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# Effect of 2-Methylaminoethyl-4,4'-Dimethoxy-5,6,5',6'-Dimethylenedioxybiphenyl-2-Carboxylic Acid-2'-Carboxylate Monohydrochloride (DDB-S) on Indocyanine Green (ICG) Clearance in Rats

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The clearance of ICG, a known hepatic blood flow marker was investigated in rats in order to examine whether DDB-S influences hepatic blood flow. The effect of DDB-S on the protein binding and blood-to-plasma partition of ICG was measured. The steady-state plasma concentration of ICG was monitored before and after co-administration of various concentration of DDB-S, and ICG clearance was estimated from the steady-state concentration and the infusion rate of ICG. There was no significant difference in protein binding and blood-to-plasma partition of ICG with and without addition of DDB-S (10, 20, and 40 μg/mL). When ICG was infused into DDB-S pretreated rats, the steady-state concentrations of ICG decreased and the calculated ICG clearance increased. However, no dose-dependency of ICG Css on DDB-S Css was observed. Since DDB-S did not affect the protein binding and blood-to-plasma partition of ICG, the increased clearance of ICG with co-administration of DDB-S seems to be due to the increased hepatic blood flow by DDB-S.

**Key words:** DDB-S, ICG, Clearance, Hepatic blood flow

## INTRODUCTION

In 1970s *Fructus Schizandrae* was found to be effective in improving the liver function and the symptoms of patients with chronic viral hepatitis B (Liu *et al.*, 1979). Schizandrin A, B, and C, Schizandrol A and B, and Schizandrer A and B were isolated from *Fructus Schizandra,* which were claimed to be responsible for hepatoprotective effect (Xie *et al.*, 1981). Dimethyldimethoxy-biphenylate (DDB, Fig. 1), a synthetic analogue of Schizandrin C, has been used against viral or chemically induced hepatic injury in Asian countries (Liu *et al.*, 1982; Lee *et al.*, 1991). Recent pharmacological studies of DDB have focused on its effect against hepatotoxic treatments such as carbon tetrachloride exposure and partial hepatectomy. DDB has been shown to protect against d-galactosamine, thioacetamide and prednisolone induced liver injuries in mice and rats, and DDB markedly improved the impaired liver functions by lowering the elevated serum alanine transferase (ALT) (Liu *et al.*, 1982; Lee *et al.*, 1991).

DDB itself is practically insoluble in water and it is considered extremely difficult to develop into an injectable form, even though there are great needs for parenteral drugs for the treatment of acute hepatitis patients, and those with decreased liver functions after surgical operations. Recently, 2-methylaminoethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2-carboxylic acid-2'-carboxylate monohydrochloride (DDB-S, Fig. 1), a water soluble DDB derivative was designed and synthesized in our laboratory in order to develop an injectable drug for hepatitis (Kim, 1996). Water solubility of DDB-S was 39 mg/mL and DDB-S did not exert any clinical toxicity in rats and Beagle dogs (Park, 1999). The pharmacokinetic parameters and elimination routes suggested that DDB-S could be suitable for the parental administration (Oh *et al.*, 1999; Suh, 2000).

The hepatoprotective action of DDB has been attributed to inhibition of lipid peroxidation (Liu and Lesca, 1982; Liu, 1989), induction of CYP450 2B1 (Li *et al.*, 1992; Kim *et al.*, 2000) and glutathione S-transferase (Li *et al.*, 1992; Liu *et al.*, 1995; Qing and Liu, 1992), and anti-inflamma-

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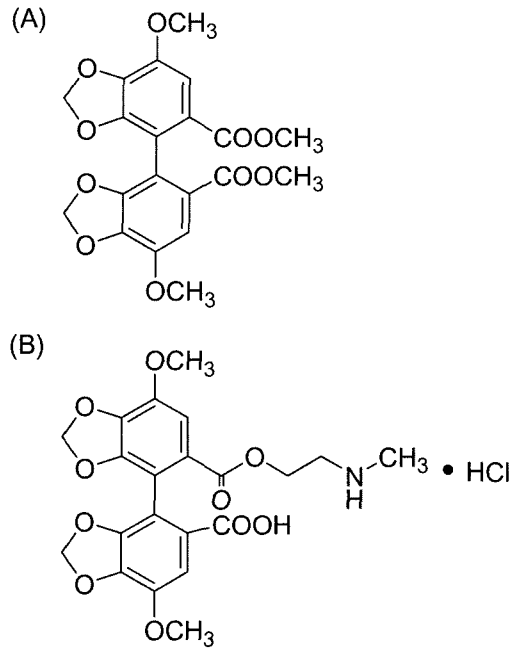


