J. Pharm. Pharmacol. 2000, 52: 1099–1103
Received February 14, 2000
Accepted April 20, 2000

Pharmacokinetics and Hepatoprotective Effects of 2-Methylaminoethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2-carboxylic acid-2′-carboxylate monohydrochloride in Rats with CCl_4-induced Acute Hepatic Failure

S.Y. OH, C.H. LEE* AND Y.S. KU

*College of Pharmacy, Ewha Womans University, 11-1 Daehyun-Dong, Seodaemun-Gu, Seoul 120-750, and *College of Pharmacy, Pusan National University, 30 Changjeon-Dong, Keumjeong-Gu, Pusan 609-735, Korea*

Abstract

The pharmacokinetics and hepatoprotective effects of 2-methylaminoethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2-carboxylic acid-2′-carboxylate monohydrochloride (DDB-S) have been investigated in rats with CCl_4-induced acute hepatic failure.

To study the pharmacokinetics of DDB-S, rats were divided into a control group and a CCl_4-intoxicated group. DDB-S 50 mg kg^{-1} was administered by intravenous bolus injection to both groups of rats. In the CCl_4-intoxicated rats the plasma concentrations of DDB-S were significantly higher, the area under the plasma concentration–time curve from time zero to time infinity was significantly greater (6·46 vs 3·34 mg min mL^{-1}), and the total body (7·74 vs 15·0 mL min^{-1} kg^{-1}), renal (2·55 vs 5·10 mL min^{-1} kg^{-1}), nonrenal (5·07 vs 9·65 mL min^{-1} kg^{-1}), and biliary (1·48 vs 2·69 mL min^{-1} kg^{-1}) clearances were significantly slower compared with the control rats. This could be due to decreased hepatic cytochrome P450 activity and impaired kidney function induced by CCl_4.

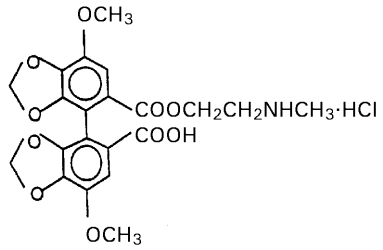
To study the hepatoprotective effects of DDB-S, rats were divided into three groups, control rats and CCl_4-intoxicated rats with or without DDB-S pretreatment (50 mg kg^{-1}, i.p.). The effects of DDB-S pretreatment on CCl_4-induced liver injury were considerable; the serum levels of alanine transaminase, aspartate transaminase, and alkaline phosphatase were significantly lower by 54·3, 44·6 and 67·2%, respectively, compared with the CCl_4-intoxicated-only group.

In an in-vitro study, rat hepatocytes were exposed to fresh medium containing 10 mM CCl_4 and various concentrations of DDB-S (10 or 100 μg mL^{-1}). The levels of alanine transaminase and aspartate transaminase in the medium were measured as an indicator of hepatocyte injury. DDB-S dose-dependently decreased the levels of alanine transaminase and aspartate transaminase compared with CCl_4-intoxication only.

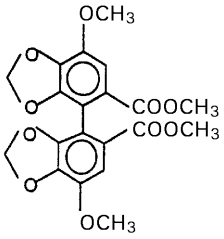
These results indicate that DDB-S has hepatoprotective activity.

2-Methylaminoethyl-4,4′-dimethoxy-5,6,5′,6′–dimethylenedioxybiphenyl-2-carboxylic acid-2′-carboxylate monohydrochloride (DDB-S, Figure 1) is a water soluble derivative of dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2,2′dicarboxylate (DDB, Figure 1) (Ham & Lee 1996a). In patients with hepatitis induced by hepatitis virus B, DDB therapy resulted in a significant decrease in the elevated alanine transaminase serum levels (Lee et al 1991; Wuon et al 1995). In Asia, DDB is currently used clinically for patients with hepatitis virus B (Wang 1984; Kim & Kang 1993). However, DDB is poorly water soluble; its solubility in various buffers (pH values ranging from 1·2 to 9·0) is 1–2 μg mL^{-1} (Park et al 1996). DDB has a low extent of absolute bioavailability after oral administration and an injectable

Correspondence: Y. S. Ku, College of Pharmacy, Ewha Womans University, 11-1 Daehyun-Dong, Seodaemun-Gu, Seoul 120-750, Korea.
E-Mail: ysku@mm.ewha.ac.kr



2-Methylaminoethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2-carboxylic acid-2′-carboxylate monohydrochloride (DDB-S; MW 485·46).



Dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybihenyl-2,2′-dicarboxylate (DDB; MW 419·96).

Figure 1. Chemical structures of DDB-S and DDB.

dosage form is required to restore the patients' liver function after an operation. Ham & Lee (1996a, b) tried to synthesize a water-soluble form of DDB and developed DDB-S. Moon et al (1997) reported that DDB-S had a therapeutic effect on hepatitis in rats and now DDB-S is being evaluated under preclinical study.

Liver disease often causes considerable change in the pharmacokinetics of drugs via change in the liver and/or kidney function. The change in pharmacokinetics of drugs depends on the hepatic disease and on the drug (Breimer 1987) . The purpose of this study was to report the pharmacokinetics and hepatoprotective effects of DDB-S in rats with CCl_4-induced acute hepatic failure (CCl_4-intoxicated rats). CCl_4 is used widely to induce hepatic disease in experimental animals as a single administration of CCl_4 can produce hepatic centrilobular necrosis (Li et al 1990).

Materials and Methods

Materials

DDB-S was donated by the Laboratory of Pharmaceutics, College of Pharmacy, Pusan National University (Pusan, Korea). β-Glucuronidase was purchased from Sigma Chemical (St Louis, MO). Other reagents were of analytical or high-performance liquid chromatographic (HPLC) grade, and therefore were used without any further purification.

Animals

The Institutional Animal Care and Use Committee, College of Pharmacy, Ewha Womans University (Seoul, Korea) approved the protocol of the animal study.

Male Sprague-Dawley rats (190–250 g; Samyuk Animal, Osan, Korea) were used after a one-week acclimatization period at a temperature of $25 \pm 2°C$ and a relative humidity of $55 \pm 5\%$ (Animal Room, College of Pharmacy, Ewha Womans University). During acclimatization the rats were allowed free access to a standard diet (Samyuk Animal) and tap water.

Pharmacokinetic studies

Rats were randomly divided into the control group and the CCl_4-intoxicated group. The CCl_4-intoxicated group received a single subcutaneous injection of $2\,mL\,kg^{-1}$ CCl_4 in olive oil (1:1, v/v). Control rats received an equivalent volume of olive oil. Each rat was fasted for 24 h (Kimura et al 1993). Under light ether anesthesia the right femoral vein and the right femoral artery were cannulated (Intramedic PE-50; Clay Adams, Parsippany, NJ) for drug administration and blood sampling, respectively. The common bile duct was also cannulated (Intramedic PE-10; Clay Adams). For the collection of urine sample, a polypropylene pipette tip (with the tip cut off) was fixed around the penis of the rat. The rats were kept in a restraining cage after the operation.

DDB-S powder was dissolved in distilled water for injection. DDB-S was administered at a dose of $50\,mg\,kg^{-1}$ (total injection volume was approximately 0·6 mL) by intravenous bolus injection over 10 s to control ($n = 4$) and CCl_4-intoxicated ($n = 4$) rats. The cannula was flushed with 0·25 mL heparinized normal saline injectable solution (25 units mL^{-1}) after drug administration to prevent blood clotting. Approximately 0·12-mL blood samples were collected at 0 (to serve as a control), 2, 5, 10, 20, 30, and 45 min, and 1, 1·5, 2, 3, and 4 h post-injection. Heparinized normal saline injectable solution (0·25 mL) was used to flush the cannula just after each blood sampling. The blood samples were centrifuged immediately, and a 50-μL plasma sample was stored at −20°C until HPLC analysis of DDB-S (Oh et al 1997). Bile and urine samples were collected between 0–0·5, 0·5–1, 1–1·5, 1·5–

2, 2–3, 3–4, and 4–6 h, and 0–3, 3–6, 6–9, 9–13, 13–16, 16–20, and 20–24 h, respectively, after intravenous administration of DDB-S.

