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The Anti-Fibrogenic Effect of a Pharmaceutical Composition of [5-(2-Pyrazinyl)-4-methyl-1,2-dithiol-3-thione] (Oltipraz) and Dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate (DDB)

Keon Wook Kang[1], Yoon Gyoon Kim[1], Choon Won Kim[2], and Sang Geon Kim[1]

[1]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Korea, and [2]Department of Clinical Pathology, Hanyang University Medical School, Seoul, Korea

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Liver fibrosis is a prepathological state wherein damaged liver tissues in chronic liver diseases, such as hepatitis, are not repaired to normal tissues, but converted to fibrous tissue. 5-(2-Pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz), a cancer chemopreventive agent, is effective against a wide variety of chemical carcinogens. Recently, we reported that oltipraz inhibits liver fibrogenesis (Kang *et al.*, 2002). In the present study, the effects of oltipraz in combination with dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate (DDB) on dimethylnitrosamine (DMN)-induced liver fibrogenesis were assessed in rats. Oltipraz (30 mg/kg body weight, po, 3 times per week for 4 weeks) was found to inhibit the increases in plasma ALT, AST and bilirubin by DMN, whereas DDB (30 mg/kg body weight, po, 3 times per week for 4 weeks) attenuated the increases in the plasma ALT and bilirubin. The lowered plasma protein and albumin contents in DMN-treated rats were completely restored by oltipraz, but not by DDB. DDB decreases liver cell injury and inflammation through inhibition of nuclear factor-kB. DMN increased the accumulation of liver collagen, as indicated by the increase in the 4-hydroxyproline content in liver homogenates, which was reduced by treatment with oltipraz, but not by DDB. Given the differential effect between oltipraz and DDB, the potential enhancement of antifibrotic efficacy by the drugs was assessed in the animal model. Despite the minimal effect of DDB on DMN-induced fibrogenesis, DDB (5-25 mg/kg), administered together with oltipraz (25-5 mg/kg), showed an additive protective effect against hepatotoxicity and fibrosis induced by DMN, which was shown by the blood chemistry parameters and histopathological analysis. The adequate composition ratio of oltipraz to DDB was 5:1. These results provide information on the pharmaceutical composition, comprising of oltipraz and DDB as the active components, for the treatment and/or prevention of liver fibrosis and cirrhosis.

Key words: Oltipraz, DDB, Fibrosis, Dimethylnitrosamine, Pharmaceutical composition

INTRODUCTION

The liver plays a key role in the metabolism of xenobiotics and endogenous substances, and is an important organ for enzymatic reactions and energy metabolism. Among the chronic diseases in Korea, liver diseases, including hepatitis, liver cirrhosis and hepatocarcinoma, are the second most widespread and life threatening next to cardiovascular diseases. Korea has a relatively large population of drinkers compared to other developed countries, and as a result liver injuries from binge drinking are fairly high, so a lot of attention has been paid to the treatment of liver diseases. Often chronic liver damage, as a result of viral infections or alcohol drinking, causes cirrhosis or liver cancer. In the United States, liver cirrhosis is the tenth leading cause of death by disease, accounting for 373,000 hospital discharge and 25,000 deaths per year (9.3 deaths per 10,000 population)(Vital and Health Statistics Series 13, No. 148).

Liver fibrosis is a prepathological state wherein damaged liver tissues in chronic liver diseases, such as hepatitis, are not repaired to normal tissues, but are converted to fibrous tissue with characteristic collagen

Correspondence to: Sang Geon Kim, Ph. D., College of Pharmacy, Seoul National University, Sillim-dong, Kwanak-gu, Seoul 151-742, Korea.
E-mail: sgk@snu.ac.kr

accumulation. Although liver fibrosis is the outcome of an *in vivo* repair process in response to tissue damage, damaged liver tissue is replaced by fibrous tissues, which can no longer function normally (e.g. *in vivo* metabolism or bile juice production). As continuous and recurring liver fibrogenesis leads to cirrhosis and eventually causes death, it is crucial to develop drugs for the treatment and prevention of liver fibrosis. However, as the precise mechanism of liver fibrogenesis is not known, appropriate curative drugs have not as yet been developed.

Various substances, including several synthetic compounds and galenic preparations, show hepatoprotective functions both *in vitro* and *in vivo* (Shimizu *et al.*, 1999). Although silymarin has a liver protective effect, inhibiting cytokine production and increasing levels of glutathione (Manna *et al.*, 1999; Shear *et al.*, 1995), the agent is not active in increasing the survival or improvement of the clinical course in liver cirrhosis (Pares *et al.*, 1998). Malotilate and its derivatives, which have been clinically used for the treatment of liver fibrosis (Igarashi *et al.*, 1986), protect the liver from toxic chemicals. The potential mechanism of action for malotilate includes the induction of phase II conjugating enzymes and the inhibition of cytochrome P450s. However, the drugs show only a preventive effect (Okuno *et al.*, 1987; Kim *et al.*, 1994). As no appropriate curative agents are currently available for the treatment of liver fibrosis, the agents mentioned above are frequently used in clinical trials.