**Fig. 1.** Chemical structures of DDB (A) and DDB-S (B)

tory action (Kim *et al.*, 2000). Typically patients with chronic liver disease such as liver cirrhosis and liver fibrosis showed decreased hepatic blood flow (Sato *et al.*, 1983). It was reported that colchicine, an antifibrotic drug for liver fibrosis, increased hepatic blood flow and showed favorable changes in hepatic biochemical tests (Tapalaga *et al.*, 1986).

In this study, the clearance of indocyanine green (ICG), a known hepatic blood flow marker, was used to investigate the effect of DDB-S on hepatic blood flow in rats (Andersen and Kuchiba, 1970). The steady-state plasma concentration of ICG was monitored before and after co-administration of various concentration of DDB-S, and ICG clearance was estimated from the steady-state concentration and the infusion rate of ICG.

## MATERIALS AND METHODS

### Materials and animals

DDB-S·HCl was synthesized in Pharmaceutical Laboratory of Pusan National University (Pusan, South Korea) as previously described (Kim, 1996; Park, 1999). ICG was purchased from Sigma Chemical Co. (St Louis, MO, USA). Heparin (50,000 IU/mL) was a product from Green Cross Pharmaceutical Co. (Seoul, Korea). HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, FRG). All other reagents were obtained from usual commercial sources and used as received.

Male Sprague Dawley (SPF strain, 250-320 g) rats were obtained from Hyo Chang Science (Dae-Gu, Korea). Rats were housed over a week in the temperature of 21-25 °C and the relative humidity of 50-60%.

### Plasma protein binding

Plasma protein binding of ICG was determined by an ultrafiltration technique using a micropartition system, MPS-1 (Amicon Corp., MA, USA). Plasma aliquots (1.0 mL) containing various concentrations of DDB-S (0, 10, 20, and 40 μg/mL) and ICG (10 μg/mL) were incubated for 30 min at 37 °C. Then, the units were centrifuged at 2000 g for 20 min. The filtrates were subject to HPLC analysis and the free fraction of ICG was determined.

### Blood-to-plasma partition

Rat blood was collected *via* heart puncture in the heparinized Vacujet tubes and was used for blood-to-plasma partition measurement. Five μL of DDB-S solution (0, 2, 4, and 8 mg/mL) and 5 μL of ICG solution (2 mg/mL) were added to 1 mL of rat blood, and mixed to obtain final concentrations of 0, 10, 20, and 40 μg/ml for DDB-S and 10 μg/mL for ICG, respectively. The mixtures were incubated for 30 min at 37 °C. Plasma was separated by centrifuging the blood samples at 2000 g for 10 min and the plasma concentration of ICG was measured by HPLC analysis. The $C_B/C_P$ value of ICG was determined by the blood concentration of ICG divided by the plasma concentration of ICG.

### Infusion of ICG and DDB-S

Rats were fasted overnight before commencement of the experiment. Each rat was kept in supine position during the whole experimental period. After anesthetizing rat with ether, one catheter (PE-50 polyethylene tubing) was cannulated into the left femoral artery for blood sampling and the other catheter into the left and right femoral veins for drug administration (Joo and Lee, 1998). After recovery from anesthesia, the $X_0$ and $k_0$ of ICG and DDB-S were administered *via* the left femoral vein according to the administration design (Fig. 2, Joo and Lee, 1998) using a Multiple syringe infusion pump (Fisher scientific, USA).