HPLC analysis of DDB-S

The concentrations of DDB-S in the biological samples were analysed by an HPLC method developed in our laboratory (Oh et al 1997). The plasma samples were used without dilution, but the urine and bile samples were diluted 20-fold with distilled water for injection. Acetonitrile (2·5 vols) was added to deproteinize the samples. The samples were then vortexed for 1 min and centrifuged for 10 min at 3000 rev min^{-1}. The supernatant (10 μL) was directly injected onto the HPLC column.

The HPLC system consisted of a pump (LC-10AD, Shimadzu, Kyoto, Japan), a UV detector (SPD-10A, Shimadzu), a reversed-phase column (μ-Bondapak C_{18}; length 30 cm, i.d. 3·9 mm; Waters Assoc., Milford, MA), and an integrator (C-R10A, Shimadzu). The mobile phase, 0·1 M KH_2PO_4–methanol–H_3PO_4 (64:36:0·05, v/v/v) was run at a flow rate of 1·5 mL min^{-1}. The column effluent was monitored by a UV detector (SPD-10A, Shimadzu) set at 241 nm. The peak of DDB-S was symmetrical and the retention time of DDB-S was approximately 8·54 min. The detection limit for DDB-S in plasma was 0·5 μg mL^{-1}. The coefficients of variation of the assay (within- and between-day) in plasma were generally low (below 6·2%).

Hepatoprotective studies

Rats were randomly divided into three groups (n = 7); control group and CCl_4-intoxicated group with or without DDB-S-pretreatment. The CCl_4-intoxicated group received a single subcutaneous injection of 2 mL kg^{-1} CCl_4 in olive oil (1:1, v/v). Control rats received an equivalent volume of olive oil. The DDB-S-pretreated group received a 50 mg kg^{-1} DDB-S intraperitoneal injection (the same solution as used in the intravenous study, total injection volume was approximately 0·6 mL) four times, 73, 49, 25 and 1 h before CCl_4 injection. At 24 h after CCl_4 injection, blood samples were collected via the right femoral artery and the levels of alanine transaminase, aspartate transaminase, alkaline phosphatase, albumin, and total proteins in serum were measured.

For the in-vitro study, rat hepatic parenchymal cells were isolated by collagenase perfusion by the modified method of Berry et al (1991). Cell viability at the beginning of the experiments was above 85%. Incubations were performed at 37°C, using 10^6 cells mL^{-1} in 10% FBS-RPMI, and gassed with carbogen. The hepatocytes were exposed to fresh medium containing 10 mM CCl_4 and various concentrations of DDB-S. After CCl_4 exposure for 60 min, the alanine transaminase and aspartate transaminase levels in the medium were measured (Transaminase kit, Asan Pharmaceutical, Korea) as an indicator of hepatocyte injury.

Pharmacokinetic analysis

The area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule-extrapolation method (Chiou 1978; Kim et al 1993). The following pharmacokinetic parameters were calculated using standard methods (Gibaldi & Perrier 1982); the first moment of AUC (AUMC), apparent volume of distribution at steady state (Vd_{ss}), mean residence time (MRT), and time-averaged total body clearance (CL), renal clearance (CL_R), non-renal clearance (CL_{NR}), and biliary clearance (CL_B).

Statistical analysis

Statistical analysis was carried out using a *t*-test (for pharmacokinetic study) or Duncan's multiple range test of posteriori analysis of variance (for the hepatoprotective study) for the unpaired mean. $P < 0.05$ was considered statistically significant. All the data are expressed as mean ± s.d.

