In Situ and in Vitro Evidence for Stereoselective and Carrier-Mediated Transport of Monocarboxylic Acids across Intestinal Epithelial Tissue

Takuo Ogihara, Ikumi Tamai, and Akira Tsuji*

Department of Pharmacobiodynamics, Faculty of Pharmaceutical Sciences, Kanazawa University, 13–1 Takara-machi, Kanazawa 920–0934, Japan. Received December 22, 1999; accepted March 10, 2000

The present study was designed to establish the significance of carrier-mediated transport in the intestinal absorption of monocarboxylic acids by examining the stereoselectivity of transepithelial transport of chiral monocarboxylic acids. The transport of l- and d-lactic acids was examined in vitro using rat intestinal tissue sheets and in situ by means of intra-jejunal administration, followed by measurement of the plasma concentration. Both the absorptive and secretory transport of l-[14C]lactic acid across the intestinal epithelial tissues of rats was significantly greater than that of the d-isomer. The secretory transport of the l-isomer was significantly greater than the absorptive transport, implying net transport in the secretory direction. When l- and d-[14C]lactic acids were administered to the rat jejunum, the absorption ratio of the l-isomer was lower than that of the d-isomer at 15 min after administration. The concentration-dependence of absorption for both l- and d-[14C]lactic acids indicated the involvement of both saturable and nonsaturable processes. The saturable process showed a higher affinity and lower capacity for l-lactic acid compared with the d-isomer, while no significant difference between the isomers was observed in the nonsaturable process. The absorption of l-lactic acid was inhibited by chiral 2-hydroxymonocarboxylic acids in a stereoselective manner. Chiral monocarboxylic acids were shown to cross the intestinal epithelial tissues and to be absorbed in a stereoselective manner after oral administration, suggesting the involvement of specific carrier-mediated transport mechanism(s) in their intestinal absorption in vivo.

Key words membrane transport; stereoselectivity; monocarboxylic acid; intestinal absorption; lactic acid

Stereoselectivity has been frequently observed in pharmacokinetic, pharmacodynamic and toxicological studies of drug–receptor interactions, drug metabolism and serum protein binding. However, little is known about the stereoselectivity in intestinal absorption.

Using rabbit and rat jejunal brush-border membrane vesicles (BBMVs), cells of a human colon carcinoma cell line, Caco-2, and gene expression in Xenopus laevis oocytes and mammalian cells, we have demonstrated that the intestinal membrane transport of monocarboxylic acids, such as acetic acid, nicotinic acid, salicylic acid, benzoic acid and pravastatin, occurs via at least two carrier-mediated processes that are driven by an inward-directed proton gradient and/or an outward-directed bicarbonate gradient.

Similar transport mechanisms for propionate and lactate in human, rabbit and rat intestinal BBMVs have been reported. These carrier-mediated forms of transport are likely to predominate over passive diffusion, according to the pH-partition hypothesis, in the absorption of monocarboxylic acids across the intestinal membrane, although no clear in vivo evidence is yet available.

Moreover, we found that l- and d-lactic acids are transported by a specific carrier-mediated transport mechanism across intestinal epithelial Caco-2 cells in a stereoselective manner. The permeation of l-[14C]lactic acid at a tracer concentration exhibited pH-dependence and its inhibition by unlabeled lactic acid was significantly greater than that of the d-isomer. The transport of both l- and d-[14C]lactic acids involved saturable and nonsaturable processes with a higher affinity and a lower capacity for l-lactic acid compared with the d-isomer. Since it is possible that the transporters discriminate with respect to the chirality of drugs, observation of differences in the permeation of enantiomers across the intestinal membrane would provide further evidence of the physiological importance of carrier-mediated transport in the intestinal absorption process.

The present study was intended to clarify the significance of carrier-mediated transport in the intestinal absorption of monocarboxylic acids by examining the stereoselectivity in the transcellular transport of chiral monocarboxylic acids by isolated intestinal tissues and in situ intestinal absorption in rats. As substrates for the present study, we chose l- and d-lactic acids, which undergo hardly any metabolism or racemic conversion during the intestinal membrane transport process.