Sulfur-containing dithiolthiones, which naturally occur in cruciferous vegetables, and several of their substituents, have liver protecting effects (Mansuy *et al.*, 1986). Of these substituents, oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] has been used clinically as a curative agent against schistosomiasis (Bueding *et al.*, 1982), and its' chemoprotective effects have been extensively studied (Ansher *et al.*, 1983; Bolton *et al.*, 1993). The expression of glutathione S-transferase (GST), which is associated with the suppression of toxicant-induced tissue injuries and carcinogenesis (Kensler *et al.*, 1987; Maxuitenko *et al.*, 1998), is increased by oltipraz in cells and animals (Clapper *et al.*, 1994; Davidson *et al.*, 1990). We have previously revealed that oltipraz exhibited a hepatoprotective effect against γ-ray irradiation (Kim *et al.*, 1997; 1998), which might result from the induction of phase II detoxifying enzymes, including GST. Recently, we reported that oltipraz suppressed dimethylnitrosamine (DMN)-induced tissue destruction and the subsequent replacement by connective tissue in rats treated with multiple doses of DMN, prevented liver fibrogenesis (Kang *et al.*, 2002).

DDB (dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate), a component derived from *Shizandrae*, is a curative agent for the treatment of hepatitis used clinically in East Asia (e.g. China and Korea). It protects liver tissue against carbon tetrachloride-, galactosamine-, thioacetamide- or prednisolone-induced injuries, and enhances antibody production (Liu *et al.*, 1982; Kim *et al.*, 1995a, 1995b). A long term randomized-controlled human study has shown DDB to substantially improve the liver function of patients with the hepatitis B virus (Lee *et al.*, 1991). We have reported that the pharmacological effect of DDB was associated with the inhibition of NF-κB activation and TNF-α production (Kim *et al.*, 2000).

In view of the protective effects of oltipraz against liver fibrogenesis, and the curative effect of DDB against a variety of hepatotoxicants, the present study was designed to determine whether oltipraz in combination with DDB was active against DMN-induced liver fibrogenesis. Furthermore, we established the adequate ratio of oltipraz to DDB as part of our efforts in developing pharmaceutical compositions active against liver fibrogenesis.

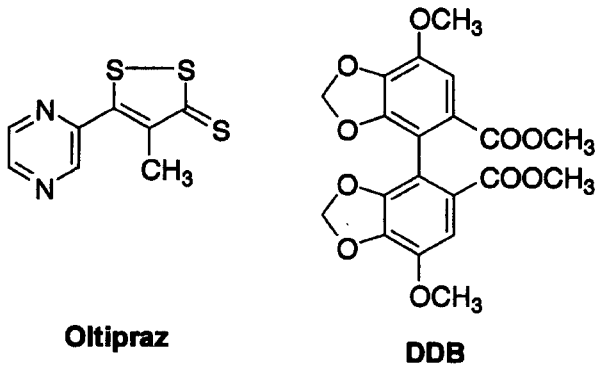
MATERIALS AND METHODS

Materials

The oltipraz was supplied by Aventis Pharma France (Vitry-sur-Seine, France, Fig 1A), the DDB was kindly provided from Taerim Pharmaceutical Co. (Seoul, Korea, Fig 1B) and the DMN other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals

All the animal studies were conducted in accordance with the institutional guidelines for care and use of laboratory animals. Sprague-Dawley rats at 6 weeks of age (140-160 g) were provided by Dai-Han Biolink (Eumsung, Korea). They were acclimatized for 1 week, and maintained in a clean room at the Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University. The animals were caged under a supply of filtered pathogen-free air, and provided with



Oltipraz **DDB**

Fig. 1. Chemical structures of oltipraz and DDB.

