completely blocked by an injection of $GdCl_3$, which is known to selectively suppress Kupffer cells (i.e. 221±120 KA unit/l) (Fig. 1). Concomitant pretreatment of rats with both retinoyl palmitate and YH439 at the daily dose of 200 mg/kg resulted in a 94% decrease in vitamin-A-potentiated hepatotoxicity (i.e. 406± 326 KA unit/l). These results provide evidence that YH439 protects the liver against chemical-induced hepatic injury by the mechanism associated with Kupffer cell inactivation.

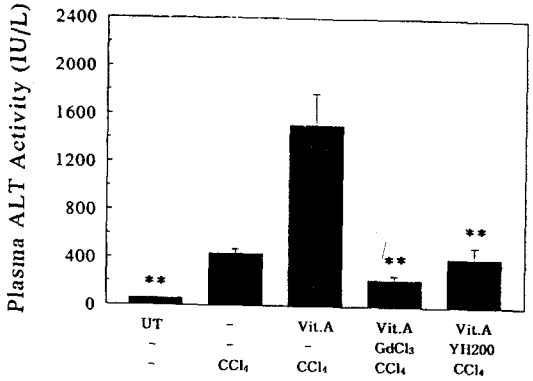

Fig. 1 Effects of YH439 or gadolinium chloride on vitamin A potentiation of CCl_4-induced liver injury. Rats were treated with Vit-A by gavage for 7 days. CCl_4 was administered at 24 h after the last dose (0.15 ml/kg, i.p.). Plasma ALT activity was monitored at 24 h after CCl_4. Data points represent mean±S.E. (N=10). Data were analyzed with one-way analysis of variance followed by Newmann-Keuls test for comparison with Vit.A+CCl_4 (** $p<0.01$); UT, untreated; Vit. A, vitamin A.

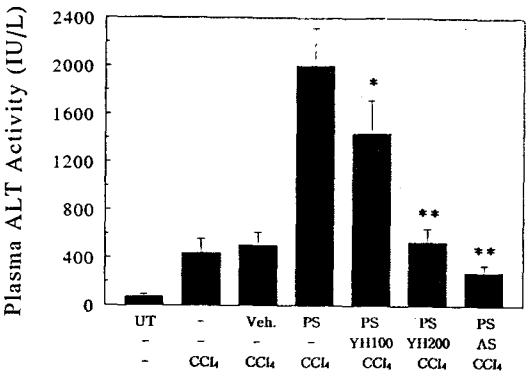

Fig. 2. Effects of YH439 on propyl sulfide-potentiated increase in plasma ALT activity induced by CCl_4. Propyl sulfide was given intraperitoneally at the daily dose of 50 mg/kg for 7 days and CCl_4 was administered at 24 h after the last dose (0.15 ml/kg, i.p.). Plasma ALT activity was monitored at 24 h after CCl_4. Data points represent mean±S.E. (N=10). Data were analyzed with one-way analysis of variance followed by Newmann-Keuls test for comparison with PS+CCl_4 (** $p<0.01$); UT, untreated; PS, propyl sulfide; AS, allyl sulfide.

Effects on the propyl sulfide-potentiated carbon tetrachloride hepatotoxicity

Other studies accomplished in this laboratory revealed that several alkyl sulfides are effective in potentiating carbon tetrachloride-induced hepatotoxicity. Propyl sulfide (PS) was the most prominent in enhancing carbon-tetrachloride-induced hepatotoxicity among the alkyl sulfides examined (Kim *et al.*, 1996). This study was designed to evaluate the protective effects of YH439 against propyl sulfide-potentiated hepatotoxicity. Whereas propyl sulfide (50 mg/kg, 7d) enhanced carbon tetrachloride-induced hepatotoxicity by >5-fold relative to CCl_4 treatment alone, concomitant treatment of animals with both propyl sulfide and YH439 blocked propyl sulfide-potentiated hepatotoxicity by 35% and 90% at the daily dose of 100 and 200 mg YH439/kg, respectively (Fig. 2). Allyl sulfide, which is an effective agent in suppressing hepatic P450 2E1 expression, also completely blocked the propyl sulfide-enhanced hepatotoxicity, indicating that propyl sulfide potentiation of carbon tetrachloride hepatotoxicity was highly associated with the induction of P450 2E1 and that YH439 reduced the propyl sulfide-enhanced hepatotoxicity through modulation of hepatic P450 2E1 expression. Whether propyl sulfide pretreatment caused activation of Kupffer cells was examined by colloidal carbon uptake in the liver. Propyl sulfide failed to increase Kupffer cell-mediated colloidal carbon uptake (data not shown).

