ENANTIOSELECTIVE LOCAL DISPOSITION OF SEMOTIADIL (R-ENANTIOMER) AND LEVOSEMOTIADIL (S-ENANTIOMER) IN PERFUSED RAT LIVER

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ABSTRACT:

The enantioselective local disposition of semotiadil (R-enantiomer) and levosemotiadil (S-enantiomer) in rat liver was investigated in the single-pass perfusion system containing 1% bovine serum albumin (BSA). After an instantaneous injection of semotiadil, levosemotiadil, or Evans Blue (a marker of BSA), each outflow time profile from the liver was analyzed by a two-compartment dispersion model. The recovery ratio, $F_H (1.88 \pm 0.28\%)$, of semotiadil was significantly smaller than that (8.99 \pm 1.40\%) of levosemotiadil. The mean transit time, $t_{\bar{M}}$ (0.146 \pm 0.014 min) of semotiadil was significantly smaller that (0.191 \pm 0.012 min) of levosemotiadil. The biliary excretion kinetics of these enantiomers was also evaluated by moment analysis. The parent compound (semotiadil or levosemotiadil) was not detected in bile, but four metabolites generated from each parent enantiomer were found in the bile. A portion (16.5 \pm 1.2%) of the drug eliminated by the liver was recovered as R-metabolites in the bile within 1 hr after an injection of semotiadil, whereas 11.2 \pm 1.6% was recovered as S-metabolites in the bile within 1 hr after an injection of levosemotiadil. This excreted percentage into the bile was significantly different between R- and S-metabolites. The mean biliary excretion time $M_{RTE}$ (19.1 \pm 2.2 min) of total R-metabolites was significantly larger than that (14.8 \pm 1.1 min) of total S-metabolites. In conclusion, stereoselectivity was suggested both at the hepatic elimination of the parent compound and at the biliary excretion of the metabolites.

Keywords: Semotiadil, Levosemotiadil, Biliary excretion, Enantioselectivity, Single-pass perfusion.

Materials and Methods

Chemicals. Semotiadil fumarate, levosemotiadil fumarate, and R- and S-metabolites (M-3, M-4, M-5, and M-7) were obtained from Santen Pharmaceutical Co., Ltd. (Osaka, Japan) (fig. 1). SA2979 and SA3006 used as internal standards for samples of the bile and the perfusate, respectively, were also obtained from Santen Pharmaceutical Co., Ltd. (fig. 1). BSA (fraction V) and $\beta$-glucuronidase were purchased from Sigma Chemical Co. (St. Louis, MO). Other reagents were of analytical or HPLC grade.

Animals. Male Wistar rats (190–200 g) were supplied by Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). Animals were allowed to have free access to water and standard rat food.

Liver Perfusion Experiment. Single-pass perfusion experiments were performed in situ according to the method of Mortimore and Tietze (31). Rats were anesthetized with diethyl ether, and the common bile duct was cannulated with a polyethylene tube (PE-10, Becton Dickinson and Co., Sparks, MD). Through the portal vein catheterized with polyethylene tube (1.67 mm o.d.), the perfusate was delivered to the liver at a constant flow rate (25 \pm 0.8 ml/min/liver) using a peristaltic roller pump (RP-N3, Furrue Science Co., Ltd., Tokyo, Japan). The perfusate of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM glucose and 1% BSA was saturated with 95% O2:5% CO2 and maintained at 37°C during the liver perfusion. The flow recovery was 99.9 \pm 0.8%. After stabilization for 20–30 min, 0.25 ml of the solution of semotiadil (25 $\mu$g/ml), levosemotiadil (25 $\mu$g/ml), or Evans Blue (4 mg/ml) dissolved in the perfusate was injected instantaneously into the liver through the portal vein catheter using a six-port rotary valve injector. Outflow perfusate...
samples were corrected at intervals of −1 sec for 50 sec from the cannula inserted into the thoracic vena cava inferior. Exact sampling times were calculated from the eluent weights. Bile samples were collected at 5-min intervals for the first 10 min and at 10-min intervals for the last 50 min.

Broadening of the injected sample in the injector and the catheters was negligible. Liver viability was confirmed by the bile flow rate (≥0.5 μl/min/g liver) and homogeneous appearance.