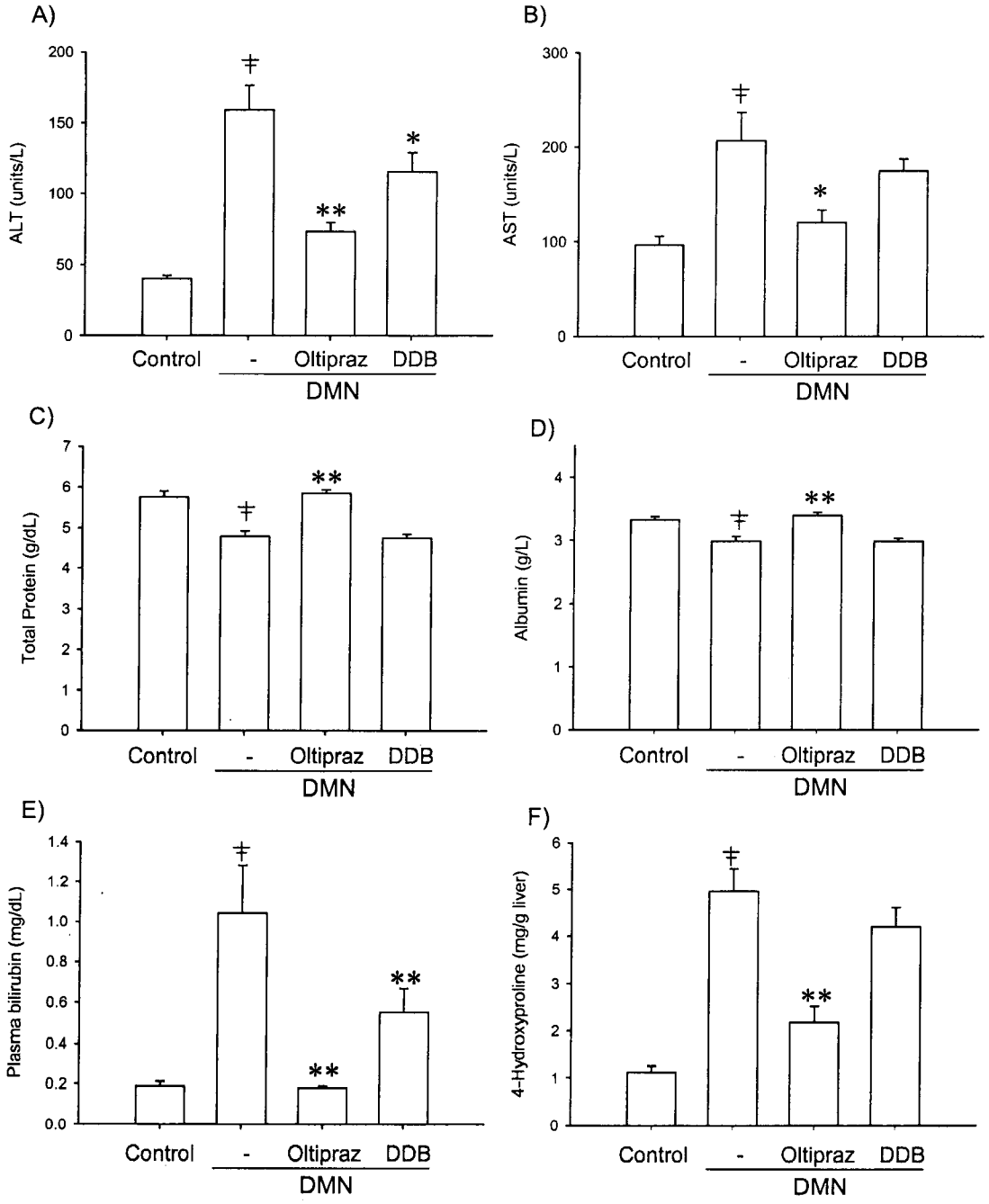


Fig. 1. The plasma alanine aminotransferase activity (A), aspartate aminotransferase activity (B), total plasma protein (C), albumin (D) and bilirubin (E) contents. The 4-hydroxyproline content (F) in the liver homogenate was measured as a marker for collagen accumulation. Animals were treated with DMN (10 μl/kg) dissolved in sterile saline (10 μl /kg body weight, ip, 3 times per week) for 3 weeks, then subjected to recovery for a week. Oltipraz (30 mg/kg), or DDB (30 mg/kg), was orally administered suspended in 40% polyethylene glycol 400 (3 times per week for 4 weeks). Blood chemistry was analyzed using an automatic blood chemistry analyzer. 4-Hydroxyproline was assayed by HPLC. The values are the mean ± SD (n=6-10). Paired Student *t* test was used to determine the significance between the two group means. One-way analysis of variance procedures were used to assess significant differences among the treatment groups (significant as compared to DMN alone, $^{*}p < 0.05$, $^{**}p < 0.01$; significant as compared to untreated control, $^{‡}p < 0.01$).

commercial rat chow (Purina, Korea) and water *ad libitum*. The cages were maintained at between 20°C and 23°C, with a 12 h light and dark cycle and relative humidity of 50% DMN, dissolved in sterile saline, was intraperitoneally injected (10 μl/kg body weight) 3 times per week for 3 weeks, and the rats were then housed for 1 week with no further treatment. The animals used as controls received a vehicle injection. Oltipraz (30 mg/kg), DDB (30 mg/kg) or

a mixture of oltipraz and DDB (25 vs. 5 mg/kg; 15 vs. 15 mg/kg; and 5 vs. 25 mg/kg), suspended in 40% polyethylene glycol 400, were orally administered 3 times per week for 4 weeks. The animals were sacrificed on day 28 under light anesthesia with diethyl ether. Blood was collected from the *vena cava*. The left lateral lobes were subjected to histopathological examination. To evaluate the effect of combination treatments, the dosing schedule of DMN was modified to 3-week treatment regimen.

