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Research Paper

Contribution of a significant first-pass effect of dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate in the liver to its poor bioavailability in rats

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Abstract

Objectives The objective of this study was to investigate the mechanism responsible for the poor oral bioavailability of dimethyl-4′,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB), a hepatoprotective agent, in rats.

Methods DDB was intravenously administered to rats at doses of 0.2–1 mg/kg. To determine the hepatic first-pass effect in rats, DDB (1 mg/kg) was administered via the pyloric vein and the femoral vein. Direct measurement of intestinal permeability was attempted using Caco-2 cell monolayers and rat intestinal epithelium.

Key findings A moment analysis indicated that the volume of distribution and clearance remained unchanged with the magnitude of the dose, indicating that DDB exhibited linear pharmacokinetics. When the area under the curve for DDB after administration to the pyloric vein was compared with that after femoral vein administration, the ratio (F_H) was found to be 0.294, indicating a significant first-pass effect for DDB. The permeability of DDB was high in the rat intestine ($1.78 \pm 0.229 \times 10^{-5}$ cm/s) and in Caco-2 cell monolayers ($6.8 \pm 0.70 \times 10^{-5}$ cm/s), suggesting that DDB, in soluble form, was readily permeable across the intestinal epithelium.

Conclusions These observations indicated that despite the fact that DDB was readily permeable to the intestinal epithelium, a significant first-pass metabolism was associated with its pharmacokinetics in rats.

Keywords Caco-2 cell monolayer; dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate; intestinal absorption; pharmacokinetics; Ussing chamber

Introduction

Dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB, Figure 1a), a synthetic analogue of schizandrin C, is one of the active components of *Schizandrae Fructus*. DDB, originally developed in China in the 1970s, has been reported to reduce alanine aminotransferase (ALT) activity in patients with chronic hepatitis.[1] In addition, it appeared to reduce liver damage in experimental systems including isolated hepatocytes obtained from rats that had liver damage and experimental hepatic failure.[2–4] Although the mechanism responsible for the hepatoprotective effect of DDB has not been fully elucidated, an anti-inflammatory effect, as the result of the inhibition of tumour necrosis factor (TNF)-α synthesis, appears to be involved.[5] In addition to its anti-inflammatory effect, it has been reported that DDB inhibits fatty liver formation, caused by chronic alcohol exposure.[6] DDB is currently in clinical use as a hepatoprotective agent in Korea, China and Vietnam.[7]

Despite the clinical utility of the drug, the oral bioavailability for DDB was found to be only 20–30% in humans.[8] The underlying reasons for this limited bioavailability are unknown, which may partly be due to the fact that a systematic pharmacokinetic characterization of DDB is unavailable. To our knowledge, only one attempt to characterize it has been reported in rats.[9] In that study, the pharmacokinetics of DDB involved the use of a dose of 10 mg/kg, not a typical dose for the drug (the conventional therapeutic dose of DDB is below 1 mg/kg). Since the aqueous solubility of DDB was limited to 2.5–4.0 μg/ml, DDB, after an oral administration

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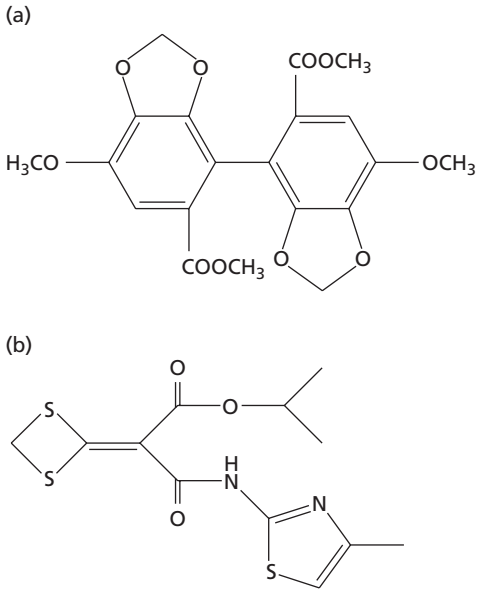

