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Effect of Biphenyl Dimethyl Dicarboxylate on Chemical-Induced Hepatotoxicity

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ABSTRACT : *To know the mechanism of biphenyl dimethyl dicarboxylate (DDB) in the protection of chemically induced hepatotoxicity, the activity of glutamic pyruvic transaminase (GPT) and the level of lipid peroxidation metabolite (malondialdehyde, MDA) and ATP content in hepatocytes were determined in serum and primarily cultured hepatocytes. For in vivo study, rats were pretreated with DDB (300 mg/kg, p.o.) for 7 days. DDB pretreatment efficiently reduced the elevation of serum GPT activity induced by carbon tetrachloride (1.6 ml/kg, s.c.) and acetaminophen administration (1500 mg/kg, i.p.). In ex vivo study, hepatocytes were isolated from the rats pretreated with DDB (300 mg/kg, p.o.) for 7 days and cultured for 12 hrs before inducing cytotoxicity with chemicals. The MDA formation and the GPT release induced by adriamycin (1×10^{-4} mg/ml) and cisplatin (2×10^{-4} mg/ml) were markedly decreased in the hepatocytes from the rats pretreated with DDB as compared to vehicle only. However, DDB pretreatment did not prevent the decrease of ATP contents of hepatocytes induced by cisplatin and adriamycin. In in vitro experiment, DDB was pretreated in primary cultured hepatocytes for 3 days. DDB enhanced the decreases of ATP contents induced by cisplatin and adriamycin. These results suggest that DDB may protect the hepatocytes from injury induced by hepatotoxicants through inhibiting the lipid peroxidation.*

Key Words : *Biphenyl dimethyl dicarboxylate, ATP, Hepatocyte.*

I. INTRODUTION

Fructus Schizandrae sinensis Bail, a traditional chinese medicine, has been shown to lower the elevated serum glutamic-pyruvic transaminase (SGPT) levels of patients suffering from chronic viral hepatitis (Lee *et al.*, 1991). A synthetic derivative compound of Schisandrin, biphenyldimethyl dicarboxylate (DDB), is now used widely in clinical fields as a hepatoprotective drug. Several studies showed that DDB efficiently decreases malondialdehyde (MDA) formation and GPT release from hepatocyte in carbon tetrachloride, D-galactosamine (Fu & Liu, 1990) and ethanol-induced hepatotoxicity (Song *et al.*, 1994). It has been suggested that DDB acts on hepatocytes by inducing hepatic microsomal drug-metabolizing enzyme system (Liu & Lesca, 1982) and promoting antioxidative effect (Song *et al.*, 1994).

However it was also reported that DDB did not prevent acute liver injury induced by carbon tetrachloride, acetaminophen, cadmium chloride and allyl alcohol, assessed by quantifying serum activities of sorbitol dehydrogenase and GPT as well as by histopathological examination in CF-1 mice (Liu *et al.*, 1994). The hepatoprotective effects of DDB against chemical toxins are in debate. But DDB is still an important candidate for the treatment of drug induced hepatotoxicity.

Viability measurements with mammalian cell systems can be divided four groups, permeability, functional, morphological and reproductive assays (Cook & Mitchell, 1989). The permeability assays include the trypan blue exclusion assay, lactate dehydrogenase release assay, GPT assay, *et al.* All being sensitive to membrane damage. The functional assays are the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide reduction assay, ATP assay *et al.* Morphological assays are used to detect changes occurring at cell surface.

Until now, the most data supporting hepa-

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toprotective effects of DDB are confinded to permeability and morphological observations as viability parameters. Thus, it is important to know whether DDB has beneficial effect on metabolic function of chemically damaged hepatocytes.

To investigate the effects of DDB on the change of metabolic function, membrane permeability and the production of lipid peroxide metabolite induced by toxic dose of several chemicals, present study was performed *in vivo* and *ex vivo*, *in vitro* using primary cultured hepatocytes.