Assay of BSA, Semotiadil, and Levosemotiadil in Outflow Perfusate. BSA labeled with Evans Blue in the outflow perfusate was determined by a spectrophotometer (UV-1200, Shimadzu) at 620 nm. The free fraction of Evans Blue in the perfusion system was negligible because of high affinity to BSA (32). The standard calibration curve for BSA labeled with Evans Blue in the perfusate was linear (r = 0.999) between 1.25 and 62.5 μg/ml.

Because no chiral inversion was noticed in the rat (33), semotiadil and levosemotiadil were determined by HPLC with ordinary reversed-phase column. Each bile sample was mixed with 0.4 ml of β-glucuronidase solution (20,000 units/ml) in 0.1 M sodium acetate buffer (pH 5.0). After incubation at 37°C for 19 hr, the internal standard solution (0.1 ml, 10 μg/ml) and 0.5 ml of 0.1 M sodium acetate buffer (pH 5.0) were added to the bile sample. The mixture was prepared to the solid-phase extraction column of BondElut PH (size: 1 ml/100 mg; Varian, Harbor City, CA) that had been washed with 1 ml of acetonitrile and 1 ml of 0.1 M sodium acetate buffer (pH 5.0). The column was washed with 1 ml of water and 1 ml of 5% water:95% acetonitrile solution (v/v). The analyte was eluted with 1 ml of a mixture of 150 mM di-n-butylamine adjusted at pH 3 with phosphoric acid and acetonitrile (5/95, v/v). The eluate was evaporated under nitrogen, and the residue was dissolved in 0.1 M potassium hydrogen sulfate (1 ml). The aqueous solution was washed once with cyclohexane (1 ml) and mixed with 1 M NaOH (0.15 ml). The mixture was extracted twice with the solvent (1 ml) composed of 80% benzene-20% cyclohexane (v/v). The organic phase was transferred to a glass tube and evaporated to dryness under nitrogen. The residue was dissolved in the mobile phase (0.2 ml) of HPLC and the aliquot (0.1 ml) was injected in HPLC.

The HPLC system consisted of controller (SCL-6A, Shimadzu), solvent-delivery pump (LC-10AS, Shimadzu), autoinjector (SIL-10A, Shimadzu), UV detector (SPD-10A, Shimadzu), integrated data analyzer (Chromatopac C-R6A, Shimadzu), and reversed-phase column of Inertsil ODS-2 (4.6 mm i.d. × 25 cm, GL Science, Tokyo, Japan) maintained at 50°C in a column oven (CTO-10A, Shimadzu). The absorbance of column eluent was monitored at 300 nm. The standard calibration lines constructed in the perfusate were linear (r > 0.999), from 2.4 to 240 ng/ml for both semotiadil fumarate and levosemotiadil fumarate.

Assay Method of Metabolites from Semotiadil and Levosemotiadil in Bile. Because four R-metabolites (or S-metabolites) (i.e. M3, M4, M5, and M7) were found exclusively in the bile after administration of semotiadil (or levosemotiadil) to rats (33, 34), the chiral inversion is negligible between R- and S-enantiomers in rats. Thus, the unchanged drugs and major biliary metabolites after oral administration were determined after deglucuronidation with β-glucuronidase using the ordinary reversed-phase column. Each bile sample was mixed with 0.4 ml of β-glucuronidase solution (20,000 units/ml) in 0.1 M sodium acetate buffer (pH 5.0). After incubation at 37°C for 19 hr, the internal standard solution (0.1 ml, 10 μg/ml) and 0.5 ml of 0.1 M sodium acetate buffer (pH 5.0) were added to the bile sample. The mixture was prepared to the solid-phase extraction column of BondElut PH (size: 1 ml/100 mg; Varian, Harbor City, CA) that had been washed with 1 ml of acetonitrile and 1 ml of 0.1 M sodium acetate buffer (pH 5.0). The column was washed with 1 ml of water and 1 ml of 5% water:95% acetonitrile solution (v/v). The analyte was eluted with 1 ml of a mixture of 150 mM di-n-butylamine adjusted at pH 3 with phosphoric acid and acetonitrile (5/95, v/v). The eluate was evaporated under nitrogen, and the residue was dissolved in 0.1 M potassium hydrogen sulfate (1 ml). The aqueous solution was washed once with cyclohexane (1 ml) and mixed with 1 M NaOH (0.15 ml). The mixture was extracted twice with the solvent (1 ml) composed of 80% benzene-20% cyclohexane (v/v). The organic phase was transferred to a glass tube and evaporated to dryness under nitrogen. The residue was dissolved in the mobile phase (0.2 ml), and the aliquot (0.1 ml) was injected in the HPLC system.