Figure 1 Chemical structure of dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (a) and YH439 (b). YH439 (isopropyl 2-(1,3-dithietane-2-ylidene)-2-(N-(4-methyl-thiazole-2yl) carbamoyl)) is the internal standard for the dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate assay.

of 10 mg/kg, may not have been completely solubilized in the intestine, which may have complicated the assessment of the bioavailability in that study.[10] Furthermore, a low permeability across the intestinal epithelium and/or a significant first-pass metabolism could be responsible for the low oral bioavailability of the drug. It has been reported that DDB was eliminated by hepatic metabolism and was converted into at least five different metabolites and that the drug was classified as being essentially insoluble in water.[11] Therefore, hepatic elimination and low permeability, as a result of low aqueous solubility, may be involved independently or in a concerted manner in the low bioavailability of DDB after its administration. Unfortunately, however, the relative contribution of these factors to its absorption has not been reported. Depending on the mechanism for its low bioavailability, strategies for improving its bioavailability could be developed and the absorption of the drug could be enhanced (e.g. solubilization or an alternative route for its administration). It appears that this aspect of the pharmacokinetics of DDB has not been examined.

The objective of this study, therefore, was to examine the kinetics associated with the systemic absorption of DDB in rats. Considering the fact that the aqueous solubility was low for the drug, the pharmacokinetics of DDB, which in the past was studied using a high dose (10 mg/kg), may not be directly proportional to that for the therapeutic dose range (below 1 mg/kg) (e.g. a different rate of dissolution of the drug in the intestine).[9] Therefore, we elected to study the pharmacokinetics of DDB at relevant doses (i.e. below 1 mg/kg). Our findings indicated that DDB, a compound that is readily permeable across the intestinal epithelium, showed a significant hepatic first-pass metabolism, thereby resulting in its limited oral bioavailability.

Materials and Methods

Materials

DDB (purity 100.6%) was obtained from the Han-Il Pharmaceutical Co. (Seoul, Korea). Cell culture reagents and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). HPLC grade acetonitrile (Fisher Scientific Korea, Seoul, Korea), chloroform (Junsei, Tokyo, Japan), foetal bovine serum (Hyclone, Logan, UT, USA) and helium (99.9995%, Fraxair Air Korea, Korea) were obtained separately. Ketamine (Ketalar, Yuhan Co., Korea) and acepromazine (Sedaject, Samu Chemical Co., Korea) were also used in this study.

Animals

Male Sprague-Dawley rats (270–310 g; Dae-Han Biolink, Korea) were used in all in-vivo experiments. The experimental protocols, particularly with respect to the ethical use of animals used in this study, were reviewed by the Animal Care and Use Committee in the College of Pharmacy, Seoul National University according to the National Institutes of Health guidelines (NIH publication number 85-23, revised 1985) of 'Principles of Laboratory Animal Care'. Except for periods during surgical procedures and blood sampling, the rats had free access to food and water at all times.

Pharmacokinetic study

Sprague-Dawley rats were anaesthetized with an intramuscular injection of ketamine (50 mg/kg) and acepromazine (10 mg/kg). After confirmation of the induction of anaesthesia, the femoral artery and vein of the rats were catheterized with polyethylene tubing (PE-50; Clay Adams, Parsippany, NJ, USA) filled with heparinized saline (25 U/ml). One hour after recovery, the DDB dosing solution (vehicle, 5% DMSO in saline) was injected (0.2 ml/kg) intravenously at doses of 0.2, 0.5, or 1 mg/kg. Blood samples (0.25 ml) were collected from the femoral artery at various times up to 240 min after the injection. The blood volume withdrawn was replaced with an equal volume of saline to compensate for fluid loss.

The first-pass metabolism of DDB was also studied in rats. For the pyloric vein administration group, the pyloric vein and femoral artery of the rats were catheterized with polyethylene tubing and the femoral vein was ligated. For the femoral vein administration group, the femoral vein and artery were catheterized and the pyloric vein ligated. DDB, dissolved in 5% DMSO, was administered at a dose of 1 mg/kg via a 15-min infusion to the pyloric vein or the femoral vein. Blood samples were collected from the femoral artery at various times up to 240 min after termination of the infusion.

