Effect of Dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2,2'-dicarboxylate (DDB) on Chemical-Induced Liver Injury

Su Nyeon KIM,^a Sung Yeon KIM,^a Hye Kyung YIM,^a Woo Young LEE,^b Kwang Su HAM,^c Sang Kyum KIM,^a Mi Young YOON,^a and Young Chul KIM^{*a}

College of Pharmacy, Seoul National University,^a San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul 151-742, Korea, Pharmaceutical & Health Science Research Institute, Pacific Corporation,^b 314-1 Bora-Ri, Yongin, Kyonggi-Do, Korea, and Pacific Pharmaceutical Co.,^c 59-6 Gungi-Ri, Ansong-Kun, Kyonggi-Do, Korea.

Received August 10, 1998; accepted October 14, 1998

The effects of orally administered dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene-dioxybiphenyl-2,2'-dicarboxylate (DDB) on the hepatotoxicity induced by carbon tetrachloride, acetaminophen or ethanol were investigated in rats and mice. Either single or repeated DDB pretreatment (50 or 200 mg/kg) did not alter the hepatotoxicity induced by carbon tetrachloride (0.2 or 1.0 ml/kg, i.p.) in female rats as indicated by increases in the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH) in serum. The hepatotoxicity of acetaminophen (350 mg/kg, i.p.) was also unaffected in male mice pretreated with DDB (50 mg/kg/d) for a week. However, DDB administration (50 mg/kg/d for 7 d) decreased the hepatic fatty degeneration induced by repeated ethanol treatment (0.75 g/kg, i.p., $\times 2$ times a day for a week) in rats as shown by the accumulation of triglycerides and cholesterol in the liver. Malondialdehyde (MDA) formation in liver homogenates was inhibited by DDB treatment. The significance of the action of DDB on alcoholic fatty liver generation in clinical settings is discussed.

Key words DDB; antihepatotoxicity; alcoholic fatty liver; lipid peroxidation

Dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2,2'-dicarboxylate (DDB) was first found as an intermediate for synthesizing schizandrin C, one of the natural compounds isolated from *Fructus Schizandrae chinensis*, which has long been used as a tonic and sedative in traditional medicine. Its alcoholic extract has also been used in treating patients with viral or chemical hepatitis.¹⁾ DDB, along with some other compounds isolated from the plant, was reported to inhibit the hepatic damage induced by carbon tetrachloride (CCl₄) or galactosamine in *in vitro* models and also to alleviate the symptoms of chronic hepatitis B in humans.^{2,3)} At present DDB is the most widely used remedy for liver injury in Korea since its first introduction in the early 1990's. Although clinical experience has shown that DDB is effective in lowering the serum alanine aminotransferase (ALT) activity in patients with chronic hepatitis B³⁾ and hepatitis resulting from chemotherapy of hepatocarcinoma,⁴⁾ the hepatoprotective action of DDB in experimental animal models has not been established yet,^{5,6)} and is still controversial. Accordingly, the mechanism by which DDB reduces hepatic damage remains unknown, partly due to a lack of appropriate *in vivo* animal models. In this study the effects of DDB administration on the toxic consequences of frequently encountered hepatotoxicants, *i.e.*, CCl₄, acetaminophen (APAP), and ethanol, were compared.

MATERIALS AND METHODS

Chemicals DDB was obtained from Pacific Pharmaceuticals Co. (Ansong, Korea). CCl₄, APAP, and ethanol was purchased from Junsei Chemical Co. (Tokyo, Japan), Sigma Chemical Co. (St. Louis, MO, U.S.A.), and Merck (Damstadt, Germany), respectively. All chemicals used in this study was reagent grade or better.

Animals and Treatments Adult female Sprague-Dawley rats and male ICR mice were obtained from the Experi-

mental Animal Breeding Center of Seoul National University. The animals were housed in temperature (22 $\pm$ 2 °C) and humidity (55 $\pm$ 5%) controlled rooms with a 12 h light/dark cycle for at least 3 weeks before use. Body weights of rats and mice were in the range of 200–250 g and 30–40 g, respectively, when used in the experiments. Rodent chow and tap water were allowed *ad libitum* unless stated otherwise. In the acute study DDB (50 or 200 mg/kg), as a suspension in 10% Tween 80, was administered orally to rats. CCl₄ (0.2 or 1.0 ml/kg) dissolved in corn oil was injected intraperitoneally 2 h after the DDB treatment. In the repeated treatment study, DDB was given orally to rats and mice at 50 or 200 mg/kg/d for a week. The rats were treated with CCl₄ (0.2 or 1.0 ml/kg, i.p.), and mice with APAP (350 mg/kg, i.p.), 24 h following the final dose of DDB. Diet, but not drinking water, was removed 18 h before treatment with CCl₄ or APAP. Ethanol was injected into rats at a dose of 0.75 g/kg, i.p., twice a day (10 a.m. and 2 p.m.), for one week. DDB, at a daily dose of 50 mg/kg, was given to rats for the same period. Rats were fasted from the last dosing of ethanol until sacrifice at 10 a.m. on the following day. Normal saline was used as a vehicle for APAP and ethanol injections.