Blood chemistry

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, total plasma proteins and albumin were analyzed using an automatic blood chemistry analyzer (Spectrum, Abbott Laboratories, Abbott Park, IL).

Assay of 4-hydroxyproline in the liver

The 4-hydroxyproline content in the liver was determined by the methods described by Kondo *et al.* (1997), with modifications. Briefly, the right lobe of the liver (0.5 g) was homogenated with deionized water, and 0.4 ml of the liver homogenate was hydrolyzed in 1 ml of 9 M HCl. The samples were incubated at 110°C for 24 h. After cooling, the hydrolysate was diluted with 1.5 ml of 0.26 M borate buffer (pH, 9.5) and centrifuged at 10,000*g* at room temperature. The pellet was discarded. The supernatant was diluted with 4 times its volume of 0.26 M borate buffer (pH, 9.5). A high-performance liquid chromatographic method was used for determination of hydroxyproline. First, the primary amino group in liver homogenate was blocked with O-phthalaldehyde, then histidinohydroxylysinonorleucine in the hydrolysate was labeled with 9-fluorenylmethyl chloroformate. The samples were then analyzed by reversed-phase high-performance liquid chromatography at 40°C [Kromasil C18 column, 25 cm, 5 μM; flow rate, 1.5 ml/min; Mobile phase A, 0.08 M NaCl with 0.3% acetic acid: acetonitrile (70:30): Mobile phase B, 0.08 M NaCl with 0.3% acetic acid: acetonitrile (30:70) with gradient] with a fluorescence detector (260, 310 nm). The retention time of 4-hydroxyproline was ~6.1 min.

Histopathology

The hepatic morphology was assessed by light microscopy. The left lateral lobe of the liver was sliced (3 slices for each rat) and fixed in 10% buffered-neutral formalin for 6 h (pH 7.4). The fixed liver tissue slices were processed and embedded in a paraplast automatic tissue processor, Citadel 2000 (Shandon Scientific, Cheshire, U.K.). Sections of 4 μm in thickness were subjected to hematoxylin, eosin and Masson's trichrome staining (Kremer *et al.*, 1989). A certified pathologist scored samples in a blinded fashion. An arbitrary scope was given to each microscopic field viewed at magnifications of 100-200 times. A minimum of 10 fields were scored per liver slice to obtain a mean value. The extent of fibrosis was graded as 0, absent; 1, trace; 2, mild; 3, moderate; and 4, severe. Extents of periportal bridging, intralobular degeneration, portal inflammation and fibrosis were also graded according to the Knodells scoring method (Moragas *et al.*, 1998).

Data analysis

Paired Student's *t* tests were used to determine any significance between the two group means. One-way analysis of variance procedures were used to assess significant differences among the treatment groups. The criterion for statistical significance was set at $p < 0.05$ or $p < 0.01$.

RESULTS

Oltipraz, but not DDB, inhibits liver fibrogenesis

In the present study, we determined the effects of oltipraz or DDB on the liver fibrogenesis induced by 3 weeks of DMN treatment. The activities of plasma ALT and AST were increased 2~4-fold in rats following DMN treatment, as compared to the controls (Fig 2A and 2B). Oltipraz, at a dose of 30 mg/kg body weight (3 times per week for 4 weeks), decreased (~80%) the plasma aminotransferase activities that had been induced by the DMN. DDB, at a dose of 30 mg/kg, moderately inhibited the activity of AST (34% inhibition), but not that of ALT.

Exposure of rats to DMN caused a ~20% decreases in the total plasma protein and albumin contents, which were restored by treatment with oltipraz, but not by DDB (Fig 2C and 2D). The total plasma protein and albumin contents reflect the extent of protein synthesis in the liver. In liver fibrosis, the total plasma protein generally decreases. Oltipraz was active in restoring the total plasma

Table 1. Effects of a combination of oltipraz plus DDB on the body weight, liver weight and survival rate of DMN-treated rats

Treatment	Body weight (g)	Liver weight (g wet)	Survival rate (live/total)
Untreated control	291 ± 25	9.8 ± 1.2	10/10
DMN (10 ml/kg)	150 ± 30‡	3.7 ± 1.5‡	6/11
DMN+oltipraz 25+DDB 5 mg/kg	220 ± 27**	8.8 ± 1.7**	10/10
DMN+oltipraz 15+DDB 15 mg/kg	201 ± 41**	7.3 ± 2.7**	9/10
DMN+oltipraz 5+DDB 25 mg/kg	205 ± 23**	6.8 ± 1.5**	10/10

Each value was determined 28 days after DMN or vehicle treatment. Values represent the mean ± SD in live animals (n=6-10). Paired Student *t* test was used to determine the significance between the two group means. One-way analysis of variance procedures were used to assess significant differences among the treatment groups (significant as compared to DMN alone, **$p < 0.01$; significant as compared to untreated control, ‡$p < 0.01$).