The resulting blood samples from the studies were immediately centrifuged at 10 000 rev/min for 1 min, and a 100-μl sample of plasma stored (–20°C) for analysis.

When necessary, the urinary and biliary recoveries of DDB were determined in rats. The surgical procedure used was similar to that for the dose-dependency study except that the

bile duct was additionally catheterized with PE-10 tubing (Clay Adams, Parsippany, NJ, USA) and the cannula secured with a suture. The three cannulae (i.e. venous/arterial and bile duct cannulae) were externalized to the back of the animal and secured. The animal was placed in a metabolic cage (Daejong Instrument, Seoul, Korea) and allowed to recover for 4 h. During the recovery and throughout the experiment, a 3% mannitol solution was infused through the venous catheter at the rate of 10 ml/h per kg to maintain a constant urine flow. DDB, dissolved in 5% DMSO in saline, was intravenously injected to the rat at a dose of 1 mg/kg (volume of administration 0.2 ml/kg). Blood was sampled via the arterial cannula at various times up to 360 min and, upon collection, immediately centrifuged at 10 000 rev/min for 1 min to obtain plasma. Bile was collected at 30-min intervals up to 120 min and 60-min intervals to the last sampling time (i.e. 240 min). Cumulative urine samples up to 6 h in the urine collection device of the metabolic cage were transferred to a fresh tube. The volumes of bile and urine were determined gravimetrically, assuming a density of 1.0 ml/g. At the end of the experiment, the metabolic cage was rinsed with 20 ml double distilled water and 20 ml methanol, and the rinse pooled with the urine sample. The amount of DDB in the blood, bile and urine was determined by means of a gas chromatography–mass spectrometry (GC-MS) assay for DDB (see below). The amounts of DDB excreted into the urine and bile were calculated by multiplying the drug concentration by the volumes of the biological fluids.

Determination of the distribution to red blood cells and plasma protein binding of DDB

Samples of blank blood (0.99 ml) and a DDB solution (10 μl, 0.5 mg/ml) were added to a heparinized tube to give a final concentration of 5 μg/ml. The resulting mixture was agitated in a shaking water-bath (SWB-10, JEIO TECH, Seoul, Korea; 200 oscillation/min) for 30 min at 37°C, and centrifuged at 3000*g* for 10 min, after which a 0.1-ml sample of the plasma was transferred to a fresh tube. The plasma concentration of DDB was determined by an HPLC assay.

Samples of rat plasma (0.980 ml) and DDB solutions (20 μl; vehicle methanol, 0.25 mg/ml) were added to heparinized tubes to give a final concentration of 5 μg DDB/ml plasma. The mixture was vortexed for 3 min, and a sample of the mixture taken for the determination of total DDB concentration. The remaining plasma was added to an ultrafiltration kit (Centricon Ultrafree-MC, Millipore, Bedford, MA, USA) and the set-up centrifuged at 37°C at 5000 rev/min for 10 min. The concentration of DDB in the plasma and the ultrafiltrate was determined by an HPLC assay. When necessary, the extent of DDB binding to the ultrafiltration kit was determined by comparison with a known concentration of DDB in phosphate-buffered saline to the concentration found in the ultrafiltrate.

In-vitro permeability assessment of DDB

Segments of the ileum were removed from Sprague-Dawley rats, rinsed twice with ice-cold saline and immediately placed in ice-cold, serum-free Dulbecco's modified Eagle's medium (pH 7.4) for 30 min with continuous oxygenation with O_2/CO_2 (95%/5%) gas. The serosal layer was stripped from a 2.5-cm segment of the ileum in the ice-cold buffer and the tissue mounted in an Ussing diffusion chamber (Navicyte Inc., San Diego, CA, USA; exposed area of 1 cm^2). Each half-cell was filled with 1 ml medium, and the temperature maintained at 37°C with continuous oxygenation. After a 30-min period, the permeability study was initiated by the addition of DDB (50 μg/ml). Samples (0.25 ml) were removed from the receiving chamber at 30-min intervals and replaced with fresh serum-free Dulbecco's modified Eagle's medium.