Production of malonedialdehyde was also monitored in the liver homogenate derived from rats treated with PS and/or YH439 followed by a carbon tetrachloride insult. Whereas propyl sulfide pretreatment increased carbon tetrachloride-induced lipid peroxidation, concomitant pretreatment of rats with both YH439 and propyl sulfide suppressed the enhanced lipid peroxidation. The suppression of lipid peroxidation by YH439 was dose-related, resulting in 50% and 75% suppression of PS-enhanced CCl_4-induced lipid peroxidation at the dose of 100 and 200 mg YH439/kg body weight, respectively (Table 1).

Table 1. Malonedialdehyde production in the liver homogenates derived from rats treated with YH439 and/or propyl sulfide followed by a single dose of CCl_4 injection

Treatment	Activity (nmol/mg tissue)	Percent change
Untreated	2.08±0.31	100
Corn oil(vehicle)	4.18±0.34	201
n-Propyl sulfide	4.96±0.35	238
n-Propyl sulfide		
+YH439 100 mg	3.55±0.48**	170
+YH439 200 mg	2.81±0.16**	135

The values are mean±S.D. (n= 10)

Rats were treated with propyl sulfide at the dose of 50 mg/kg and YH439 was administered at the dose of 100 or 200 mg/kg for 7 days. Liver homogenates were prepared at 24 h after CCl_4 injection (0.15 ml/kg, i.p.).

Data were analyzed with one-way analysis of variance followed by the Newmann-Keuls test for comparisons of multiple group means. Comparison with vehicle (** $p<0.01$).

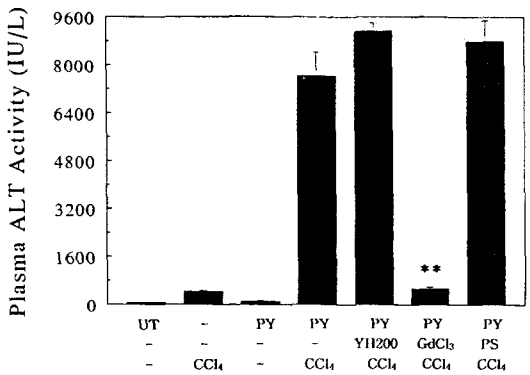

Fig. 3. Effects of YH439 on pyridine potentiation of CCl_4-induced hepatotoxicity. Pyridine was given intraperitoneally to rats at the daily dose of 50 mg/kg for 7 days, and CCl_4 was administered after the last dose (0.15 ml/kg, i.p.). Plasma ALT activity was monitored at 24 h after CCl_4. Data points represent mean± S.E. (N=10). Data were analyzed with one-way analysis of variance followed by Newmann-Keuls test for comparison with PY+ CCl_4 (** $p<0.01$).; UT, untreated; PY, pyridine; PS, propyl sulfide.