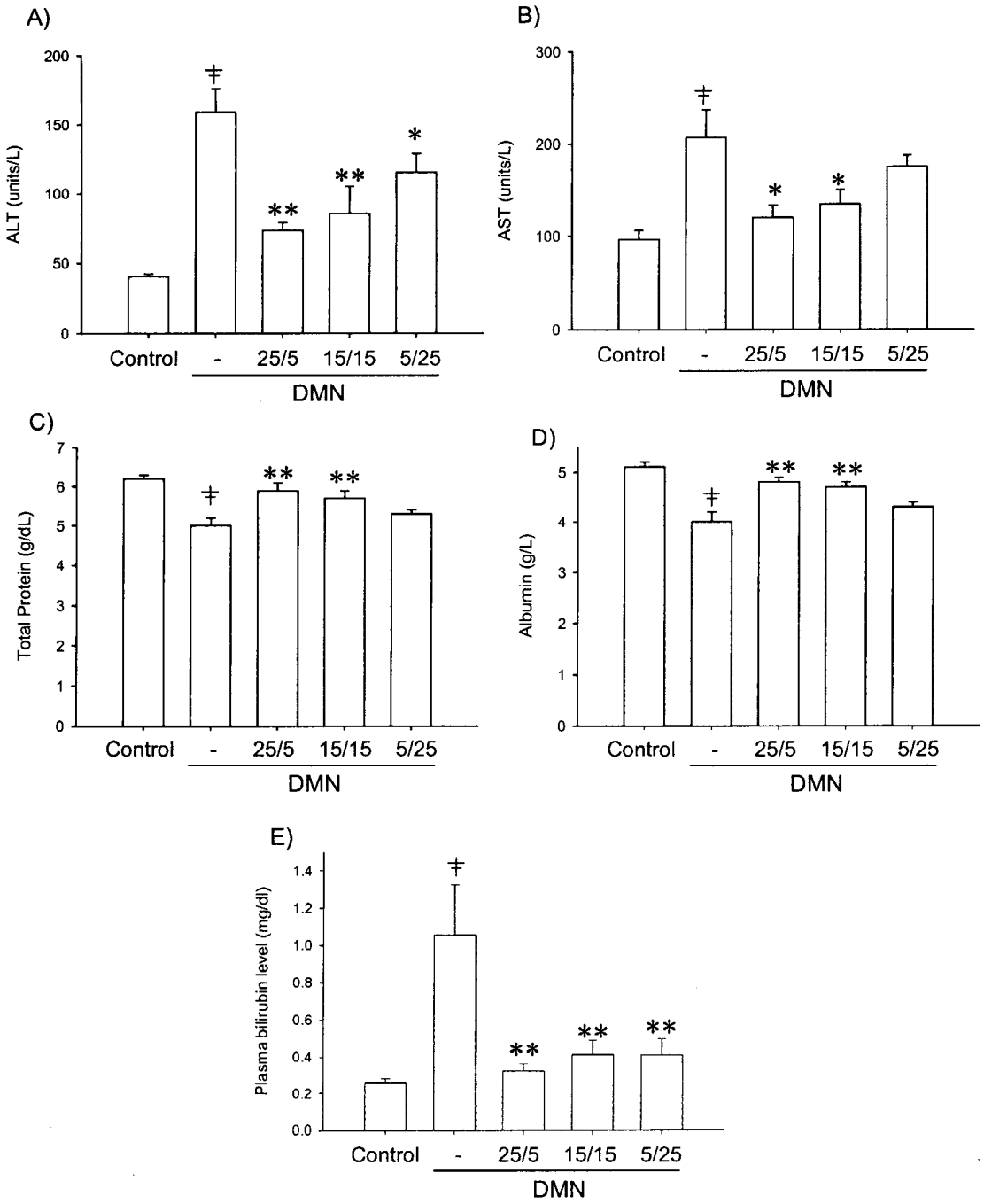


Fig. 3. Parameters for liver function. Blood chemistry was analyzed using an automatic blood chemistry analyzer. Values are the mean ± SD (n=6-10 live animals). Paired Student *t* test was used to determine the significance between the two group means. One-way analysis of variance procedures were used to assess significant differences among the treatment groups (significant as compared to DMN alone, *$p < 0.05$, **$p < 0.01$; significant as compared to untreated control, ‡$p < 0.01$). 25/5, 25 mg/kg oltipraz+5 mg/kg DDB; 15/15, 15 mg/kg oltipraz+15 mg/kg DDB and 5/25, 5 mg/kg oltipraz+25 mg/kg DDB (orally 3 times per week for 4 weeks).

protein content, whereas DDB was ineffective. Both oltipraz and DDB significantly inhibited the increase in the total bilirubin content caused by DMN (Fig 2E).