Results and Discussion

Pharmacokinetic studies

The mean arterial plasma concentration–time profiles of DDB-S following its intravenous administration (10 mg) to control and CCl_4-intoxicated rats are shown in Figure 2. Table 1 lists the relevant pharmacokinetic parameters. The plasma concentrations of DDB-S declined in a polyexponential fashion for both groups of rats, with higher levels in the CCl_4-intoxicated rats than in control rats (Figure 2). As a result, the AUC was significantly greater (87% increase) and this could be due to a significantly slower CL (48% decrease) in CCl_4-intoxicated rats (Table 1). In CCl_4-intoxicated rats, the slower CL was due to significantly slower CL_R (48% decrease) and CL_{NR} (48% decrease) (including CL_B which showed a 43% decrease) (Table 1). The slower CL in CCl_4-intoxicated rats resulted in a significantly longer terminal half-life (32% increase) and MRT (82% increase). However, the values of Vd_{ss} were comparable between

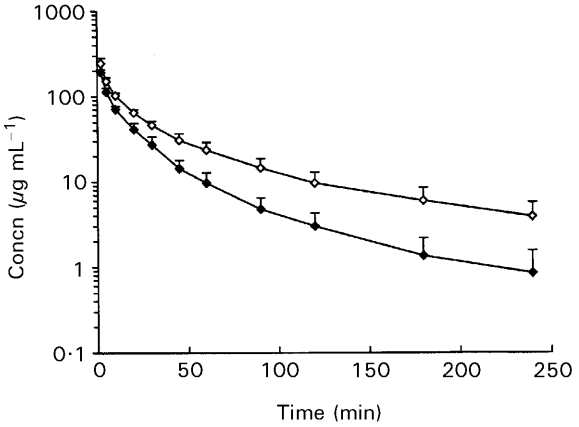


Figure 2. Mean plasma concentration–time profiles of DDB-S following its intravenous administration (50 mg kg^{-1}) to control (◆, n = 4) and CCl_4-intoxicated (◇, n = 4) rats. Vertical bars represent s.d.

both groups of rats. The slower CL_{NR} (including CL_B) in CCl_4-intoxicated group could be due, at least partly, to slower metabolism of DDB-S in the liver. It is known that the activity of hepatic cytochrome P450 is markedly decreased by the hepatic failure induced by CCl_4 (Hoyumpa & Schenker 1991). The slower CL_R of DDB-S in CCl_4-intoxicated rats could be due to CCl_4-induced kidney impairment (Konig et al 1960).

Hepatoprotective studies

Table 2 shows the pathophysiological parameters in control and CCl_4-intoxicated rats with or without pretreatment with DDB-S. It was observed that serum levels of alanine transaminase (280% increase), aspartate transaminase (139% increase), and alkaline phosphatase (72% increase) in CCl_4-intoxicated rats without DDB-S pretreatment were significantly higher compared with control rats. Schiff & Schiff (1982) have explained the mechanism of CCl_4-induced liver injury. The effects of DDB-S pretreatment on CCl_4-induced liver injury were considerable; the serum levels of alanine transaminase, aspartate transaminase, and alkaline phosphatase were significantly lower by 54·3, 44·6 and 67·2%, respectively, compared with the CCl_4-intoxicated group. The levels of serum albumin and total proteins, however, were comparable among the three groups of rats.

Liu (1989) reported the hepatoprotective effects of DDB. In the in-vitro study, DDB-S showed hepatoprotective activity against CCl_4-induced hepatocyte injury. The extent of liver cell injury was expressed in terms of alanine transaminase and aspartate transaminase levels measured in the medium by the treatment of CCl_4 (10 mM). The decreases in the alanine transaminase and aspartate transaminase levels were dependent on DDB-S

Table 1. Pharmacokinetic parameters of DDB-S following its intravenous administration (50 mg kg^{-1}) to control and CCl_4-intoxicated rats.