Effects on the pyridine-potentiated carbon tetrachloride-induced hepatotoxicity

Previous studies have shown that YH439 is effective in selectively suppressing constitutive and inducible expression of P450 2E1. Pyridine is a potent inducer of P450 2E1 (Kim *et al.*, 1986). The role of P450 2E1 induction in potentiating CCl_4 toxicity was further evaluated using pyridine as a P450 2E1 inducer. Pyridine pretreatment substantially enhanced the toxicity of carbon tetrachloride by 23-fold, relative to CCl_4-treated animals (Fig. 3). Concomitant treatment of animals with both propyl sulfide and pyridine slightly further increased hepatotoxicity, representing that the two compounds may, at least in part, share common mechanism for the enhancement of hepatotoxicity. A single dose of gadolinium chloride injection to pyridine-treated rats resulted in almost complete blocking of CCl_4-potentiated hepatotoxicity (i.e. 97%), which suggests that Kupffer cells must be involved in the step of potentiation of hepatotoxicity. In contrast to the observation that YH439 efficiently blocked retinoyl palmitate-induced hepatotoxicity, YH439 failed to reduce the pyridine-potentiated toxicity, which suggests either that the other form (s) of cytochrome P450 inducible by pyridine may also play a role in potentiating CCl_4-induced hepatotoxicity or that the potentiation of hepatotoxicity by pyridine supercedes the YH439 blocking effect.

The levels of hepatic triglycerides and total cholesterol were assessed in the pyridine-pretreated rats insulted with CCl_4. Gadolinium chloride blocked pyridine-enhanced increases in hepatic triglycerides and total cholesterol contents by 80% and 70%, respectively. YH439 at the dose of 200 mg/kg caused 42% and 24% decreases in pyridine-enhanced increases of hepatic triglycerides and cholesterol, respectively (Table 2).

Protective effects of YH439 on cadmium chloride-induced toxicity

The protective effects of YH439 on metal-induced toxicity were assessed in ICR mice. A single administration of cadmium chloride caused 5- to 6-fold increases in plasma ALT

and AST activities. The plasma obtained at 6 h after treatment of rats with YH439 followed by a single dose of cadmium chloride failed to exhibit protective effects (Table 3). Interestingly, the plasma obtained at 12 h after the treatment resulted in significant improvement in plasma AST activity (Table 3). Thus, YH439 was also effective in blocking metal-induced organ toxicity. Nonetheless, the decrease in ALT activity was not statistically significant (i.e. by 87%).

When ICR mice were insulted with galactosamine (700 mg/kg, i.p.) and lipopolysaccharide (2 μg/kg, i.v.), plasma ALT activity was elevated by ~23-fold (data not shown). Three day pretreatment of mice with 200 mg/kg YH439 failed to significantly reduce galactosamine+lipopolysaccharide-induced liver toxicity.

Liver fibrosis was induced in Female Wistar

Table 2. Hepatic triglycerides and total cholesterol contents in rats treated with pyridine (50 mg/kg,i.p.,7d) and/or YH439 followed by administration with a subtoxic dose of CCl_4 (0.15 ml/kg, i.p.)

Treatment		Triglycerides (mg/g tissue)	Cholesterol (mg/g tissue)
Untreated		9.6±0.96**	4.9±0.20**
Pyridine		24.9±2.09	12.3±1.09
Pyridine			
+$GdCl_3$	10 mg	4.8±0.23**	3.8±0.16**
+YH439	200 gm	14.4±1.26**	9.3±0.61**

Pyridine was daily administered at the dose of 50 mg/kg for 7 days to induce P450 2E1, followed by the injection of carbon tetrachloride.

The values are mean±S.E. (n= 10).

Data were analyzed with one-way analysis of variance followed by the Newmann-Keuls test for comparisons of multiple group means. Comparison with pyridine+CCl_4 treated group (** $p<0.01$)

Table 4. The effect of YH439 on the thioacetamide-induced hepatic fibrosis

Treatment		Hydroxyproline (mg/g tissue)	Percent Change
Untreated		0.086±0.010	100
Thioacetamide		0.138±0.021*	160
Thioacetamide			
+YH439	200 mg	0.135±0.033**	157

Female Wistar rats were treated with thioacetamide in drinking water at the concentration of 0.03 % for 3 months. After fibrosis formation, rats were daily treated with YH439 at 100 mg/kg for two weeks and liver firbosis formation was assessed by hydroxyproline levels.