In order to achieve the steady-state plasma concentration (Css) of DDB-S at approximately 10, 20, and 40 μg/mL and $C_{SS}$ of ICG at approximately 10 μg/mL, the initial dose ($X_0$, μg/kg) and the maintenance dose ($k_0$, ug/kg/min) of ICG and DDB-S were calculated by the following equations (Skak and Keiding, 1987):

$$X_0 = V_{dSS} \cdot C_{SS} \quad (1)$$

$$k_0 = Cl_T \cdot C_{SS} \quad (2)$$

where $V_{dSS}$ (ml/kg) and $Cl_T$ (mL/kg/min) are the volume of distribution at the terminal phase and total body clearance, respectively. The $V_{dSS}$ and $Cl_T$ of ICG in rats were reported to be 378 mL/kg and 16.8 mL/kg/min (Joo and Lee,

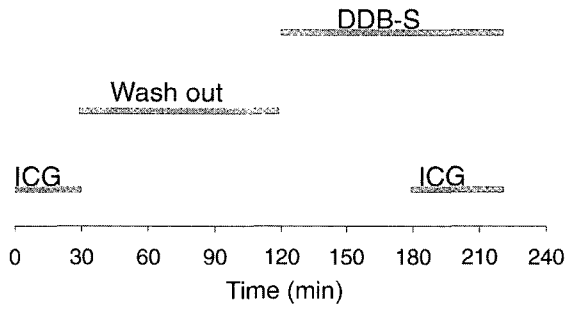


**Fig. 2.** Administration design of ICG and DDB-S

1998), thus the calculated $X_0$ and $k_0$ of ICG to maintain 10 μg/mL of Css was 3,780 μg/kg and 168 μg/kg/min, respectively. DDB-S was administered into animals at 6,870, 13,740, and 27,480 μg/kg of $X_0$ and 153, 306, and 612 μg/kg/min of $k_0$ for 10, 20, and 40 μg/mL of Css, respectively, based on the $V_{dSS}$ (687 mL/kg) and $Cl_T$ (15.3 mL/kg/min) of DDB-S in the literature (Oh *et al.*, 1999).

At 20, 25, and 30 min after $X_0$ administration of ICG, 200 μL of blood sample was withdrawn via the left femoral artery and the plasma concentration of ICG was determined. Following a 90 min washout period, the $X_0$ and $k_0$ of DDB-S were administered via the right femoral vein. At 60, 65, and 70 min after $X_0$ of DDB-S, 200 μL of blood sample were withdrawn via the left femoral artery and the plasma concentrations of DDB-S were determined. During the steady state of DDB-S, the second $X_0$ and $k_0$ of ICG were administered via the left femoral vein. At 20, 25, and 30 min after the second $X_0$ of ICG, 200 μL of blood sample was withdrawn into heparinized tubes *via* the left femoral artery and the plasma concentration of ICG was measured. During the experiment, the body temperature of rats was maintained at 37 °C.

### Estimation of total blood clearance ($Cl_T^B$) of ICG

Plasma clearance ($Cl_T$) was estimated from the steady-state plasma concentration ($C_{SS}$) of ICG produced during the femoral infusion based on that hepatic clearance is the main pathway of ICG removal from the body (Fleck and Braunlich, 1986):

$$Cl_T = \frac{k_0}{C_{SS}} \quad (3)$$

Since the ICG removal rate (clearance) is independent of blood or plasma, it becomes that:

$$Cl_T^B \cdot C_B = Cl_T \cdot C_P \quad (4)$$

$$Cl_T^B = \frac{Cl_T \cdot C_P}{C_B} \quad (5)$$

### HPLC assay

The plasma was separated from blood samples by centrifugation at 2000 g for 10 min. One hundred μL of plasma were combined with 100 μL of internal standard in methanol solution (p-amino benzoic acid for DDB-S and protriptyline for ICG) and 125 μL of acetonitrile. The mixed samples were centrifuged at 2000 g for 10 min, and the supernatant was subject to HPLC analysis.

