SHORT COMMUNICATION

Inhibition of vinyl carbamate-induced hepatotoxicity, mutagenicity, and tumorigenicity by isopropyl-2-(1,3-dithietane-2-ylidene)-2-[N-(4-methylthiazol-2-yl)carbamoyl]acetate (YH439)

Seong Gon Kim1, Young-Joon Surh2,5, Yeowon Sohn3, Joong-Keun Yoo4, Jong Wook Lee4, Amy Liem1 and James A. Miller1

1McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, WI 53706, USA, 2College of Pharmacy, Seoul National University, Seoul 151-742, 3Department of Biotechnology, KFDA, Seoul 122-020 and 5Yuhan Research Center, Gunpo-si, South Korea

To whom correspondence should be addressed
Email: surh@plaza.snu.ac.kr

Isopropyl-2-(1,3-dithietane-2-ylidene)-2-[N-(4-methylthiazol-2-yl)carbamoyl]acetate (YH439) is a novel dithioylidene malonate derivative developed for the treatment of hepatic injury. The compound has been found to down-regulate the expression of hepatic cytochrome P-450 2E1 (CYP2E1) at the transcriptional level (8). Certain organosulfur compounds present in garlic elicit protective effects on chemically induced carcinogenesis and mutagenesis and their chemopreventive activities are associated in part with inhibition of CYP2E1. As part of a program to determine the likely chemopreventive potential of YH439, we initially examined its effects on hepatotoxicity induced by vinyl carbamate (VC), a proximate carcinogen that is preferentially bioactivated by CYP2E1. A single i.p. injection of VC (125 mg/kg body wt) to male Sprague–Dawley rats resulted in severe hepatic lesions as demonstrated by elevated levels of serum enzymes such as alanine aminotransferase and aspartate aminotransferase. Histopathological evaluation of liver sections from VC-treated animals revealed that the hepatic damage mainly consisted of centrilobular necrosis with sinusoidal congestion. Oral administration of YH439 (200 mg/kg body wt) to male Sprague–Dawley rats 2 days, 1 day and 4 h prior to VC completely prevented the hepatic damage caused by this carcinogen. In another experiment, rat hepatic microsomal-mediated bacterial mutagenicity of VC was suppressed by YH439 in a dose–related manner. Furthermore, pretreatment of female CD-1 mice with YH439 by gastric intubation resulted in diminution of VC-induced skin carcinogenesis.

Malotilate (diisopropyl-1,3-dithiol-2-ylidenemalonate; structure shown in Figure 1) is a synthetic agent that has been reported to prevent the progress of experimental liver injury induced by various hepatotoxins, such as carbon tetrachloride, dimethylnitrosamine, bromobenzene, and acetaminophen (1–5). Using malotilate as a lead compound, a series of structural derivatives were synthesized for clinical use in the treatment of chronic liver diseases. Of these new hepatotrophic compounds, isopropyl-2-(1,3-dithietane-2-ylidene)-2-[N-(4-methylthiazol-2-yl)carbamoyl]acetate (YH439*; see the structure in Figure 1) has been found to retain the most remarkable hepatoprotective activity with no apparent toxicity (6). The compound is currently under phase II clinical trial for use in humans as a hepatoprotectant. Both malotilate (7) and YH439 (8) have recently been found to markedly suppress the expression of cytochrome P450 2E1 (CYP2E1) which is responsible for metabolism of many low molecular weight drugs and carcinogens (9). Since CYP2E1 is inducible by ethanol consumption, this isoform has been implicated in the intoxication of chronic alcoholics who are much more susceptible to liver injury induced by certain drugs including acetaminophen (10).

In consideration of roles of cytochrome P-450-dependent mixed-function oxidases in metabolic activation as well as detoxification of chemical carcinogens of diverse categories, compounds that modulate the activity of these enzymes may influence processes of chemically-induced tumorigenesis. As part of our study directed towards evaluating possible cancer chemopreventive properties of YH439, we initially sought to examine its effect on hepatotoxicity, mutagenicity, and tumorigenicity of vinyl carbamate (VC), a proximate hepatocarcinogen that is known to be preferentially activated by CYP2E1 (11) to an ultimate electrophilic and tumorigenic epoxide (12).