When necessary, Caco-2 cells (passage number 29–32) were used to examine the transport of DDB across the human intestinal epithelium. Standard procedures were used for feeding and subculturing.[12–14] For the determination of DDB transport across a Caco-2 cell monolayer, cell culture media was replaced by transport media (pH 7.4, Milli-Q water containing HBSS, 25 mM HEPES and 25 mM glucose). The transport medium in the apical side was removed by aspiration and replaced with transport medium (37°C) containing DDB at concentrations of 0.25, 0.5, 1, 2.5, or 5 μM. The Transwell set up was incubated (37°C) and agitated (85 oscillation/min) in a shaking water-bath, and the insert transferred to a fresh well at 5-min intervals for periods of up to 120 min.

In the in-vitro studies, experiments were run for 120 min and the DDB concentration in the medium determined. The transport rate was plotted against the initial concentration of DDB in the apical side and the apparent permeability calculated using the standard method. In this study, the system was considered to have an adequately tight junction when the mannitol permeability coefficient was less than 5×10^{-6} cm/s in the Ussing chamber study and less than 0.4%/h per cm^2 in the Caco-2 study.

Determination of DDB concentration

When it was necessary to determine DDB concentrations in studies of red blood cell partitioning/plasma protein binding and to evaluate permeability, an HPLC-UV assay for DDB, essentially as described by Lee *et al.*,[15] was used. The HPLC system consisted of a Hitachi LC system (Tokyo, Japan), L-7100 pump, L-7400 UV detector and L-7500 integrator. Samples were injected via a Hitachi L-7200 autoinjector equipped with a 100-μl loop. Separations were performed on an octadecylsilica column (250 × 4.5 mm, 4 μm particle size, YMC, Kyoto, Japan) at ambient temperature.

To determine the DDB levels in the rat plasma, a GC-mass selective detector (MSD) assay for DDB, essentially as described by Bae *et al.*,[9] was used. In this study, YH-439 (isopropyl 2-(1, 3-dithietane-2-ylidene)-2-(*N*-(4-methylthiazole-2yl) carbamoyl), Figure 1b) was used as an internal standard. The GC-MSD system consisted of an HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), an HP5973 mass selective detector and an HP6890 series autosampler. Separation was achieved by the use of an Rtx-5MS GC capillary column (60 m length, 250 μm i.d., 0.25 μm film thickness; Restek, Bellefonte, PA, USA).

Data analysis

A standard moment analysis was used to determine the elimination clearance ($CL_{elimination}$) and steady state volume of distribution (Vd_{ss}) for DDB. The area under the DDB

concentration in the plasma versus time curve from time zero to infinity ($AUC_{0\to\infty}$) and the area under the respective first moment time curve from time zero to infinity ($AUMC_{0\to\infty}$) were calculated using the linear trapezoidal method and standard area extrapolation technique. Equations 1 and 2 were then used to calculate the clearance and volume for DDB.

$$CL_{elimination} = \frac{Dose}{AUC_{0\to\infty}} \quad (1)$$

$$Vd_{ss} = \frac{AUMC_{0\to\infty}}{AUC_{0\to\infty}} \times CL_{elimination} \quad (2)$$

One-way analysis of variance was used to compare the means between the treatments. $P < 0.05$ was accepted as denoting statistical significance. Data were expressed as the mean ± standard deviation (SD).

Results

GC-MSD assay for DDB in the rat plasma

DDB and the internal standard were readily separated from other endogenous peaks; the retention times for DDB and the internal standard were found to be 11.7 and 12.8 min, respectively. The calibration curve for DDB was linear ($r^2 = 0.9993 \pm 0.0001$ for three calibration runs) in the concentration range studied. In a preliminary study, a number of typical extraction solvents (i.e. ether, ethylacetate, chloroform and hexane) were tested. Among these, chloroform was found to have the highest extraction efficiency (approximately 70%) for DDB and the resulting chromatogram had a stable baseline. Based on results from the preliminary experiments, chloroform was selected as the extraction solvent and was used in all subsequent studies.