The values are mean±S.D. (n= 6)

Data were analyzed with one-way analysis of variance followed by the Newmann-Keuls test for comparison of multiple group means. Comparison with vehicle (* $p<0.05$, ** $p<0.01$)

Table 3. Protective effects of YH439 against cadmium chloride in mice

Pretreatment	IU/L			
	6h after $CdCl_2$	% Change	12 h after $CdCl_2$	% Change
ALT activity				
Untreated	93±20	100	–	–
$CdCl_2$	616±186	662	728±294	782
$CdCl_2$+YH439 100 mg	923±248	992	172±21	184
AST activity				
Untreated	198±25	100	–	–
$CdCl_2$	1703±250	860	1232±176	622
$CdCl_2$+YH439 100 mg	1846±217	932	752±84*	380

The values are mean±S.E. (n= 10)

ICR mice were daily treated with 100 mg/kg of YH439 for 3 days (p.o). Animals were sacrificed at 6 hr or 12 h after a single dose of $CdCl_2$ injection (6 mg /kg, i.v.).

Data were analyzed with one-way analysis of variance followed by the Newmann-Keuls test for comparison of multiple group means. Comparison with $CdCl_2$-treated group (* $p<0.05$)

rats by providing 0.03% thioacetamide in drinking water for 3 months, as supported by elevated hepatic hydroxyproline content. YH439 treatment of the rats for 2 weeks (100 mg/kg/d) after thioacetamide administration failed to reduce thioacetamide-induced liver fibrosis (Table 4).

DISCUSSION

Studies have shown that expression of P450 2E1 is greatly enhanced by treatment with organic chemicals including ethanol, acetone or pyridine (Casazza *et al.*, 1984; Johansson and Ingelman-Sundberg, 1988; Kim *et al.*, 1988; Koop *et al.*, 1985; Patten *et al.*, 1986). Organic agents such as ethanol, pyrazine and pyridine, which are substrates for P450, serve as P450 2E1 inducers. Certain compounds simultaneously induce P450 2E1, glutathione S-transferases and microsomal epoxide hydrolase. A number of studies have shown that P450 2E1 induction is highly associated with hepatotoxicity (Seeff *et al.*, 1986; Tindberg and Ingelman-Sundberg, 1989; Yoo *et al.*, 1987). It has been shown that diallylsulfide protects the liver against carbon tetrachloride or acetaminophen intoxication via inhibition of P450 2E1-mediated metabolism of the agents (Brady *et al.*, 1988, 1991). Increases in phase II detoxification enzymes such as microsomal epoxide hydrolase and glutathione S-transferases have also been implicated in the protective effects by the organosulfur compounds against toxicant-induced liver injury (Kim *et al.*, 1994, 1996). Their chemoprotective effects would be associated with modulation of P450 2E1 levels (Hayes *et al.*, 1987). Previous studies also revealed that allyl sulfide, allyl methylsulfide and allyl mercaptan inhibit P450 2E1-mediated metabolic activities and suppress the expression of P450 2E1 (Kwak *et al.*, 1994). Recently, we have shown that YH439, a experimental synthetic hepatoprotectant, inhibits the expression of constitutive and inducible P450 2E1 expression (Kim *et al.*, 1996). Nonetheless, no appropriate experimental method for assessing hepatotoxicity in association with P450 2E1 expression nor the underlying mechanism has been established yet.

Activation of Kupffer cells contributes to hepatotoxicity induced by a variety of toxicants (ElSisi 1993a, 1993b and 1993c). The present study was initiated to determine whether YH439 was effective in suppressing chemical-induced potentiation of hepatotoxicity in association with Kupffer cell activation. We were interested in the effects of YH439 in the potentiated hepatotoxic animal models.

Activated Kupffer cells have been shown to release chemical mediators that can be toxic to hepatocytes. Thus, the release of reactive oxygen species from vitamin-A-activated Kupffer cells could increase lipid peroxidation and potentiate liver injury following CCl_4 administration. In order to examine the effect of YH439 on the blocking of Kupffer cell activation, animals were pretreated with vitamin-A along with YH439 followed by an injection of a subtoxic dose of CCl_4. The observation that YH439 blocks the vitamin-A effect on carbon tetrachloride-induced hepatotoxicity in this study supports the hypothesis that YH439 might inhibit Kupffer cell phagocytosis and the associated oxygen burst. One of the other working hypotheses was associated with potential increases in P450 2E1 levels by propyl sulfide, which might have further accelerated the production of reactive metabolic intermediates from carbon tetrachloride. Pretreatment of animals with gadolinium chloride prior to the injection of alkyl sulfide, however, failed to block propyl sulfide-potentiated hepatotoxicity, as evidenced by plasma ALT activity. The effectiveness of YH439 in blocking propyl sulfide-potentiated hepatotoxicity was consistent with the capability of YH439 in suppressing P450 2E1 expression.