YH439 was provided by the Yuhan Research Center (Kunpo-si, Korea). VC was synthesized as described previously (12). Triocetanoin was obtained from the Pfaltz and Bauer, Inc. (Stamford, CT). 12-O-Tetradecanoylphorbol-13-acetate (TPA) was supplied from the Chemsyn Science Laboratory (Lenexa, KS). Reagents for determination of serum enzymes were purchased from the Sigma Chemical Co. (St Louis, MO). Male Sprague–Dawley rats (Teklad/Halan Sprague–Dawley, Madison, WI) of ~4 weeks of age were gavaged with YH439 (200 mg/kg body wt) suspended in triocetanoin 2 days, 1 day and 4 h prior to a single i.p. injection of VC (125 mg/kg) in saline or the vehicle alone. Blood was collected 24 h after VC dosing and the activities of serum enzymes were measured using kits from the Sigma Chemical Co., according to the instructions provided. Liver biopsy specimens were fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histopathological examination. Statistical differences in serum enzyme activities between treated and untreated groups were determined by Student’s unpaired t-test. The Salmonella-microsome assay was performed according to the liquid preincubation method of Maron and Ames (13), using Salmonella typhimurium TA1535 as a tester strain. The data were corrected for the numbers of spontaneous revertants. To conduct the two stage skin carcinogenesis experiment, the dorsal region of female CD-1 mice (~7 weeks old) was shaved with an
Table I. Effect of YH439 pretreatment on serum enzyme levels following administration of VC

<table>
<thead>
<tr>
<th>YH439</th>
<th>VC</th>
<th>Serum enzyme activities (units/liter)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>SGOT 6.5 ± 0.6</td>
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<tr>
<td></td>
<td></td>
<td>SGPT 23 ± 2</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>SGOT 54 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGPT 22 ± 1</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>SGOT 341 ± 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGPT 241 ± 37</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>SGOT 51 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGPT 24 ± 1</td>
</tr>
</tbody>
</table>

*YH439 suspended in trioctanoin was administered by gastric intubation to male Sprague–Dawley rats at a dose of 200 mg/kg body wt 2 days, 1 day and 4 h before VC administration (125 mg/kg, i.p.) in saline or the vehicle alone. Control animals were pretreated with trioctanoin alone. Serum enzyme activities were measured as described in the text.*

**Each number represents the mean ± SD from duplicate analysis of 4 animals.**

**Significantly different from the rats treated with VC alone (P < 0.0001).**

The effect of YH439 on the mutagenic activity of VC was examined in *S. typhimurium* TA1535. As summarized in Table III, YH439 exhibited dose–related protection against VC-induced *his*+ reversion in these bacteria. Oral administration of YH439 (150 mg/kg body wt) 1 day and 2 h before the topical application of VC (11.5 µmol) resulted in ~60% reduction in formation of mouse skin tumors at 22 weeks after
Table II. Effect of YH439 on mutagenicity of VC in S.typhimurium TA1535

<table>
<thead>
<tr>
<th>VC (µmol)</th>
<th>YH439 (µmol)</th>
<th>No. of his(^{+}) revertants/plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.45</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>331 ± 36</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>132 ± 32</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>81 ± 15</td>
</tr>
<tr>
<td>2</td>
<td>0.23</td>
<td>76 ± 4</td>
</tr>
</tbody>
</table>

VC was incubated with the bacteria (1–2×10\(^6\)) and microsomes (1 mg protein) from male Sprague–Dawley rats in a final vol. of 0.6 ml 0.1 M potassium phosphate buffer (pH 7.4) in the absence or presence of the given amount of YH439. After preincubation at 37 °C for 30 min, the mixtures were diluted with soft agar, plated onto minimal glucose agar plates and further incubated for 48 h to allow the development of his\(^{+}\) revertant colonies.

Table III. Chemopreventive effects of YH439 and diallyl sulfide against VC-induced mouse skin carcinogenesis\(^{a}\)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>At 22 weeks</th>
<th>% Tumor-bearing mice</th>
<th>No. of papillomas/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trioctanoin</td>
<td>Acetone 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>YH439</td>
<td>Acetone 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Diallyl sulfide</td>
<td>Acetone 4</td>
<td>0.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Trioctanoin</td>
<td>VC 58</td>
<td>1.2 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>YH439</td>
<td>VC 37</td>
<td>0.5 ± 0.9(^{b})</td>
<td></td>
</tr>
<tr>
<td>Diallyl sulfide</td>
<td>VC 29</td>
<td>0.6 ± 1.1(^{b})</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Young female CD-1 mice were pretreated with YH439 (150 mg/kg), diallyl sulfide (200 mg/kg) or vehicle (trioctanoin) by gavage as described in the text. Skin papilloma was initiated by a single topical application of 11.5 µmol VC in acetone and promoted with a topical dose (2.5 µg) of TPA given twice weekly until termination of the experiment.

\(^{b}\)Significantly different when compared with the value observed in control animals given VC alone (P < 0.01).

promotion, which was comparable to that attained with diallyl sulfide pretreatment (Table III). Under these experimental conditions, none of the mice given YH439 alone developed tumors. Activating mutations in codon 61 of H-ras gene were identified in all of the DNA samples from randomly selected VC-induced tumors. YH439 pretreatment did not alter the frequency or spectrum of H-ras mutations induced by VC (data not shown).