II. MATERIALS AND METHODS

1. Animals

Male Sprague Dawley rats, weighing 200-250 g, were used throughout the experiment.

2. *In vivo* Test

After the acclimation period, the rats were divided into 6 groups. Rats of 3 groups (I, II, III) received DDB (300 mg/kg, p.o.) and normal diet for 7 days before the treatment with hepatotoxicants. Rats of 3 groups (IV, V, VI) received normal diet and normal drinking water. On 7th day, groups I and IV received acetaminophen (1500 mg/kg, i.p.), groups II and V received carbon tetrachloride (1.6 ml/kg, s.c.). Either normal saline or corn oil vehicle was used for the administration of the hepatotoxicants as indicated in Fig. 1. After 24 hrs, for the determination of hepatotoxicity, blood was obtained from rat orbit and serum GPT activiy was assayed using Sigma Chemical Co. kit # 59 uv.

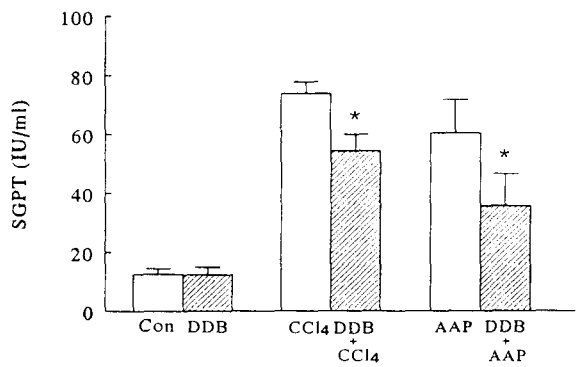

Fig. 1. Effects of DDB on serum glutamic pyruvic transaminase activities in *in vivo* experiment. CCl_4; carbon tetrachloride, AAP; acetaminophen, *P < 0.05.

3. *Ex vivo* Test

Rats were divided into DDB (300 mg/kg, p.o.) or vehicle (normal saline, p.o.) treated group, and received once a day for 7 days before the isolation of hepatocytes. Hepatocytes were isolated by the two-step collagenase perfusion method (Seglen, 1976). Briefly, animals were first anesthetized by intraperitoneal administration of 90 mg/kg phenobarbital. The liver was cannulated through the portal vein, and was washed with Ca^{2+} and Mg^{2+}- free Hanks' balanced salt solution, pH 7.4, for 15-20 min at a flow rate of 30 ml/min at first. It was then perfused with the same buffer containing 0.02% collagenase (Type IV, Worthington) and 0.075% $CaCl_2$ for 15 min at a flow rate of 15 ml/min. After enzymatic perfusion, the organ was excised and placed in a petri dish. The Glisson's capsule was disrupted, and the cells were dispersed in DMEM medium containing 0.1% bovine serum albumin. The cell suspension was filtered through gauze, allowed to sediment for 20 min, and finally, washed three times in medium by centrifugation at 50×g. Cell viability was estimated by the trypan blue exclusion test. Isolated hepatocytes from DDB pretreated (300 mg/kg, p.o.) or vehicle only for 7 days were seeded (0.6×10^6 cells/ml) on rat tail collagen-coated 6-well plates. The seeding medium consisted of MEM supplemented with 10% FBS, 0.1% BSA, 10^{-7} M hydrocortisone, 1 μg/ml insulin. Cells were allowed to attach for 2 hrs before changing the medium to serum-free DMEM/Ham's F-12 supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), selenium (10^{-7} M), hydrocortisone (10^{-7} M). After the acclimation period (12 hrs), the hepatocytes, each of groups (DDB pretreated or vehicle), received adriamycin (1×10^{-4} mg/ml), cisplatin (2×10^{-4} mg/ml). After 24 hrs, for the estimate of hepatotoxicity, GPT release, MDA formation (Yagi *et al.*, 1978), ATP contents (Leach, 1981) of hepatocytes were assayed.