The validation results for the GC-MSD assay for DDB are summarized in Table 1. The inter- and intra-day variations for the assay were acceptable down to 20 ng/ml (i.e. less than 20% for 20 ng/ml, and 15% for 50–500 ng/ml). Considering this, the limit of quantification (LOQ) of DDB in rat plasma by the GC-MSD assay was determined to be 20 ng/ml. The accuracy of the assay was 17.9% for LOQ and less than 10% for other concentrations. These observations indicated that the assay was valid for a DDB concentration range of 20–500 ng/ml.

Table 1 Intra-day and inter-day precision and accuracy for the gas chromatography-mass selective detector assay of dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate in rat plasma

Concn (ng/ml)	Intra-day (n = 3)		Inter-day (n = 5)	
	REM (%)	CV (%)	REM (%)	CV (%)
20	7.76	12.6	17.9	16.5
50	8.41	5.57	6.19	5.58
100	1.23	4.41	4.14	3.48
200	0.211	1.89	5.24	3.93
500	0.400	0.31	0.746	1.06

REM, relative error of the mean; CV, coefficient of variation.

Systemic pharmacokinetics of DDB in rats

When DDB was given to rats via an intravenous bolus administration at 0.2, 0.5 or 1 mg/kg, the concentration of the drug in plasma decreased with time in a multi-exponential manner (Figure 2). Table 2 summarizes the results of the moment analysis for the dose range studied. In particular, $CL_{elimination}$ and Vd_{ss} were independent of the dose in the range tested (as determined by one-way analysis of variance) (Table 2). These observations indicated that DDB exhibited linear pharmacokinetics in the dose range studied.

The terminal-phase slope for DDB concentration in the plasma for the lowest dose appeared to be slightly different (Figure 2). To determine whether the pharmacokinetic estimation was affected by the terminal phase in the case of the lowest dose, the AUC from time zero to infinity for the lowest dose was estimated, assuming that the terminal phase slope was identical to the slopes obtained for the case of the two higher doses (a slope shallower than the observed slope). Under these conditions, the AUC from time zero to 90 min (the last sampling time with the measurable concentration in the sample) was over 80.1% ± 6.63% of the estimated AUC from time zero to infinity, indicating that the AUC value from time zero to 90 min accounted for the majority of the AUC. Therefore, the terminal-phase slope estimated from the temporal profile for the lowest dose was used in the calculation of the pharmacokinetic parameters for the case of a dose of 0.2 mg/kg.

After the intravenous administration of DDB at a dose of 1 mg/kg, cumulative biliary and urinary excretion for periods of up to 6 h were 118 ± 19.2 and 132 ± 75.7 ng, respectively. The area under the plasma–concentration time curve for periods up to 90 min accounted for more than 85% of the area under the plasma–concentration time curve to infinity. Consistent with this finding, DDB was not detected in bile samples collected after 2 h, suggesting that a 6-h collection period was adequate for the determination of DDB recovery to the bile and urine. Taken together, the cumulative excretion of DDB into the bile and urine was likely to be less than 0.1% of the total dose.

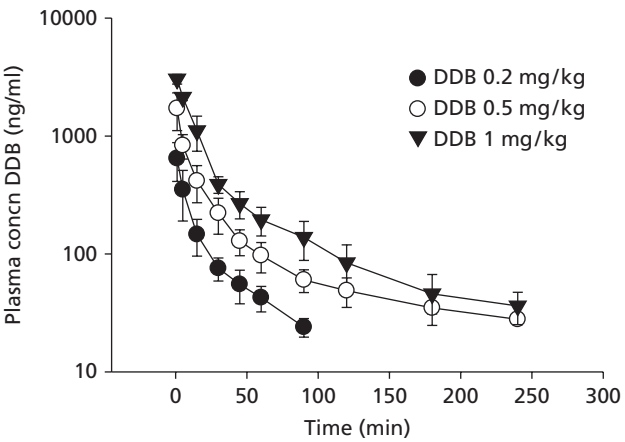

Figure 2 Temporal profiles for dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate concentration in rat plasma. Rats were administered 0.2, 0.5, or 1 mg/kg dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB) intravenously. Values are mean ± SD, n = 5 for each dose level.