MATERIALS AND METHODS

Materials l-[14C]Lactic acid (5.55 GBq/mmol) and d-[14C]lactic acid (2.04 GBq/mmol) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO), and [1H]mannitol (1110 GBq/mmol) was purchased from New England Nuclear (Boston, MA). Pentobarbital sodium (Nembutal™) was obtained from Abbott Laboratories (North Chicago, IL). All other chemicals were commercial products of reagent grade.

Intestinal Tissue Transport Experiments The animal study was performed according to the Guidelines for the Care and Use of Laboratory Animals on the Takara-machi Campus of Kanazawa University and approved by the Committee of Ethics of Animal Experimentation of Kanazawa University, Takara-machi Campus. Details of the conditions of each experiment are described in the figure legends. A typical experiment for l-lactic acid transport was performed as follows. Intestinal tissues were isolated from the jejunum of male Wistar rats (8 weeks old, 190–220 g, Nihon SLC). The isolated tissues were washed several times with Hank's balanced salt solution (HBSS, 1.2 mm CaCl2, 0.6 mm KH2PO4, 1.2 mm MgSO4, 119 mm NaCl, 2.4 mm K2HPO4, 10 mm D-glucose and 21 mm NaHCO3, pH 7.4; osmolality

* To whom correspondence should be addressed. e-mail: tsuji@kenrouku.kanazawa-u.ac.jp
© 2000 Pharmaceutical Society of Japan
315 mOsm/kg) and attached to the diffusion chamber system (Costar, Bedford, MA) with a low-volume chamber for rat jejunum (area of exposed tissue: 0.64 cm²). To initiate the transport experiments, 1.5 ml Krebs-Ringer phosphate buffer (KRFB, 128 mm NaCl, 5.1 mm KCl, 1.4 mm CaCl₂, 1.3 mm MgSO₄, 1.3 mm KH₂PO₄, 21 mm NaHCO₃, 10 mm sodium phosphate buffer, pH 7.4, 37 °C) was put into the serosal side (receiver side) and KRFB without bicarbonate and chloride (HCO₃⁻, Cl⁻-free KRFB, 149 mm sodium gluconate, 5.1 mm K gluconate, 1.4 mm Ca gluconate, 1.3 mm MgSO₄, 1.3 mm KH₂PO₄, 10 mm sodium phosphate buffer, pH 6.0, 37 °C) containing L-[¹⁴C]lactic acid as a substrate was loaded into the mucosal side (donor side). The tissues were incubated at 37 °C and the solution was saturated with 5% CO₂/95% O₂ on the serosal side and 100% O₂ on the mucosal side by continuous bubbling at the rate of 5 ml/min. At designated times, a 0.5 ml aliquot was removed from the receiver side and replaced with an equal volume of fresh KRFB (pH 7.4, 37 °C). The amount of radio-labeled substrates transported across the intestinal membrane was estimated from the radioactivity and expressed as a permeability (µl/min/mm²) by dividing the transported amount by the initial concentration in the donor medium. Radioactivity was determined in a liquid scintillation counter (LS6000TA, Beckman, Fullerton, CA). Each result is the mean ± S.E.M. of three experiments.

**Plasma Levels and Absorption Ratio** L- or D-[¹⁴C]lactic acid (1 ml/kg), diluted to a concentration with non-labeled L- or D-lactic acid and HCO₃⁻, Cl⁻-free KRFB (pH 6.0), was directly administered to a 5 cm loop of jejunum from male Wistar rats (Nihon SLC) under anesthesia induced by intraperitoneal administration of pentobarbital sodium (50 mg/kg). At designated times after administration of L- or D-[¹⁴C]lactic acid, blood samples were withdrawn from the jugular and portal veins using a heparinized syringe, and rats were killed by exsanguination under anesthesia. The isolated intestinal loop with its remaining contents was dissolved in Solvable™ (Packard, Meriden, CT) and bleached with 30% hydrogen peroxide. The bleached solution was mixed with Atomlight™ (NEN, Boston, MA) to measure the radioactivity. Plasma samples were mixed with Aquasol™-2 (Packard) and the radioactivity was measured.