The inhibition of liver fibrogenesis caused by oltipraz was confirmed by the decrease in the 4-hydroxyproline content in the liver, which correlated well with the reduction in the hepatic collagen accumulation. The 4-hydroxyproline content was increased ~5-fold in the liver of rats treated for 3 weeks with DMN compared to healthy control rats (i.e. 5.0 vs. 1.1 mg/g liver). Oltipraz treatment suppressed the DMN-

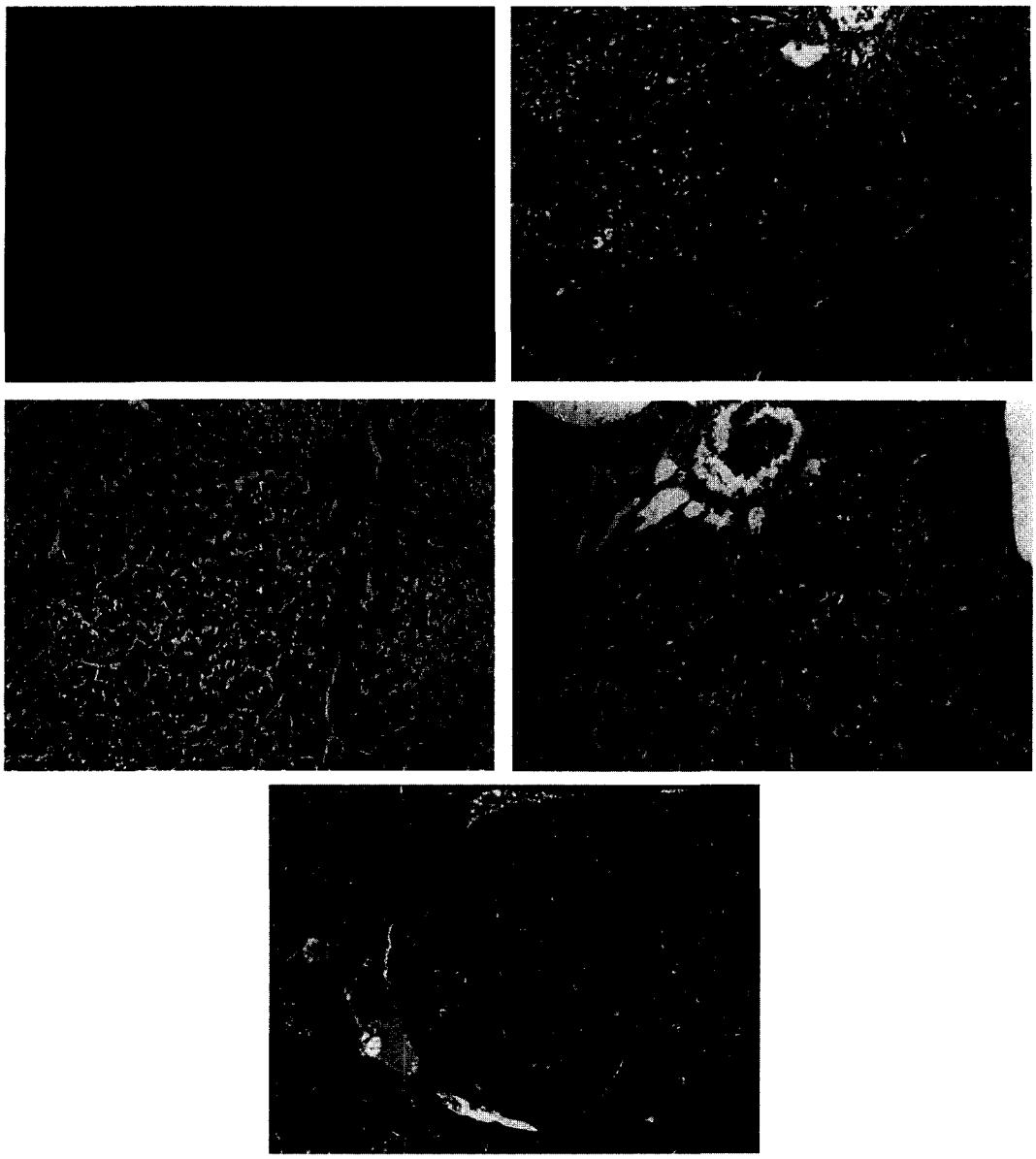


Fig. 4. Representative microphotographs. Shown above are Masson's trichrome staining of liver sections from rats treated with vehicle (A), DMN (10 μl/kg, 3 times per week for 3 weeks) (B), oltipraz 25 mg/kg+DDB 5 mg/kg+DMN (C), oltipraz 15 mg/kg+DDB 15 mg/kg+DMN (D) or oltipraz 5 mg/kg+DDB 25 mg/kg+DMN (E). A, Low power view of normal control liver section showing no pathological changes (× 100); B. Low power view of liver section showing nodular appearance surrounded by thick fibrous bands (× 100); C. Low power view of liver section showing nodular appearance surrounded by thick fibrous bands (× 100); D. Low power view of liver section showing a thin fibrous band (× 100); E. Low power view of liver section showing a well preserved central vein (× 100).