4. *In vitro* Test

Hepatocytes were isolated from normal rats. Ex-

perimental design was composed of DDB (0.01 mM, for 3 days) or vehicle (0.5% ethanol) group. After pretreatment, each of groups (DDB pretreated or vehicle) received adriamycin (1×10^{-4} mg/ml), cisplatin (2×10^{-4} mg/ml). After 24 hrs, for the measurement of hepatotoxicity, adenosine triphosphate (ATP) content of hepatocytes were assayed.

III. RESULTS & DISCUSSION

The mechanism of SGPT lowering effect of DDB is not known but several reports suggested that DDB has an hepatoprotective and membrane stabilizing action (Li, 1991). Fig. 1 showed 7 days pretreatment of DDB significantly reduced the increases of SGPT in acetaminophen-and carbon tetrachloride-induced hepatotoxicity. These result agrees with previous reports (Fu & Liu, 1990). Because DDB did not decrease the basal level of SGPT of normal rats, it can be suggested that DDB did not inhibit GPT production and did not increase GPT degradation rate. These suggestion supported with previous report where DDB did not accelerate the disappearance of GPT activity from circulation of intravenously injected exogenous serum with high GPT activity.

Similar experiments were performed with primarily cultured hepatocytes isolated after DDB or vehicle pretreatment for 7 days. Even after 12 hrs primary culture, DDB pretreatment inhibited the releasing of GPT induced by cisplatin and adriamycin (Fig. 2).

As adriamycin (Bagchi *et al.*, 1995) and cisplatin (Torii *et al.*, 1993) are reported to increase free radical production in liver and many chemicals whose hepatotoxicity are proved to be ameliorated with DDB treatment are known to increase free radical production. We investigated the antioxidative effects of DDB on cisplatin-, adriamycin-induced lipid peroxidation. Fig. 3 showed that DDB significantly decreased cisplatin- and adriamycin- induced MDA formation in *ex vivo* test.

Assessing the effects of DDB on the changes of energy metabolism induced by cisplatin and adriamycin, we measured ATP contents of hepatocytes isolated after DDB or vehicle pretreatment for 7 days. Cisplatin and adriamycin markedly decreased ATP contents of hepatocytes and such ATP decrements were not prevented by DDB pretreatment (Fig. 4). In *in vitro* test, DDB pretreatment for 3 days did not reduce the decreases of ATP contents induced by cisplatin and adriamycin also (Fig. 5).

According to *ex vivo* data, it can be postulated

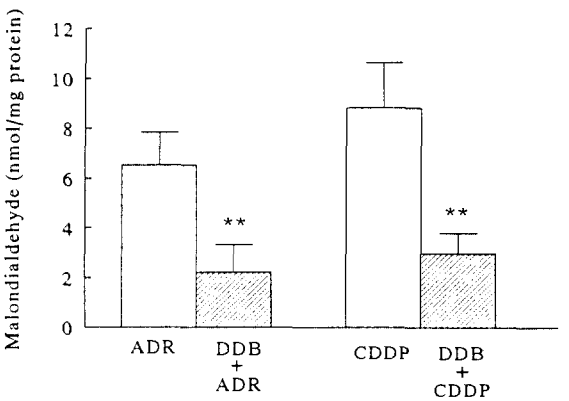

Fig. 3. Effects of DDB on malondialdehyde formation in *ex vivo* test. ADR; adriamycin, CDDP; cisplatin, **P < 0.01.

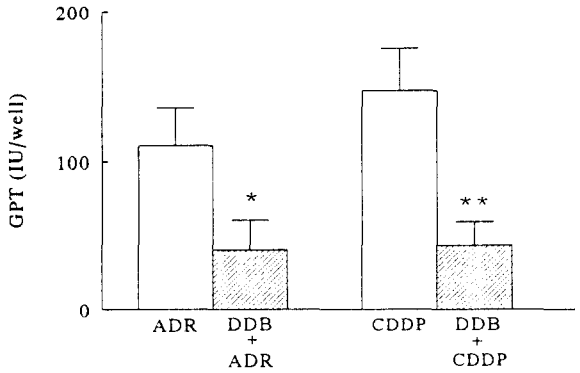

Fig. 2. Effects of DDB on glutamic pyruvic transaminase activities released to culture medium in *ex vivo* test. ADR; adriamycin, CDDP; cisplatin, *P < 0.05, **P < 0.01.