Table 2 Summary of the pharmacokinetic parameters for dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate

	Dose (mg/kg)		
	0.2	0.5	1
AUC (ng min/ml)	10 200 ± 3460	31 800 ± 8350	65 200 ± 11700
AUMC (ng min^2/ml)	373 000 ± 171 000	2 390 000 ± 1 360 000	3 620 000 ± 1 540 000
MRT (min)	35.1 ± 7.66	73.5 ± 34.2	53.7 ± 16.4
$CL_{elimination}$ (ml/min per kg)	21.6 ± 7.79	16.7 ± 4.81	15.8 ± 3.25
Vd_{ss} (l/kg)	0.719 ± 0.161	1.19 ± 0.531	0.815 ± 0.155

Values are mean ± SD, $n = 5$ for each dose level. *AUC*, area under the curve; *AUMC*, area under the first moment time curve; *MRT*, mean residence time; $CL_{elimination}$, elimination clearance; Vd_{ss}, volume of distribution at steady state.

Pyloric vein administration study

When DDB was administered via a short-term infusion to the pyloric vein at a dose of 1 mg/kg, the concentration of the drug in the plasma was significantly lower than that for the femoral vein infusion group (Figure 3). As a result, the AUC for the femoral vein administration group was determined to be 35 400 ± 14 900 ng min/ml while, for the pyloric vein, the value was 10 400 ± 464 ng min/ml. The ratio (F_H) of the AUC for pyloric vein administration to the AUC for femoral vein administration was 0.294, and, thus, the hepatic extraction ratio for the drug was approximately 70.6%, indicating that DDB was subject to a significant hepatic first-pass effect.

Red blood cell partition and plasma protein binding of DDB

The haematocrit was 44.2 ± 0.631% and 44.1 ± 0.470% in blank rat blood and the blood sample containing 5 μg/ml DDB, respectively, indicating that the presence of the drug did not lead to haemolysis. The concentration of DDB was found to be 5.05 ± 0.257 μg/ml in the rat plasma (the haematocrit was 44.1 ± 0.470%), and, thus, the estimated concentration of DDB in the red blood cells would be 4.94 ± 0.326 μg/ml (i.e. the partition coefficient for DDB between plasma and red blood cells would be 0.982 ± 0.112). Therefore, the plasma concentration of DDB was essentially identical to the blood concentration and the pharmacokinetic parameter obtained for the plasma concentration (Table 1) should be close to or the same as that for the blood concentration.

Based on the measured DDB concentration in the plasma and filtrate, the percentage binding of the drug to plasma proteins was estimated to be 74.2 ± 4.54%. This indicated that the compound bound highly to plasma proteins. The binding of the drug to the ultrafiltration kit was 7.8 ± 1.6%, indicating that nonspecific binding of the drug to the experimental system was a minor factor.

In-vitro estimation of intestinal permeability

When the apical to basal transport of DDB across a Caco-2 monolayer was examined, the rate was found to be proportional to the initial DDB concentration, in the range 0.25–5 μM (Figure 4). Consistent with this observation, the Eadie–Hofstee transformation indicated that DDB transport was mediated by linear kinetics (Figure 4, inset). The calculated permeability for DDB across Caco-2 monolayers was determined to be (6.8 ± 0.70) × 10^{-5} cm/s. In this study, an extension of the transport study with a higher DDB