The dose regimen of 7-day pyridine treatment followed by an insult of a subtoxic dose of carbon tetrachloride caused a super-potentiation of CCl_4-induced intoxication. Plasma ALT activity was elevated 70-fold in rats treated with pyridine prior to CCl_4 administration, as compared to untreated animals. To examine whether propyl sulfide and pyridine shares common molecular mechanism for the toxicant-induced potentiation of hepatotoxicity, rats were treated with both propyl sulfide and pyri-

dine, resulting in a minimal additive increase. The present study clearly demonstrated that YH439-mediated suppression in P450 2E1 expression is likely to be involved in propyl sulfide-potentiated hepatotoxicity whereas the agent is not sufficiently effective in blocking pyridine-potentiated carbon tetrachloride-induced hepatotoxicity. These results support the conclusion that the extent of hepatic P450 2E1 induction highly represents the degree of potentiated hepatotoxicity and the hepatoprotective effects of YH439 is at least in part mediated with modulation of P450 2E1 expression. It is also possible that other metabolic detoxification pathways contribute to the hepatoprotective effects of YH439. It has also been reported that metabolism of acetaminophen is enhanced by treatment of rats with YH439 through increases in the production of conjugated metabolites of acetaminophen, supporting the hypothesis that modulation in phase II enzymes is partly associated with the detoxification by YH439 (Cho *et al.*, 1996). YH439 was also effective in blocking $CdCl_2$-induced liver toxicity, although this agent failed to block galactosamine and LPS-induced liver toxicity. YH439 was ineffective in reducing liver fibrosis induced by chronic administration of thioacetamide, indicating that this agent primarily intervents the step of toxicant activation.

These results demonstrated that YH439 efficiently blocks Kupffer cell-mediated vitamin A-enhanced carbon tetrachloride hepatotoxiciy, and that suppression in P450 2E1 levels by YH439 is associated with blocking of propyl sulfide-mediated hepatotoxicity. Thus, the hepatoprotective effects of YH439 are highly associated with modulation of Kupffer cell activity as well as suppression of P450 2E1 expression. The molecular mechanism of reducing $CdCl_2$-induced toxicity after YH439 treatment should be established.

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REFERENCES

Brady JF, Li D, Ishizaki H and Yang CS: *Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. Cancer Res 48: 5937E-5940, 1988*

Brady JF, Wang M-H, Hong J-Y, Xiao F, Li Y, Yoo J-S H, Ning SM, Lee M-J, Fukuto JM, Gapac JM and Yang CS: *Modulation of rat hepatic microsomal monooxygenase enzymes and cytotoxicity by diallyl sulfides. Toxicol Appl pharmacol 108: 342-354, 1991*

Braughler JM, Duncan LA and Chase RL: *The involvement of iron in lipid peroxidation: importance of ferric to ferrous ratios in initiation. J Biol Chem 261: 10282-10289, 1986*

Casazza JP, Felver ME and Veech RL: *The metabolism of acetone in rat. J Biol Chem 259: 231-236, 1984*

Cho E, Jang SH, Lee JW, Kim ND and Lee MG: *Metabolic changes of acetaminophen after intravenous administration to rats pretreated with a hepatoprotective agent, YH439 Res. Commun Mol Pathol Pharmacol 91: 3-16, 1996*

Choi EY. Kim SG, Lee JW, Yoo JK, Shin JK and Kim ND: *Suppression of rat hepatic CYP2E1, by YH439, an experimental hepatoprotectant: Protective role against hepatic injury Biochem Pharmacol submitted.*