HPLC apparatus was the same as that used for the assay of the concentrations in the perfusate. A reversed-phase column, Develosil ODS-T-5 (4.6 mm i.d. × 25 cm; Nomura Chemicals, Seto, Japan), was used with the mobile phase of a mixture of 0.05 M phosphoric acid adjusted at pH 4.5 using sodium hydroxide and acetonitrile (62/38, v/v). The column temperature was 40°C. The flow rate of mobile phase was maintained at 0.8 ml/min for the first 15 min after the sample injection was increased to 1.5 ml/min according to the gradient program for the next 10 min and then was held at 1.5 ml/min for the last 7 min.
The absorbance of the eluent was monitored at 300 nm. The standard calibration lines constructed with the blank bile sample were linear \((r > 0.999)\) from 2 to 500 ng for M-5, and from 5 to 200 ng for M-3, M-4, and M-7, respectively.

**Data Analysis of Time Profiles of Outflow Perfusate.** The outflow time profiles of semotiadil and levosemotiadil were analyzed by the curve-fitting based on one- and two-compartment dispersion models (22). The pharmacokinetic parameters in dispersion models were estimated by MULTI(FILT) on an IBM-PC compatible computer. The outflow time profiles were well described by the two-compartment dispersion model, on the basis of Akaike’s information criterion. A two-compartment dispersion model with central elimination is given by eq. 1 in the Laplace-transformed form,

\[
C(s) = \frac{M}{Q} \exp \left[ \frac{Q \cdot V_B}{2D_c} \left( 1 - \sqrt{1 + \frac{4D_c}{Q} \left( s + k_{12} + k_e - \frac{k_{12} \cdot k_{21}}{s + k_{21}} \right)} \right) \right],
\]

where \(M\) is the amount injected into the portal vein, \(Q\) is the flow rate of the perfusate, \(D_c\) is the corrected dispersion coefficient, \(V_B\) is the volume of blood space that actually means the distribution volume of central compartment, \(k_e\) is the elimination (or irreversible transfer) rate constant from the perfusate into the hepatic tissues, and \(k_{12}\) and \(k_{21}\) are the forward and backward transfer rate constants, respectively. The partition ratio \((k' = k_{21}/k_{12})\) is an index of the nonequilibrium distribution (or reversible transfer) between the blood space and the hepatic tissues. It has been demonstrated that the central elimination model is kinetically equivalent to the peripheral elimination model (22).

These parameters are correlated to distribution volume \((V_B)\), dispersion number \((D_n)\), and efficiency number \((R_n)\) as

\[
V_H = V_B(1 + k') \quad (2)
\]
\[
D_n = D_c/Q \cdot V_B \quad (3)
\]
\[
R_n = k_e \cdot V_B/Q. \quad (4)
\]

The moment parameters of hepatic local disposition \([i.e. the recovery ratio (F_H) and the mean transit time (\(\bar{t}_H\))]\) were correlated with the parameters in the dispersion model by the following equations.

\[
F_H = \exp \left[ \frac{Q \cdot V_B}{2D_c} \left( 1 - \sqrt{1 + \frac{4D_c}{Q} \cdot k_e} \right) \right], \quad (5)
\]
\[
\bar{t}_H = \frac{V_B(1 + k')}{Q \sqrt{1 + \frac{4D_c}{Q} \cdot k_e}}. \quad (6)
\]

**Data Analysis of Excretion Time Profiles into Bile.** The excretion of metabolites into the bile were analyzed by the moment analysis. The excretion ratio \((A_e)\) and the mean residence time \((MRT)\) were calculated by the trapezoidal integration of excretion rates with the extrapolation to infinite time.

**Statistics.** The statistical differences among the kinetic parameters of semotiadil, levosemotiadil, and BSA in the output perfusate were tested by ANOVA at 5% significant level. The statistical differences between the moment of the metabolites generated from semotiadil and levosemotiadil in the bile were also tested at 5% significant level.