induced increase in the 4-hydroxyproline content by 73%. In contrast, DDB failed to decrease the 4-hydroxyproline content in the liver (Fig. 2E). Thus, oltipraz, but not DDB, was effective in reducing the DMN-induced liver fibrogenesis. DDB was active in improving the liver function.

Oltipraz/DDB inhibits DMN-induced liver fibrogenesis

The anti-fibrotic mechanism of oltipraz involves the inhibition of TGF-β expression. Semi-quantitative RT-PCR analysis revealed that oltipraz completely inhibited the expression of TGF-mRNA caused by lipopolysaccharide or DMN (Kang *et al.*, 2002), but induces hepatic phase II detoxifying enzymes (Kim *et al.*, 1997; Kensler *et al.*, 1987). Conversely, DDB failed to increase the expression of phase II enzymes (Kim *et al.*, 2000). Hence, it is plausible that DDB may be complementary to oltipraz and enhance its anti-fibrogenic effect. The anti-fibrotic activity of oltipraz/DDB in combination was evaluated in subsequent experiments.

The body weight of rats decreased to 52% of that of the controls following DMN treatment (291 ± 25 g vs. 150 ± 30 g mean ± SD, n=7-10, $p < 0.01$). Oltipraz/DDB at the ratios of 25:5, 15:5 and 5:25 mg/kg significantly prevented the decrease in the body weight of rats treated with DMN (Table 1). The liver weight was also decreased by DMN to 38% of that of the controls, but this was restored by oltipraz/DDB treatment (Table 1). In particular, the combination ratio of 25:5 (oltipraz:DDB) increased the liver weight up to 70% of that of the controls.

DMN-induced increases in the activities of plasma transaminase were significantly inhibited by treatment of rats with oltipraz/DDB ratios of 25:5, 15:5 or 5:25 (mg/kg). Comparing the efficacies of the combination component ratios, the animals administered oltipraz and DDB at the dosage ratios of 15:15 and 5:25 mg/kg showed significant inhibition in the plasma activities of ALT and AST, with the degree of this inhibition being smaller than those of the animals administered with a dosage ratio of 25:5 mg/kg [49% (15:15) and 34% (5:25) vs. 57% inhibition (25:5)](Fig 3A and 3B).

The plasma total protein and albumin levels were assessed as representative indices of liver function. One week after administering DMN for a 4-week period, the total protein and the plasma albumin contents significantly decreased in treated animals, whereas the contents recovered to normal control group levels in the animal groups administered oltipraz and DDB at dosage ratios of 25:5 or 15:5 mg/kg (Fig 3C and 3D).

The bilirubin content in plasma is used as a representative indicator of hepatic functionality. In rats administered with oltipraz and DDB at dosage ratios of 25:5, 15:5 and 5:25 mg/kg over a 4-week period, the increases in the bilirubin content caused by DMN were inhibited by a statistically significant degree (Fig 3E). Similar to the results of the ALT and AST values, the increases in the bilirubin content were almost completely inhibited (~90%) in the animals administered with oltipraz and DDB at a dosage ratio of 25:5 mg/kg.

The extent of liver fibrosis was histopathologically examined to further reveal the anti-fibrotic effects of the pharmaceutical compositions of oltipraz and DDB. No pathological changes were observed in the liver of the control rats (Fig 4A). Masson's trichrome staining, which was used to assess the extracellular matrix, revealed that rats treated with DMN exhibited cirrhotic liver morphology, such as multiple fibrotic nodules. Distinct fibrotic bands in the hepatic tissue were observed in rats one week after administering DMN for 3-weeks, 3 times a week (Fig 4B). In the animals administered with oltipraz and DDB at dosage ratios of 25:5 and 15:15 mg/kg for 4-weeks (3 times a week), one day after the administration of DMN and then treated for an additional week (3times) on the fourth week, the intensity and incidence of fibrotic band extensions in the hepatic tissue were significantly reduced compared to when DMN was administered alone (Fig. 4C and 4D). Especially, the animal group administered with oltipraz and DDB at a dosage ratio of 25:5 mg/kg (Fig. 4C) showed superior efficacy of the drug compared to that of the dosage ratios of 15:15 mg/kg (Fig. 4D) and 5:25 mg/kg (Fig. 4E), and strongly inhibited the progress of hepatic fibrosis. The degree of hepatic fibrosis was also determined by evaluating the fibrosis and Knodell scores, which show degrees of liver damage and fibrosis. Multiple analyses confirmed that the extent of liver fibrosis was significantly reduced by treatment at all oltipraz and DDB dosage ratios compared to that caused by DMN alone (Table 2).