The results of this study demonstrate that pretreatment of male Sprague–Dawley rats with YH439 by gavage completely protects against the acute hepatotoxic effects of VC. The attenuation by YH439 of VC-induced hepatic injury appears to be related to its ability to inhibit CYP2E1 that mediates the activation of this carcinogen to an ultimate electrophilic and tumorigenic metabolite, vinyl carbamate epoxide (VCO). In line with this possibility, Kim and his co-workers (7) reported that malotilate, when given to male Sprague–Dawley rats by gavage, decreased the levels of CYP2E1 protein in liver microsomes. Subsequent work by Jeong et al. (8) has shown that oral administration of the structurally related malotilate derivative YH439 leads to rapid and specific suppression of CYP2E1-linked catalytic activity in rat liver microsomes with a concomitant decrease in the hepatic CYP2E1 mRNA level.

Recently, certain organosulfur-containing compounds have been found to be potent and effective cancer chemopreventive agents. Examples are diallyl sulfide in garlic (15–18) and sulforaphane (4-methylsulfinylbutyl isothiocyanate) isolated from broccoli (19). One of the plausible mechanisms of chemoprotective action of diallyl sulfide has been ascribed to its selective inhibition of CYP2E1 (20). Sulforaphane was found to induce phase II detoxification enzymes, such as NAD(P)H:quinone oxidoreductase and glutathione S-transferase (21), thereby facilitating the removal of carcinogens or their active metabolites. In addition, sulforaphane has been reported to inhibit cytochrome P450 2E1 (CYP2E1) (22), an important isoform that is responsible for activation of a wide array of carcinogens and mutagens. Besides naturally occurring organosulfur compounds, certain synthetic sulfur-containing substances have been shown to suppress experimental carcinogenesis. Of particular interest are sulindac and oltipraz. Sulindac, which was originally developed as a non-steroidal antiinflammatory drug, has been found to inhibit experimental colon and lung tumorigenesis in rodents (23,24). Because of its ability to induce regression of colonic adenomas (25), chemoprevention trials with sulindac have been underway for patients with familial adenomatous polyposis. The antiinflammatory drug oltipraz also exhibits striking chemopreventive activities against chemically induced carcinogenesis in various animal models (26–28). It also ameliorates hepatic damage induced by aflatoxin B\(_1\), acetaminophen, or carbon tetrachloride (29–31). The chemoprotective properties of oltipraz have been attributed primarily to the induction of glutathione S-transferase and NAD(P)H:quinone reductase (32), which play key roles in protection against chemically-induced toxicity and carcinogenesis. Furthermore, recent studies have shown that oltipraz can inhibit phase I xenobiotic metabolizing enzyme (33,34), such as cytochrome P450 1A2 and 3A4 isoforms that catalyze the activation of aflatoxin B\(_1\) to an electrophilic epoxide metabolite. Sulforaphane and oltipraz can thus influence the metabolism of carcinogens via inhibition of phase I or induction of phase II enzymes, which may account for their chemopreventive or chemoprotective effects. By analogy, YH439 has a dual effect on the carcinogen metabolism. In addition to inhibiting CYP2E1 (8), oral administration of this synthetic dithiol malonate to rats gave rise to significant elevation of the phase II enzymes: in our preliminary studies, the activities of hepatic glutathione S-transferase and quinone reductase were increased ~32% and 76%, respectively in rats after administration of YH439 (200 mg/kg) by gavage for 30 days (unpublished data). Taken together, the capability of YH439 to retard the metabolic activation of carcinogens and/or to facilitate their removal from the body would provide rationale for application of this compound to cancer chemoprevention (35).

VC can interact with target cell DNA to form covalently bond adducts via metabolic activation to the ultimate electrophilic and carcinogenic epoxide VCO, which may lead to mutations in certain protooncogenes. Activation of H–ras protooncogene through mutation in a specific codon has been frequently observed in a wide range of human cancers as well as in chemically-induced tumors in experimental animals. The types of ras gene mutations are generally associated with the known metabolic activation pathways and DNA binding characteristics of carcinogens, and can be affected by specific chemopreventive agents. YH439 pretreatment did not alter the frequency or spectrum of H–ras mutations induced by VC. Since the pattern of ras mutations is determined in part by the nature of the ultimate electrophilic form of a given carcinogen, the above results suggest that the observed chemopreventive effects of YH439 against VC are mediated through suppression of experimental liver injury.
of their activation and subsequent DNA binding without deviating the metabolic pathways involved.

In conclusion, YH439 pretreatment produced substantial protection against VC-induced liver damage, mutagenesis and tumorigenesis, presumably through repression of CYP2E1 responsible for activating this hepatocarcinogen and/or via induction of phase II detoxification enzymes. Further investigations should follow to evaluate the chemopreventive activity of YH439 in diverse animal models.

Acknowledgements

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References


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