The concentrations of DDB-S and ICG were determined using a HPLC system (Waters, LC module I plus, U.S.A.) equipped with a LC module I pump, waters 486 tunable UV/visible absorbance detector and Waters 746B data module. A μ-Bondapak $C_{18}$ column (3.9 × 300 mm ID, Waters, MA, U.S.A.) was used as an analytical column. DDB-S was resolved well from p-amino benzoic acid in the mobile phase of 60% 0.05M $KH_2PO_4$-$NaHPO_4$ buffer (pH 6.0) and 40% methanol at a flow rate of 0.9 mL/min. The UV detector was set at 260 nm. For ICG detection, the mobile phase consisted of 53% 0.05M $KH_2PO_4$-$NaHPO_4$ buffer (pH 6.0) and 47% acetonitrile (Awani and Bakker, 1989). Solvent was delivered at a flow rate of 0.8 mL/min, and the UV detector was set at 225 nm. Retention time of ICG was 9.5 minutes.

### Statistical analysis

The Students *t*-test was used to assess statistical differences between the control period and each treatment period, and $p<0.05$ was selected as the criterion of significance. All values are reported as mean ± standard deviation.

## RESULTS AND DISCUSSIONS

The effect of DDB-S on the protein binding and blood-to-plasma partition of ICG was measured and the result was listed in Table I. There was no significant difference in protein binding and blood-to-plasma partition of ICG with and without addition of DDB-S (10, 20 and 40 μg/mL).

The steady-state concentrations (10, 20 and 40 μg/mL) of DDB-S were well achieved within 60 min after the correspondent $X_0$ and $k_0$ of DDB-S (Fig. 3). The steady-state concentration of ICG (10 μg/mL) was established within 20 min after the $X_0$ and $k_0$ (Fig. 4, Before DDB-S). When ICG was infused into DDB-S pretreated rats, the measured steady-state concentrations of ICG ranged from 5.8 to 7.2 μg/mL (Fig. 4, After DDB-S). Table II summarized the steady-state plasma and blood clearance

**Table I.** Effect of DDB-S on blood-to-plasma partition and plasma free fraction of ICG (n > 3)

| | DDB-S concentration (μg/mL) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 40 |
| $C_B/C_P$ | 0.63 ± 0.06 | 0.69 ± 0.14 | 0.64 ± 0.06 | 0.69 ± 0.07 |
| Free fraction | 0.024 ± 0.001 | 0.025 ± 0.003 | 0.021 ± 0.002 | 0.024 ± 0.003 |

of ICG upon co-administration of DDB-S. ICG clearance increased with DDB-S administration, although it did not achieve a statistical significance at p = 0.05. No dose-dependency of ICG Css on DDB-S Css was observed.

ICG is excreted nearly exclusively via liver (Fleck and Braunlich, 1986), so its systemic clearance is represented by the hepatic clearance. Hepatic clearance is a function of intrinsic hepatic clearance, protein binding, and hepatic blood flow. The hepatic clearance of low extraction compounds (ER< 0.3) depends on their free fraction and intrinsic hepatic clearance, while the hepatic blood flow is the determining factor of the elimination process for high extraction compounds (ER > 0.7). The hepatic extraction ratio of ICG is about 0.4 (Joo and Lee, 1998; Klaassen and Plaa, 1969), therefore the hepatic clearance of ICG is a function of hepatic intrinsic clearance and hepatic blood flow.

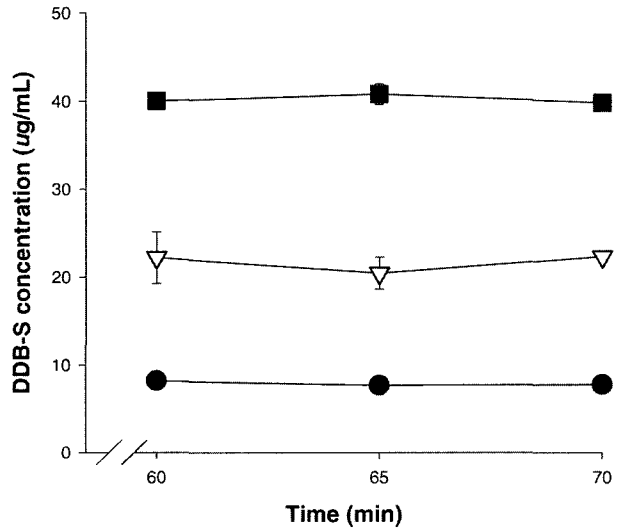


**Fig. 3.** The steady-state plasma concentration of DDB-S with $X_0$ (μg/kg) and $k_0$ (μg/kg/min) of 6,870 and 153 (●), 13,740 and 306 (▽), and 27,480 and 612 (■). Data were represented as mean ± SD (n=6).