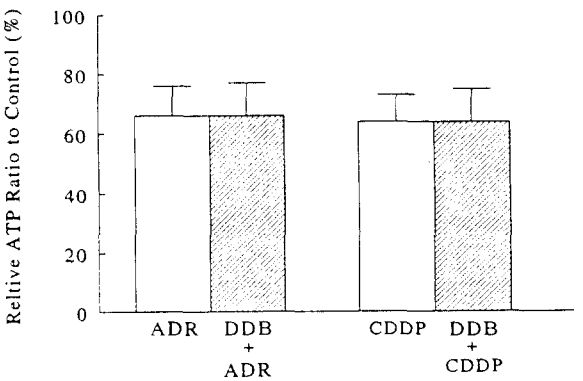

Fig. 4. Effects of DDB on intracellular ATP level in *ex vivo* test. ADR; adriamycin, CDDP; cisplatin.

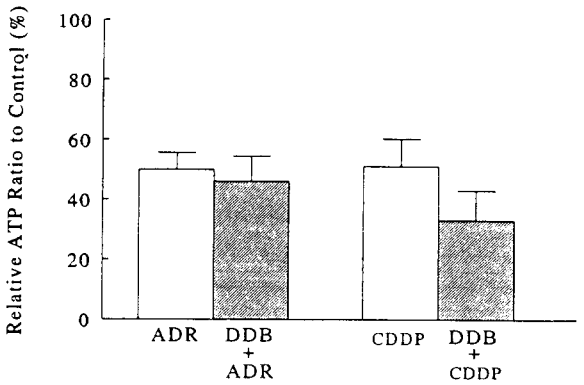

Fig. 5. Effects of DDB on intracellular ATP level in *in vitro* test. ADR; adriamycin, CDDP; cisplatin.

that DDB makes change in hepatocyte toward more resistant to cytotoxic chemicals. Such a change persisted after removal of DDB at least 12 hrs. Induction of microsomal drug-metabolizing enzymes (Liu *et al.*, 1982), menbrane stabilization (Chen & Chen, 1989) and increased radical scavenging activity (Song *et al.*, 1994) can be suggested. Because DDB did not prevent the decrease of ATP contents in hepatocytes, the assumption that increased drug detoxicification reduced the hepatotoxicity is not feasible. Gomisin A, another lignan component of shizandra fruits with very similar chemical structure to DDB, was reported to inhibit the elevation of serum aminotransferase activity and hepatic lipoperoxides contents but did not affect the decrease in liver glutathione contents induced by acetaminophen administration (Yamada *et al.*, 1993). It was suggested that gomisin A protects the liver from injury after administration of acetaminophen through the suppression of lipid peroxidation. Lipid peroxidation can result as a consequence of a primary increase in the generation of free radicals or as a consequence of a decrease in the activity of one or more of antioxidant system (Chance *et al.*, 1979). Song *et al.* reported that DDB pretreatment did not increase antioxidant enzyme activities, such as catalase, superoxide dismutase, glutathion peroxidase. In future study, the effect of DDB on other enzymes and moleclcules that related with generation or scavenging of free radical should be done.

Considering another possible postulation that lipid membrane is changed to be more resistant to radical or other insults, there were reports that DDB affected cell membrane fluidity (Chen & Chen, 1989). To know more detail membrane stabilizing effect of DDB, the more studies should be done including the effect of DDB on the lipid composition of cell membrane.