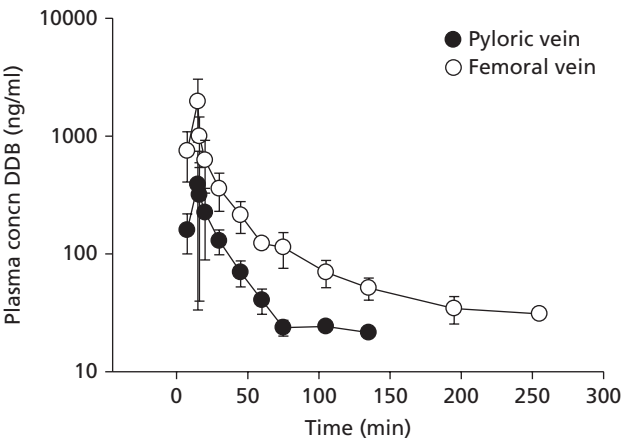

Figure 3 Temporal profiles for dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate in rat plasma after pyloric or femoral vein administration. Rats were administered 1 mg/kg dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB). Values are mean ± SD, $n = 3$ for each group.

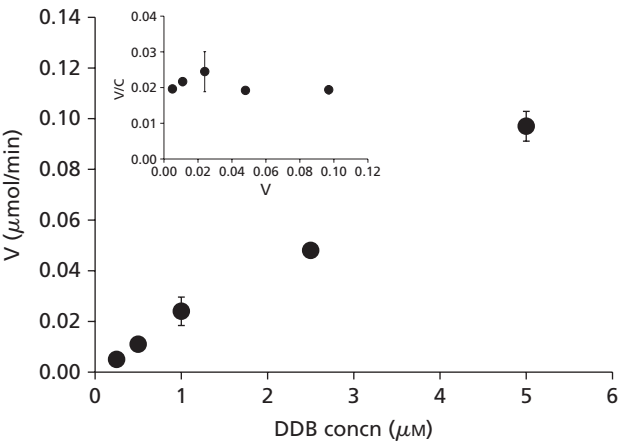

Figure 4 Rate of dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate transport across Caco-2 cell monolayers. Rate of dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB) transport is given as a function of DDB concentration in the medium. Each value represents mean ± SD of triplicate runs. The inset indicates the Eadie–Hofstee transformation of the concentration dependency data. V, velocity; C, concentration.

concentration was not possible, because the highest concentration used (i.e. 5 μM) approached the practical solubility limit of the drug (i.e. 5.98 μM). The leakage of mannitol in the Caco-2 experimental system was approximately 0.295 ± 0.0205%/h per cm^2, indicating that the integrity of the Caco-2 cell monolayer was maintained reasonably well.[16]

In addition to DDB permeability in Caco-2 cell monolayers, the intestinal permeability of the drug was found to be $(1.78 \pm 0.229) \times 10^{-5}$ cm/s in the rat intestinal epithelium. Mannitol permeability in the Ussing chamber set-up was found to be less than $(3.9 \pm 0.28) \times 10^{-6}$ cm/s, suggesting that the integrity of the rat intestinal epithelium used in the study was adequate.

Discussion

The findings herein indicated that the systemic pharmacokinetics of DDB were mediated by linear elimination kinetics in rats with an estimated elimination clearance of approximately 15.8–21.6 ml/min per kg (Table 2; i.e. 3.95–5.40 ml/min per rat assuming the body weight of a rat to be 250 g). It was found that urinary and biliary recovery were less than 0.1% for DDB, indicating that the drug was primarily eliminated via its metabolism. To determine the hepatic first-pass effect directly, DDB was administered to the pyloric vein and the AUC compared with that from the femoral vein administration study. The hepatic extraction was found to be approximately 70.6%, suggesting that a significant portion of DDB was presystemically eliminated.

In this study, the AUC for a dosage of 1 mg/kg in rats that had been subjected to surgery without opening the abdomen (Table 2) was found to be approximately 66 800 ng min/ml, a significantly higher value than that obtained for the femoral vein administration group. In the pyloric administration study, the drug was given to the rats via a 15-min infusion; the half life for the initial phase after an intravenous bolus administration was approximately 15.1 min, and, as a result, a significant portion of the drug may have been eliminated during the infusion period (i.e. 15 min). Therefore, the significantly lower AUC value for the femoral vein administration group compared with the typical dosing may be attributed to the method used for DDB administration.