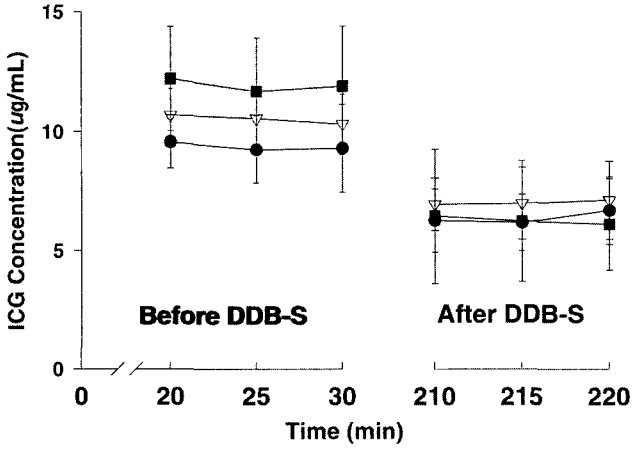


**Fig. 4.** Plasma concentration-time profiles of ICG; DDB-S 10 μg/mL (●), DDB-S 20 μg/mL (▽), and DDB-S 40 μg/mL (■). Data were represented as mean ± SD (n=6).

**Table II.** Effect of DDB-S concentrations on the plasma and blood clearance (mL/kg/min) of ICG (n=6)

| | Before DDB-S | Css of DDB-S (μg/mL) | | |
|---|---|---|---|---|
| | | 10 | 20 | 40 |
| $Cl_T$ | 16.45 ± 2.99 | 26.35 ± 5.81 | 23.86 ± 4.94 | 29.01 ± 9.76 |
| $Cl_T^B$ | 25.95 ± 4.71 | 38.46 ± 8.48 | 37.04 ± 7.67 | 41.98 ± 14.12 |

Clearance can be altered due to drug-drug interactions *via* protein binding change or metabolic enzyme induction or inhibition. DDB-S did not change ICG protein binding values (Table I), so the altered clearance by protein binding change should be excluded. The treatment of DDB-S was acute, so it is hard to expect to have ICG metabolic enzyme induction by DDB-S if any, since it is believed that intact ICG is excreted into bile. When the metabolism or elimination of ICG was inhibited, the observed clearance should be lower than before the DDB-S treatment, which does not agree with the result in Table II. Therefore, assuming that the capacity of ICG elimination did not change, it was implied that ICG clearance increased due to the increased hepatic blood flow by DDB-S.

In conclusion, DDB-S did not affect the protein binding and blood-to-plasma partition of ICG. The increased clearance of ICG with co-administration of DDB-S seems to be due to the increased hepatic blood flow by DDB-S. However, further investigation is necessary to conclude that the increase in the hepatic blood flow contributes to the hepatoprotective action of DDB-S.

## REFERENCES

Andersen, M. N. and Kuchiba, K., Measurement of acute change in liver function and blood flow. *Arch. Surg.*, 100, 541-545 (1970).

Awani, W. M. and Bakker, L. J., Antipyrine, indocyanine green, and lorazepam determined in plasma by high-pressure lipuid chromatography. *Clin. Chem.*, 35, 2124-2126 (1989).

Fleck, C. and Braunlich, H., Relation between renal and hepatic excretion of drugs. II. Age-dependence of phenol red excretion in comparison with those of p-aminohippurate and indocyanine green. *Exp. Pathol.*, 29, 235- 247 (1986).

Joo, E. H. and Lee, Y. B., No effect of Diltiazem on the hepatic clearance of Indocyanine green in the rats. *Arch. Pharm. Res.*, 21, 411-417 (1998).

Kim, S. G., Kim, H. J., Choi, S. H., and Ryu, J. Y., Inhibition of lipopolysaccharide-induced I-kappaB degradation and tumor necrosis factor-alpha expression by dimethyl-4.4-dimethoxy-5,6,5,6-dimethylene dioxybiphenyl-2,2-dicarboxylate (DDB): minor role in hepatic detoxifying enzyme expression. *Liver,*

20, 319-329 (2000).