In conclusion, our study suggests that DDB may protect the hepatocytes from injury induced by hepatotoxicants through the suppression of membrane lipid peroxidation.

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REFERENCES

Bagchi, D., Bagchi, M., Hassoun, E.A., Kelly, J. and Stohs, S.J. (1995): Adriamycin-induced hepatic and myocardial lipid peroxidation and DNA damage, and enhanced excretion of urinary lipid metabolites in rats. *Toxicology*, **95**(1), 1-9.

Chance, B., Sies, H. and Boveris, A. (1979): Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.*, **59**, 527-605.

Chen, L. and Chen W.W. (1989): The effect of biphenyl dimethyl dicarboxylate (DDB) on the fluidity of human erythrocyte membrane. *Acta Pharmaceutica Sinica*, **24**(1), 1-4.

Cook, J.A. and Mitchell, J.B. (1989): Viability measurements in mammalian cell systems. *Anal. Biochem.*, **179**, 1-7.

Fu, T. and Liu, G. (1990): Protective effects of biphenyl dimethyl dicarboxylate on damage in isolated rat hepatocytes induced by carbon tetrachloride and D-galactosamine. *Chin. Med. J.*, **70**(4), 201-204.

Leach, F.R. (1981): ATP determination with firefly luciferase. *J. Appl. Biochem.*, **3**, 473-517.

Lee, H.S., Kim, Y.T., Jung, H.C., Yoon, Y.B., Song, I.N. and Kim, C.Y. (1991): Prospective, randomized, controlled trial with diphenyl-dimethyl-dicarboxylate in chronic active liver diseases: The effect on lowering serum alanine aminotransferase levels. *Kor. J. Intern. Med.*, **40**(2), 172-178.

Li, X.Y. (1991): Bioactivity of neolignans from fructus Schizandrae. *Memorias do Instituto Oswaldo Cruz*, **86**(Suppl 2), 31-37.

Liu, K.T., Cresteil, T., Columelli, S. and Lesca, P. (1982): Pharmacological properties of dibenzo[a,c] cyclooctene derivatives isolated from Fructus Schi-

zandrae chinensis II. Induction of phenobarbital-like hepatic monooxygenases. *Chemic-Biol. Interactions*, **39**(3), 315-330.

Liu, K.T. and Lesca, P. (1982): Pharmacological properties of Dibenzo[a,c]cyclooctene derivatives isolated from Fructus Schizandrae Chinensis III. Inhibitory effects on carbon tetrachloride-induced lipid peroxidation, metabolism and covalent binding of carbon tetrachloride to lipids. *Chemic-Biol. Interactions*, **41**(1), 39-47.

Liu. J., Liu. Y. and Klaassen, C.D. (1994): The effect of chinese hepatoprotective medicines on experimental liver injury in mice. *J. Ethnopharmacol.*, **42**(3), 183-191.

Ohkawa, H., Ohishi, N. and Yagi, K. (1978): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95**, 351-358.

Seglen, P.O. (1976): Preparation of isolated rat liver cells. *Methods Cell Biol.*, **13**: 30-78.

Song, H.Y., Ha, K.R., Koh, H.C., Shin, I.C. and Suh, T. K. (1994): Effects of biphenyldimethyl dicarboxylate (DDB) on the lipid peroxidation, oxygen free radical scavenging enzyme activities and hepatic functions in ethanol-induced hepatotoxic rats. *Kor. J. Pharmacol.*, **30**(2), 217-225.

Torii, Y., Mutoh, M., Saito, H. and Matuki, N. (1993): Involvement of free radicals in cisplatin-induced emesis in Suncus murinus. *Eur. J. Pharmacol.*, **248**(2), 131-135.

Yamada, S., Murawaki, Y. and Kawasaki, H. (1993): Preventive effect of gomisin A, a lignan component of shizandra fruits, on acetaminophen-induced hepatotoxicity in rats. *Biochem. Pharmacol.*, **46**(6), 1081-1085.