Table 2. Fibrosis and Knodell scores in the livers of DMN-induced fibrotic rats treated with oltipraz/DDB.

Treatment	Fibrosis score	Knodell score
Untreated control	0	0
DMN (10 ml/kg)	2.9 ± 0.9‡	11.7 ± 1.8‡
DMN+oltipraz 25+DDB 5 mg/kg	0.3 ± 0.5**	2.0 ± 1.5**
DMN+oltipraz 15+DDB 15 mg/kg	0.3 ± 0.5**	4.0 ± 1.2**
DMN+oltipraz 5+DDB 25 mg/kg	1.2 ± 0.6**	6.0 ± 1.3**

The values represent the mean ± SD (n=6-10 live animals). Paired Student t test was used to determine the significance between the two group means. One-way analysis of variance procedures were used to assess significant differences among the treatment groups (significant as compared to DMN alone, **$p < 0.01$; significant as compared to untreated control, $p < 0.01$). Extents of fibrosis were graded as 0=no increase; 1=slight increase; 2=moderate increase; 3=distinct increase and 4=severe increase. Extents of periportal bridging (severe=10), intralobular degeneration (severe=4), portal inflammation (severe=4) and fibrosis (severe=4) were also graded according to the Knodells scoring method. The score represents the sum of each severity score.

DISCUSSION

The present study shows that a pharmaceutical composition containing oltipraz and DDB is effective in the inhibition of liver fibrosis. The protective effects of oltipraz/DDB combinations on hepatic fibrosis were observed with various ratios in rats administered with DMN. The tests were conducted with the weight ratios of 25:5, 15:15 and 5:25 (mg/kg) of oltipraz:DDB. In the results, when DMN was administered (observation was conducted 1 week after 3 weeks of administration), ALT, AST, bilirubin, fibrosis score and Knodell score were significantly increased compared to those of the control group. In contrast, these values decreased in the rats treated with the oltipraz/DDB combinations. Oltipraz (25 mg/kg)/DDB (5 mg/kg) showed the most inhibitory effect against hepatic fibrosis. These results prove that oltipraz and DDB show synergism in

suppressing liver damage and fibrosis, with an optimal ratio of 5:1.

Furthermore, the administration of a combination of oltipraz/DDB seemed to reduce the blood biochemical indices, liver fibrosis score and Knodell score to a greater extent than oltipraz alone. In our previous study, oltipraz at a dose of 5 mg/kg did not prevent the defective functionality and fibrosis in the liver of rats treated with DMN for 4 weeks (Kang *et al.*, 2002). We have now demonstrated that administration of oltipraz and DDB at a dosage ratio of 1:5 significantly decreases the Knodell and liver fibrosis scores. This complementary anti-fibrotic effect of the compositions may result from the activation of the distinct pharmacological targets for oltipraz and DDB. We have demonstrated that the pharmacological effect of DDB resulted from the inhibition of NF-κB activation and TNF-α production (Kim *et al.*, 2000). Since NF-κB is known as a transcription factor mediating the inflammatory response, an inhibitor of NF-κB would be capable of inhibiting the systemic inflammatory response. DDB inhibits the inflammatory response and hepatocyte injuries induced by DMN (Kim *et al.*, 1997). Oltipraz suppresses the expression of TGF-β and increases the expression of antioxidant enzymes, with the subsequent elevation of cellular GSH (Kang *et al.*, 2002; 3; Stohs *et al.*, 1986). In addition, it was reported that DDB suppresses the CYP3A metabolic activity in human liver microsomes (Kim *et al.*, 2001). Hence, it is likely that DDB inhibits the metabolism of oltipraz. Hence, low doses of oltipraz in combination with DDB may exert this antifibrotic effect for an extended period of time. By this mechanism, the concomitant treatment of oltipraz with DDB maximizes the preventive and therapeutic effects for the treatment of liver fibrosis. Accordingly, the clinical application of oltipraz with DDB may reduce the potential side effects, such as disorders of the gastrointestinal tract and the paraesthesia of the hands and feet, which may result from the admini-stration of larger doses of oltipraz (Pendyala *et al.*, 2001; Zhang *et al.*, 1997).

In summary, oltipraz prevented liver fibrosis induced by DMN, while DDB was marginally effective in the restoration of liver function. Concomitant treatments with oltipraz/DDB at the dosage ratios of 25:5, 15:5 and 5:25 mg/kg effectively inhibited liver fibrogenesis. A pharmaceutical composition containing oltipraz and DDB may be safely used for the long-term treatment and prevention of liver fibrosis and cirrhosis.

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