Pharmacokinetic parameter	Control rats	CCl_4-intoxicated rats
Area under the plasma concentration–time curve from time zero to time infinity (mg min mL^{-1})	3·34 ± 0·542	6·46 ± 1·33**
Apparent volume of distribution at steady state (mL kg^{-1})	616 ± 269	645 ± 69·1
Terminal half-life (min)	80·8 ± 43·3	107 ± 14·0
Time-averaged total body clearance (mL min^{-1} kg^{-1})	15·0 ± 2·94	7·74 ± 1·52**
Time-averaged renal clearance (mL min^{-1} kg^{-1})	5·10 ± 2·04	2·55 ± 1·02*
Time-averaged nonrenal clearance (mL min^{-1} kg^{-1})	9·65 ± 1·20	5·07 ± 0·908**
Time-averaged biliary clearance (mL min^{-1} kg^{-1})	2·69 ± 1·08	1·48 ± 0·214*

Values are mean ± s.d., n = 4. *$P < 0·05$, **$P < 0·01$.

Table 2. Effects of DDB-S on the pathophysiological changes caused by CCl_4-intoxication.

Characteristics	Group		
	Control	CCl_4 alone	DDB-S-pretreated with CCl_4
Liver weight/100 g body weight	3·14 ± 0·171	4·07 ± 0·393	3·62 ± 0·190[a]
Serum alanine transaminase (int. units L^{-1})	38·9 ± 7·56	148 ± 49·5[b]	80·3 ± 13·8
Serum aspartate transaminase (int. units L^{-1})	195 ± 32·1	466 ± 194[b]	208 ± 57·1
Serum alkaline phosphatase (int. units L^{-1})	140 ± 28·0	241 ± 54·1[b]	162 ± 16·1
Serum albumin (g L^{-1})	2·11 ± 0·107	2·03 ± 0·121	2·11 ± 0·107
Serum total protein (g dL^{-1})	5·43 ± 0·256	5·28 ± 0·223	5·37 ± 0·297

Values are mean ± s.d., n = 7. [a]Each group was significantly different ($P < 0·05$). [b]CCl_4 alone was significantly different ($P < 0·05$) from control rats and rats pretreated with DDB-S before CCl_4 administration.

dose (10 or 100 $\mu g\,mL^{-1}$). The alanine transaminase and aspartate transaminase levels after 1 h in the CCl_4-intoxicated-only group were $200 \pm 28{\cdot}3$ and $281 \pm 34{\cdot}9$ Karmen unit mL^{-1}, respectively. The corresponding levels at 10 $\mu g\,mL^{-1}$ DDB-S were $160 \pm 15{\cdot}0$ and $242 \pm 24{\cdot}1$ Karmen unit mL^{-1}, respectively, and with 100 $\mu g\,mL^{-1}$ DDB-S were $139 \pm 5{\cdot}63$ and $207 \pm 19{\cdot}4$ Karmen unit mL^{-1}, respectively; the values were significantly different compared with the CCl_4-intoxicated-only group (Duncan's multiple comparison test, $\alpha = 0{\cdot}05$).

These results clearly indicate that DDB-S has hepatoprotective activity.

Acknowledgements

This study was supported in part by a Research Grant from the Alumni of Graduate School of Ewha Womans University (1997), Seoul, Korea.

References

Berry, M. N., Edwards, A. M., Barritt, G. J. (1991) Isolated hepatocytes preparation, properties and applications. In: Burdon, R. H., Knippenberg, P. H. (eds) Laboratory Techniques in Biochemistry and Molecular Biology. Elsevier, Amsterdam

Breimer, D. D. (1987) Pharmacokinetics in liver disease. Pharm. Weekbl. Sci. 9: 79–84

Chiou, W. L. (1978) Critical evaluation of potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. J. Pharmacokinet. Biopharm. 6: 539–546

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics. 2nd edn, Marcel Dekker, New York

Ham, T. J., Lee, C. H. (1996a) A study on the physicochemical properties of water soluble DDB derivative. The 26th Annual Academic Convention of the Korean Society of Pharmaceutics, Seoul, Korea, Abstract 11, p. 78

Ham, T. J., Lee, C. H. (1996b) A study on the biological activities of water soluble DDB derivative. The 26th Annual Academic Convention of the Korean Society of Pharmaceutics, Seoul, Korea, Abstract 33, p. 102.