**Results and Discussion**

Figure 2 shows the typical time profiles of semotiadil, levosemotiadil, and BSA in the outflow perfusate and the predicted time courses. Table 1 presents the local disposition parameters and the local moments of semotiadil, levosemotiadil, and BSA estimated by curve-fitting. The parameter for elimination \((k_e \text{ or } R_n)\) of semotiadil was significantly larger than that of levosemotiadil. Consequently, \(F_H\) (1.88 ± 0.28%) of semotiadil was significantly smaller than that of levosemotiadil (8.99 ± 0.03%), respectively, in 1% BSA solution (pH 7.4; total drug concentration, 50 \(\mu\)M) (35). Therefore, the difference of bindings with BSA in the perfusate is possibly one of the factors attributable to the difference in the hepatic elimination of these enantiomers. \(\bar{t}_H\) (0.146 ± 0.014 min) of semotiadil was significantly smaller than that (0.191 ± 0.012 min) of levosemotiadil. \(V_B(\%)\) values of semotiadil and levosemotiadil were ~30%, which are much larger than 20% of BSA, the marker of blood space. \(k'\) values of semotiadil and levosemotiadil were greater than unity. In the hepatic perfusion study using \(\beta\)-lactam antibiotics (ampicillin, oxacillin, and cefixime), \(V_B(\%)\) values were close to that of BSA (22, 24). \(k'\) values of these \(\beta\)-lactam antibiotics were also close to that of BSA. \(V_B\) of BSA is almost close to the volume of blood space, because BSA has slight interaction with hepatic tissues (36–38). The small \(F_H\) and the large \(\bar{t}_H\) values of semotiadil and levosemotiadil show that these enantiomers were effectively eliminated by the liver, and a small portion of each enantiomer returns to the perfusate as the intact form after a considerable long residence in/on the hepatic tissues. Thus, \(k'\) is an index for the extent of nonequilibrium distribution between the blood space and the hepatic tissues. The unusually large \(V_B\) values of semotiadil and

![Fig. 2. Typical outflow time profiles of semotiadil (R-enantiomer) (A), levosemotiadil (S-enantiomer) (B), and BSA (C) after instantaneous injection into perfused rat liver.](image-url)
where $V_C$ is the volume of distribution in the central compartment, and $k'$ is the partition ratio between the central and peripheral compartments. It is noted that eq. 7 is similar to eq. 2. It has been known that $V_C$ is usually greater than that of the blood space and includes the term of equilibrium distribution.

$k_{21}$ is an index for nonequilibrium distribution. As $k_{21}$ increases to infinity, a two-compartment dispersion model in eq. 1 reduces to a one-compartment dispersion model. In this study, $k_{21}$ values of semotiadil and levosemotiadil were almost the same as that of BSA. $D_N$
is an index for eddy mixing in the blood space and is predicted to be independent of the properties of drug. Actually, $D_v$ values of semotiadil and levosemotiadil were almost the same as that of BSA.

Figure 3 shows the time profiles of cumulative amounts of metabolites excreted into the bile, and table 2 presents moment values of biliary excretion of metabolites. In both cases of $R$- and $S$-enantiomers, the biliary-excreted amount of $M$-3 and $M$-4 within 1 hr were much larger than those of $M$-5 and $M$-7. The difference of $Ae(t) - F_p(t)$ was statistically significant between $M$-4 enantiomers, between $M$-7 enantiomers, and between total $R$- and $S$-metabolites, which demonstrates that the extent of metabolism from parent drug to metabolites or the extent of biliary excretion of metabolites is enantioselective. $MRT_v$ of three $R$-metabolites ($M$-4, $M$-5, and $M$-7), except $M$-3, were significantly greater than those of $S$-metabolites. Consequently, $MRT_v$ of total $R$-metabolites was significantly greater than that of total $S$-metabolites, which demonstrates that the rate of metabolism from parent drug to metabolites or the rate of biliary excretion of metabolites is enantioselective.

In conclusion, the present study for the hepatic local disposition of semotiadil and levosemotiadil demonstrated the enantioselectivity both at the uptake and at the metabolic transformation (or the biliary excretion) by the liver, and $V_g$ of semotiadil and levosemotiadil is predicted to be actually the sum of the blood space in the liver and the volume of an equilibrium distribution between the blood space and hepatic tissues.

References