Cowper KB, Currin RT, Dawson TL, Lindert KA, Lemasters JJ and Thurman RG: *A new method to monitor Kupffer-cell function continuously in the perfused rat liver. Biochem J 266: 141-147, 1990*

Edwards MJ, Keller BJ, Kauffman FC and Thurman RG: *The involvement of Kupffer cells in carbon tetrachloride toxicity. Toxicol Appl Pharmacol 119: 275-279, 1993*

ElSisi AED, Earnest DL and Sipes IG: *Vitamin A potentiation of carbon tetrachloride hepatotoxicity: Role of liver marcophages and active oxygen species. Toxicol Appl Pharmacol 119: 295-301, 1993a*

ElSisi AED, Earnest DL and Sipes IG: *Vitamin A potentiation of carbon tetrachloride hepatotoxicity: Enhanced lipid peroxidation without enhanced biotransformation 119: 289-294, 1993b*

ElSisi AED, Pauline H, Sim W-L W, Earnest DL

and Sipes IG: *Characterization of Vitamin A potentiation of carbon tetrachloride-induced liver injury 119: 280-288, 1993c*

English JC and Anders MW: *Evidence for the metabolism of N-nitrosodimethylamine and carbon tetrachloride by a common isozyme of cytochrome P-450. Drug Metab Dispos 13: 449-452, 1985*

Gutteridge JM: *Free-radical damage to lipids, amino acids, carbohydrates and nucleic acids determined by thibarbituric acid reactivity. Int J Biochem 14: 649-653, 1982*

Hayes MA, Rushmore TH, and Goldberg MT: *Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil. Carcinogenesis (Lond.) 8: 1155-1157, 1987*

Johansson I and Ingelman-Sundberg M: *Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450 (2E1) in rat and rabbit liver microsomes. Cancer Res 48: 5387-5390, 1988*

Kim, ND, Kim SG and Kwak MK: *Enhanced expression of rat microsomal epoxide hydrolase gene by organosulfur compounds. Biochem Pharmacol 47: 541-547, 1994*

Kim SG, Williams DE, Schuetz EG, Guzelian PS and Novak RF: *Pyridine induction of cytochrome P-450 in the rat: Role of P-450j (alcohol-inducible form) in pyridine N-oxidation. J Pharmacol Exp Therap 246: 1175-1182, 1988*

Kim SG, Chung HJ and Cho JY: *Molecular mechanism for alkyl sulfide-modulated carbon tetrachloride-induced hepatotoxicity: The role of cytochrome P450 2E1, P450 2B and glutathione S-transferase expression J Pharmacol Exp Therap 277: 1058-1066, 1996*

Koop DR, Crump BL, Nordbloom GD and Coon MJ: *Immunochemical evidence for induction of the alcohol-oxidizing cytochrome P-450 of liver microsomes by diverse agents: Ethanol, imidazole, trichloroethylene, acetone, pyrazole and isoniazid. Proc Nat Acad Sci USA 82: 4065-4069, 1985*

Kwak MK, Kim SG, Kwak JY, Novak RF and Kim ND: *Inhibition of P450 2E1 expression by organosulfur compounds allylsulfide, allylmercaptan and allylmethylsulfide in rats Biochem Pharmacol 47: 531-539, 1994*

Lewis JG, Stewart W and Adams DO: *Role of oxygen radicals in induction of DNA damage by metabolites of benzene. Cancer Res 48: 4762-4765, 1988*

Nelson SD, Mitchell JR, Timbrell JA, Snodgrass WR and Corcoran GB: *Isoniazid and iproniazid activation of metabolites to toxic intermediates in man and rat. Science 193: 901-903, 1976*

Patten CJ, Ning SM, Lu AYH and Yang CS: *Acetone-inducible cytochrome P-450: Purification, catalytic activity and interaction with cytochrome b5. Arch Biochem Biophys 251: 629-638, 1986*

Rajakumar DV and Rao MNA: *Dehydrozingerone and isoeugenol as inhibitors of lipid peroxidation and as free radical scavengers. Biochem Pharmacol 46: 2067-2072, 1993*