Assays Rats and mice were sacrificed 24 h after the CCl₄ or APAP treatment. Blood samples were collected by cardiac puncture on animals under light ether anesthesia. The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST),⁷⁾ and sorbitol dehydrogenase (SDH)⁸⁾ in serum was assessed to determine the liver injury. In the ethanol study rats were sacrificed 20 h following the last dosing of ethanol. Their livers were excised after exsanguination and processed for determination of triglyceride,⁹⁾ cholesterol,¹⁰⁾ glutathione (GSH),¹¹⁾ and malondialdehyde (MDA) levels to estimate lipid peroxidation.¹²⁾

Statistical Analysis All results expressed as means $\pm$ S.E. were analyzed by a two-tailed Student's *t*-test. The acceptable level of significance was established at *p*<0.05.

* To whom correspondence should be addressed.

RESULTS AND DISCUSSION

In rats CCl_4 was significantly hepatotoxic at the doses used as shown by the increase in SDH, ALT and AST activity in serum (Table 1). DDB (50 or 200 mg/kg) given 2 h prior to CCl_4 did not affect the hepatotoxicity of this halogenated hydrocarbon. A daily dose of DDB (200 mg/kg) administered repeatedly for a week did not down-modulate the elevation in hepatotoxic parameters induced by a single dose of CCl_4 . An i.p. dose of APAP (350 mg/kg) resulted in a marked increase in serum SDH, ALT and AST activity (Table 2). DDB administered for a week at a daily dose of 50 mg/kg prior to APAP challenge did not modify these hepatotoxic parameters.

The protective effect of DDB on chemically-induced hepatic damage in animal models is still a matter in controversy. Liu *et al.*¹³⁾ reported that DDB, along with other compounds isolated from *Fructus Schizandrae*, decreased the CCl_4 hepatotoxicity in mice. It was also shown that DDB inhibited CCl_4 -induced lipid peroxidation and binding of CCl_4 metabolites to the microsomal lipids.¹⁴⁾ On the other hand, other investigators failed to demonstrate any protective effects of DDB in mice and rats against damage induced by model hepatotoxicants such as CCl_4 , APAP, cadmium chloride and allyl alcohol.^{5,6)} The results of our study are in agreement with the observations made by the latter investigators.

The effect of DDB administration on fatty liver induced by ethanol was examined in rats. Ethanol given intragastrically twice daily, at a dose of 0.75 g/kg each, for a week induced fatty degeneration as indicated by the increased level of triglycerides and cholesterol in hepatic tissue (Table 3). MDA formation was also enhanced significantly. When a daily dose of 50 mg/kg DDB was administered concomitantly during the period of ethanol treatment, the hepatic level of triglycerides and cholesterol was reduced to the normal range and MDA formation was decreased. Lu and Liu¹⁵⁾ reported that structural analogues of DDB, schizandrin B and C, along with other dibenzo[a,c]cyclooctene lignans isolated from *Fructus Schizandrae*, inhibited Vit C/NADPH-induced lipid peroxidation in rat liver microsomes. Oral administration of schizandrin B to mice also markedly reduced hepatic MDA formation induced by ethanol and increased the activity of hepatic superoxide dismutase and catalase. Thus, the decrease in induction of alcoholic fatty liver by DDB observed in this study appears to be associated with inhibition of lipid peroxidation *via* its direct antioxidative activity and/or effect on antioxidant enzyme systems in the liver.

The results of our study indicate that DDB may not inhibit the hepatic damage induced by necrogenic doses of chemicals such as CCl_4 or APAP, but is capable of preventing the reversible damage induced by ethanol in rodent models. The discrepancy between the positive clinical experience with DDB as a hepatoprotective agent and the controversial results in animal models appears to be related to these observations. In general chemical-induced hepatic injury in experimental animals is extremely severe compared with the injury observed in human patients. If the hepatoprotective effect of DDB is due mainly to its antioxidative action, it may not be effective enough when necrosis develops rapidly following a massive dose of potent hepatotoxicant. On the other hand,

Table 1. Effect of DDB Administration on CCl_4 -Induced Hepatotoxicity in Rats

	DDB (mg/kg, p.o.)	CCl_4 (ml/kg, i.p.)	SDH	ALT	AST
			(units/ml)		
Exp. I	0	0	4±2	28±5	81±5
	0	0.2	1596±541	451±157	740±112
	50	0.2	1963±506	445±130	799±125
	200	0.2	1363±202	514±162	750±132
	0	1.0	4481±537	2343±289	2952±210
	200	1.0	4303±440	2335±305	3092±171
Exp. II	0	0	13±2	35±2	68±4
	0	0.2	1601±368	372±53	584±61
	200	0.2	2025±400	498±91	615±82
	0	1.0	4641±212	2137±211	2061±116
	200	1.0	4851±234	1808±174	2093±130