Although hepatic metabolism has not been reported for schizandrin C, schizandrin B, a structural analogue of schizandrin C and DDB, was found to be metabolized by murine cytochrome P450 (CYP)2E1.[17] In contrast, DDB was found to be metabolized by CYP1A2 (O-dealkylation of the carboxymethyl group), CYP3A4 and CYP2C9 (demethylation of the methylenedioxyphenyl moiety).[11,18] Therefore, the presystemic elimination of DDB may also have been mediated by these CYP isozymes. Taken together with the assessment for the hepatic first effect, these observations indicated that up to 70% of DDB was presystemically eliminated by first-pass metabolism, probably by CYP isozymes in the liver.

Since the solubility of DDB in water is extremely low (2.5–4.0 μg/ml), it was possible that DDB was not completely solubilized in the intestinal fluid (approximately 2.5 ml/250 g rat), thereby leading to an inadequate absorption.[10,19] Alternatively, intestinal permeability may not have been adequate thereby leading to a low bioavailability in humans. To address this issue, we attempted to directly measure intestinal permeability. We found that solubilized DDB was, in fact, highly permeable (i.e. $(1.78 \pm 0.229) \times 10^{-5}$ cm/s for the rat intestinal epithelium; $(6.8 \pm 0.70) \times 10^{-5}$ cm/s for the Caco-2 cell monolayer). With regards to the permeability study with Caco-2 cell monolayers, the value of $(6.8 \pm 0.70) \times 10^{-5}$ cm/s suggested that it was likely that the drug was nearly completely bioavailable.[20] Regarding the permeability study with rat intestinal epithelium, the effective surface area of the small intestine was approximately 1×10^4 cm^2 for rats and, thus, the DDB absorption clearance may have been close to 0.178 ml/s (10.7 ml/min).[21] Since the typical intestinal fluid volume of a rat was found to be 0.4–1.0 ml/100 g, the absorption rate constant (k_a) for DDB was calculated to be 4.28–10.7/min for a 250 g rat, indicating that the drug permeated rapidly across the epithelium.[19] Theoretically, bioavailability (F) in the absence of presystemic first-pass elimination may be estimated from the equation $F = 1 - e^{-k_a \times intestinal\ transit\ time}$. Therefore, assuming that the drug underwent rapid permeation throughout the intestinal tract and that the average intestinal transit time was 24 h (i.e. 1440 min), the theoretical bioavailability would be expected to be complete. Therefore, based on the permeability assessment, our data indicated that solubilized DDB would be completely bioavailable, if first-pass metabolism was not the primary factor in its bioavailability.

It has been reported that schizandrin C, a chemical analogue of DDB, may be a substrate for efflux transporters such as multidrug resistance proteins and the P-glycoprotein in Caco-2 cell monolayers.[22] In contrast, transport in the absorptive direction was apparently directly proportional to the DDB concentration (Figure 4), suggesting that the involvement of efflux transporters was not functionally important for DDB. The discrepancy may have been due in part to a significant difference in the chemical structures.

Based on literature reports, the oral bioavailability of DDB in humans was approximately 30%.[8] Therefore, the significant first-pass metabolism of the drug, coupled with its extremely low solubility in water (i.e. 2.5–4.0 μg/ml), may have contributed to the problematic absorption of the drug by the systemic circulation.[10]

Conclusions

The elimination and distribution of DDB appeared to be mediated by a linear kinetic process in rats. The drug exhibited significant first-pass metabolism, while solubilized DDB was readily permeable to the rat intestinal epithelium and to Caco-2 cell monolayers. The kinetics for the intestinal epithelium appeared to be mediated by a linear process. Therefore, these observations indicated that a significant first-pass metabolism for DDB was the primary determinant for its low bioavailability. Secondary to first-pass metabolism, limited solubility of the drug in the intestine may also be involved in the problematic absorption of the drug into the systemic circulation.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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