Kim, Y. K., Synthesis and physicochemical properties of water-soluble DDB derivative. *Master of Science Thesis*, Pusan National University (1996).

Klaassen, C. D. and Plaa, G. L., Plasma disappearance and biliary excretion of indocyanine green in rats, rabbits, and dogs. *Toxicol. Appl. Pharmacol.*, 15, 374-84 (1969).

Lee, H. S., Kim, Y. T., Jung, H. C., Yoon, Y. B., Song, I. S., and Kim, C. Y., Protective randomized controlled trial with diphenyl dimethyl dicarboxylate in chronic active liver diseases: the effect on lowering serum alanine aminotransferase levels. *Kor. J. Int. Med.*, 40, 173-178 (1991).

Li, Y., Paranawithana, S. R., Yoo, J. S., Ning, S. M., Ma, B. L., Lee, M. J., Liu, G. T., and Yang, C. S., Induction of liver microsomal cytochrome P-450 2B1 by dimethyl diphenyl bicarboxylate in rats. *Zhongguo Yao Li Xue Bao,* 13, 485-490 (1992).

Liu, G. T., Pharmacological actions and clinical use of fructus schizandrae. *Chin. Med. J (Engl).,* 102, 740-749 (1989).

Liu, K. T. and Lesca, P., Pharmacological properties of dibenzo [a,c]cyclooctene derivatives isolated from Fructus Schizandrae chinensis. I. Interaction with rat liver cytochrome p-450 and inhibition of xenobiotic metabolism and mutagenicity. *Chem. Biol. Interact.,* 39, 301-314 (1982).

Liu, K. T., Wang, G. F., Wei, H. L., Bao, T. T., and Song, Z. Y., A comparison of the protective action of biphenyl dimethyl dicarboxylate, *trans*-stilbene alcoholic extracts of Fructus shiznadrae and ganoderma against experimental liver injury in mice. *Acta. Pharm. Sinica*, 14, 598-604 (1979).

Liu, K. T., Wei, H. L., and Song, Z. Y., Further studies on the protective action of diphenyl dimethyl dicarboxylate (DDB) against experimental liver injury in mice. *Acta. Pharm. Sinica*, 17, 101-105 (1982).

Oh, S. Y., Lee, C. H., and Ku, Y. S., Pharmacokinetics and hepatoprotective effect of 2-methylaminoethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2-carboxylic acid-2-carboxylate monohydrochloride in rats with CCl4-induced acute hepatic failure. *J. Pharm. Pharmacol.,* 52, 1099-1103 (2000).

Park, A. J., Synthesis of a water-soluble derivative of dimethyl dimethoxy Biphenylate and its preventive and therapeutic effect on liver cirrhosis. *Ph. D. Thesis*, Pusan National University (1999).

Qing, W. and Liu, G., Protective effect of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate (DDB) against carcinogen-induced rat liver nuclear DNA damage. *Biomed. Environ. Sci.*, 5, 201-207 (1992).

Sato, N., Hayashi, N., Kawano, S., Kamada, T., and Abe, H., Hepatic hemodynamics in patients with chronic hepatitis or cirrhosis as assessed by organ-reflectance spectrophotometry. *Gastroenterology*, 84, 611-616 (1983).

Skak, C. and Keiding, S., Methodological problems in the use of indocyanine green to estimate hepatic blood flow and ICG clearance in man. *Liver,* 7, 155-162 (1987).

Suh, I. S., Studies on the metabolism and pharmacokinetics of dimethoxy biphenyl monocarboxylate HCl. *Master of Science Thesis*, Pusan National University (2000).

Tapalaga, D., Dumitrascu, D., Cotul, S., Stanciu, L., and Tamas, S., Colchicine treatment effects on liver cirrhosis assessed by liver blood flow measurements. *Med. Interne.*, 24, 69-73 (1986).

Xie, J. X., Zhou, J., Zhang, C. Z., Yang, J. H., Jin, H. Q., and Chen, J. X., Synthesis of Schizandrin C analogs. *Pharm. Factory Shanghai,* 15, 306 (1981).