Seeff LB, Cuccherini BA, Zimmerman HJ, Adler E and Benjamin SB: *Acetaminophen hepatotoxicity in alcoholics. Ann Intern Med 104: 399-404, 1986*

Tindberg N and Ingelman-Sundberg M: *Cytochrome P-450 and oxygen toxicity, oxygen-dependent induction of ethanol-inducible cytochrome P-450 (2E1) in rat liver and lung. Biochemistry 28: 4499-4504, 1989*

Yoo JH, Cheung RJ, Patten CJ, Wade D and Yang CS: *Nature of N-nitrosodimethylamine demethylase and its inhibitors. Cancer Res 47: 3378-3383, 1987*

=국문초록=

상승적 화학적 간독성에 미치는 YH439의 영향

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간독성물질인 CCl_4의 대사에서 반응성이 높은 대사중간체의 증가가 P450 2E1의 활성 및 발현 증가와 관련된다. YH439는 랫트에서 사염화탄소에 의하여 유발된 간 손상에 보호효과가 탁월하였고, 각종 독성물질에 의하여 발생하는 간염을 억제하며 P450 2E1의 발현을 억제하는 것으로 나타났다. P450 2E1의 발현억제가 YH439의 간장보호작용의 일부기전으로 해석되나 free radical 공격의 제어, 방어과정에 관련된 탐식세포의 역할등 간장독성에 관련된 YH439의 영향 및 관련된 기초연구는 완전히 확립되어 있지 않다. 본 연구에서는 상승적인 화학적 독성에 대한 YH439의 보호효과를 관찰하였다. Retinoyl palmiate (Vit-A)를 전처리하고 YH439를 처리한 rat의 경우 CCl_4 단독투여군에 비하여 혈장 alanine aminotransferase (ALT)활성이 5배로 증가하여 CCl_4에 의한 간독성을 현저히 강화시켰으나, YH439와 Vit-A를 동시에 전처리한 rats에 있어서는 Vit-A에 의하여 강화된 독성이 94% 감소하였다. Vit-A에 의한 혈장 ALT 활성 증가는 Kupffer cell 활성을 선택적으로 억제하는 $GdCl_3$의 투여에 의해 완전히 차단되어 YH439가 Kupffer cell 활성억제를 매개로 상승적 간손상에 대하여 보호효과가 있음을 지지한다. Propyl sulfide의 전처치는 CCl_4에 의해 유도되는 간독성을 CCl_4 단독투여와 비교했을때 5배 이상 증가시켰으나, Propyl sulfide와 YH439를 병용투여할 경우 propyl sulfide에 의해 강화되는 간독성이 YH439의 투여용량에 의존적으로 감소하였고, propyl sufide와 CCl_4에 의한 지질과산화의 증가가 YH439에 의하여 용량의존적으로 억제되는 것으로 나타났다. Propyl sulfide에 의하여 강화된 간독성의 차단은 YH439가 P450 2E1 발현조절을 통하여 간독성을 제어함을 지지한다. 그러나 YH439는 pyridine과 CCl_4에 의한 독성을 억제시키지 못하였다. 이는 Pyridine에 의해 유도되는 다른 형의 P450발현이 YH439에 의해 억제되지 못하는 이유로 해석된다. 중금속에 의해 유도되는 간독성에 대한 YH439의 보호효과를 ICR mice에서 관찰하였을 때 $CdCl_2$를 1회 투여할때 ALT와 aspartate aminotransferase (AST)활성이 5~6배 증가하였으나 YH439를 투여한 후 $CdCl_2$를 투여한 동물에 있어서는 투여후 6시간에 AST의 증가가 유의성 있게 억제되었다. 그러나 YH439는 thioacetamide에 의하여 유발된 liver fibrosis에는 개선효과가 없는 것으로 나타났다. 이러한 결과는 YH439가 Kupffer cell 불활성화를 통하여 Vit-A에 의해 유도되는 간독성을 효과적으로 방어하고, YH439에 의한 P450 2E1의 발현억제는 propyl sulfide를 경유하는 간독성 차단과 관계되며, YH439는 중금속으로 유도된 조직독성에 방어효과가 있음을 지지한다.