Hoyumpa, A. M., Schenker, S. (1991) Is glucuronidation truly preserved in patients with liver disease? Hepatology 13: 786–795

Kim, D. W., Kang, B. K. (1993) Clinical study for low dose and short-term therapy of biphenyl dimethyl dicarboxylate (DDB) in the chronic hepatitis patients with elevated serum aspartate aminotransferase and alanine aminotransferase levels. Kor. J. Clin. Pharm. 3: 45–53

Kim, S. H., Choi, Y. M., Lee, M. G. (1993) Pharmacokinetics and pharmacodynamics of furosemide in protein-calorie malnutrition. J. Pharmacokinet. Biopharm. 21: 1–17

Kimura, T., Nakayama, S., Yamao, T., Kurosaki, Y., Nakayama, T. (1993) Pharmacokinetics of indocyanine green in rats with experimentally induced hepatic diseases. Biol. Pharm. Bull. 16: 1140–1145

Konig, E., Lohmeyer, D., Dahme, E. (1960) The course of enzymic and histological changes in the rat kidney poisoned by single doses of carbon tetrachloride. Z. Ges. Exp. Med. 133: 442–454

Lee, H. S., Kim, Y. T., Jung, H. C., Yoon, Y. B., Song, I. S., Kim, C. Y. (1991) Prospective randomized controlled trial with diphenyl dimethyl dicarboxylate in chronic active liver disease: the effect on lowering serum alanine aminotransferase levels. Kor. J. Int. Med. 40: 172–178

Li, C., Nakayama, S., Kurosaki, Y., Nakayama, T., Kimura, T. (1990) Pharmacokinetics of cefpiramide in rats acutely intoxicated with carbon tetrachloride. J. Pharmacobio-Dyn. 13: 186–194

Liu, K. T. (1989) A new drug for chronic hepatitis: diphenyl dimethyl dicarboxylate, DDB. J. Biomed. Lab. Sci. 2: 230–235

Moon, C. O., Kim, S. H., Chung, K. Y., Park, S. K., Yang, J. H., Yang, H. S., Kim, N. D., Lee, C. H. (1997) Therapeutic effect of water-soluble DDB derivative on hepatitis in rat. The 46th Annual Academic Convention of the Pharmaceutical Society of Korea, Seoul, Korea, Abstract PB41, p. 365

Oh, S. Y., Lee, C. H., Ku, Y. S. (1997) Pharmacokinetics, tissue distribution, and biliary excretion of a new water soluble DDB (dimethyl dimethoxy biphenylate) derivative after intravenous administration to rats. The 46th Annual Academic Convention of the Pharmaceutical Society of Korea, Seoul, Korea, Abstract PB28, p. 358

Park, G. B., Chung, C. K., Lee, K. P. (1996) Solubilization and formulation as soft gelatine capsule of biphenyldimethyldicarboxylate. J. Kor. Pharm. Sci. 26: 1–11

Schiff, L., Schiff, E. R. (1982) Disease of the Liver. 5th edn, Lipponcott, Toronto, p. 621

Wang, C. F. (1984) Clinical effects of DDB pillules on 209 cases of viral hepatitis and cirrhosis. Beijing Med. J. 6: 350–356

Wuon, J. E., An, S. G., Cha, G. M., Chung, Y. M., Kim, H. S., Tchah, H., Park, H. J. (1995) Prospective, controlled trial with diphenyl-dimethyl-dicarboxylate in children with chronic liver diseases: the effect on lowering serum aminotransferase levels. J. Kor. Ped. Soc. 38: 1378–1383