A single dose of DDB (50 or 200 mg/kg, p.o.) was administered 2 h prior to CCl_4 treatment (0.2 or 1.0 ml/kg, i.p.) in Exp. I. In Exp. II, DDB (200 mg/kg/d, p.o.) was administered repeatedly for a week and CCl_4 was injected 24 h following the last dose of DDB. Values represent means±S.E. for 6 to 8 animals.

Table 2. Effect of DDB Administration on APAP-Induced Hepatotoxicity and Mortality in Mice

	SDH	ALT	AST	Mortality
	(units/ml)			(No. of dead/No. of animals used)
Control	26±3	45±2	78±4	0/8
APAP	4656±1172	4995±1325	3507±986	4/12
DDB+APAP	6700±821	5499±1263	4426±668	4/8

DDB (50 mg/kg/d, p.o.) was administered repeatedly for a week and APAP (350 mg/kg, i.p.) was injected 24 h following the last dose of DDB. Values represent means±S.E. for surviving animals.

Table 3. Effect of DDB Administration on Ethanol-Induced Hepatic Fatty Degeneration in Rats

	Triglyceride	Cholesterol	Glutathione	MDA formation
	(mg/g liver)	(mg/g liver)	(μmol/g liver)	(nmol/g liver)
Control	10.4±1.2	4.9±0.5	5.40±0.51	199.0±38.8
Ethanol	26.8±3.1	7.1±0.2	3.75±0.15	538.5±80.2
DDB+Ethanol	12.6±2.0**	6.0±0.3*	2.88±0.37	185.8±14.0**

Ethanol was given at an intraperitoneal dose of 0.75 g/kg each, twice a day, repeatedly for one week. DDB was administered orally at 50 mg/kg/d for one week. Values represent means±S.E. for 6 animals. An asterisk denotes a significant difference from the group treated with ethanol only (*, $p<0.05$; **, $p<0.01$, Student's *t*-test).

when hepatic injury proceeds gradually as in the case of chronic viral infection and/or chronic ethanol consumption in humans, DDB could be effective in attenuating the retarded induction of damage in hepatocytes by inhibiting the aggravating effect of oxidative damage. The protective effect of DDB against ethanol-induced fatty liver has been observed for the first time in our study. Considering the fact that alcohol ingestion is the predominating cause of hepatic disorders in humans, the applicability of DDB is likely to increase and further investigations are needed to elucidate its underlying mechanism of action.

Acknowledgement This work was supported in part by a grant from Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University.

REFERENCES

- 1) Bao T. T., Liu G. T., Xu G. F., Sung C. Y., *Chinese Med. J. (Peking)*, **54**, 275—277 (1974).
- 2) Fu T., Liu G., *Biomed. Environ. Sci.*, **5**, 185—194 (1992).
- 3) Liu G. T., *Proc. CAMS and PUMC*, **2**, 123—126 (1987).
- 4) Yu H. Q., Yang X. U., Zhang Y. X., Shi J. Z., *Chinese Med. J. (Peking)*, **100**, 122—123 (1987).
- 5) Liu J., Liu Y., Klaassen C. D., *J. Ethnopharmacol.*, **42**, 183—191 (1994).
- 6) Kim S. G., Nam S. Y., Chung H. C., Hong S. Y., Jung K. H., *J. Pharm. Pharmacol.*, **47**, 678—682 (1995).
- 7) Reitman S., Frankel S. K., *Am. J. Clin. Pathol.*, **28**, 56—63 (1957).
- 8) Gerlach U., "Method in Enzymatic Analysis," ed. by Bergmeyer H.U., A. E. Harper, New York, 1965, pp. 761—764.
- 9) Van Handel E., Zilversmit D. B., *J. Lab. Clin. Med.*, **50**, 152—157 (1957).
- 10) Zlatis A., Zak B., *Anal. Biochem.*, **29**, 143—148 (1969).
- 11) Griffith O. W., *Anal. Biochem.*, **106**, 207—212 (1980).
- 12) Ohkawa H., Ohishi N., Yagi K., *Anal. Biochem.*, **95**, 351—358 (1979).
- 13) Liu G. T., Wang G. F., Wei H. L., Bao T. T., Song Z. Y., *Acta Pharm. Sinica*, **14**, 598—604 (1979).
- 14) Liu K. T., Lesca P., *Chem. Biol. Interact.*, **41**, 39—47 (1982).
- 15) Lu H., Liu G. T., *Chem. Biol. Interact.*, **78**, 77—84 (1991).