**Data Analysis** The permeability coefficient (µl/min/mm² membrane) was determined from the slope of the initial linear portion of the permeability (µl/mm² membrane) versus time (min) curves by linear regression analysis. For pharmacokinetic parameters, the plasma curves were analyzed by the nonlinear least-squares regression computer program (WinNonlin™, SCI Inc., Apex NC),

\[ C = A \exp(-k_1 \cdot t) + B \exp(-k_2 \cdot t) \]  

(1)

where \( C \), \( k_1 \), \( k_2 \), and \( t \) represent the plasma concentration, absorption rate constant, excretion rate constant and time after administration, respectively. The area under the concentration-time curve (\( AUC_{0-\infty} \)) was calculated by applying the trapezoidal rule.

The absorption ratio was calculated from the recovered radioactivity in the intestinal loop and contents,

\[ \text{absorption ratio} = 1 - \frac{\text{recovered radioactivity}}{\text{dosing radioactivity}} \]  

(2)

Absorption rate (nmol/min/kg) was defined as the value obtained by multiplying the absorption ratio per minute by the dose.

In order to estimate the kinetic parameters for the saturable transport, the transport rate (J) was fitted to the following equation consisting of both saturable and apparently nonsaturable-linear terms by using WinNonlin™,

\[ J = J_{\max} \cdot S \left( K_s + S \right)^{-1} + k_d \cdot S \]  

(3)

where \( J_{\max} \) and \( K_s \) are the maximum transport rate and half-saturation constant (Michaelis constant) for the carrier-mediated process, \( S \) is the concentration of substrate, and \( k_d \) is the first-order rate constant for the apparently nonsaturable process.

Statistical analysis was performed using Student's two-tailed t-test. A difference between means was considered to be significant when the p-value was less than 0.05.

**RESULTS**

**In Vitro Intestinal Tissue Transport Study** Figure 1 shows the mucosal-to-serosal (Panel A) and serosal-mucosal (Panel B) transport of L- and D-lactic acids at a concentration of 0.5 µM across the rat jejunum. The permeation of L,D-[¹⁴C]lactic acids increased linearly with time after an initial lag period of a few minutes in both cases. The permeability coefficients of the mucosal-to-serosal transport of L-[¹⁴C]lactic acid, 1.19 ± 0.04 µl/min/cm², was slightly but significantly higher than that of the D-isomer, 1.01 ± 0.05 µl/min/cm². When the secretory serosal-to-mucosal transport of the L- and D-isomers was measured in the same manner, the permeability coefficients were 1.60 ± 0.06 and 1.03 ± 0.19 µl/min/cm², respectively. The secretory transport of the L-isomer was slightly but significantly higher than the absorptive permeation, whereas these values were comparable for the D-isomer. Furthermore, the secretory permeation of the L-isomer was significantly higher than that of the D-isomer.

**In Situ Absorption Study** (1) Plasma Concentrations and Pharmacokinetic Parameters: The plasma concentrations of total radioactivity after a single administration of 1 µM (1 mmol/ml/kg) L- and D-[¹⁴C]lactic acids into the jejunal of male rats are shown in Fig. 2. After administration, the plasma concentration of total radioactivity in the portal vein reached a maximum (\( C_{\text{max}} \)) of 1.47 ± 0.15 ng/ml at 30 min for L-[¹⁴C]lactic acid and 1.70 ± 0.32 ng/ml at 15 min for D-lactic
acid. The absorption rate constant ($k_a$) of the $d$-isomer was 0.301 min$^{-1}$, being 3.7 times greater than that of the $l$-isomer (0.081 min$^{-1}$), whereas the elimination rate constants ($k_e$) were not significantly different between the isomers, 0.017 and 0.012 min$^{-1}$ for $l$- and $d$-isomers, respectively. The $AUC_{0-15\text{min}}$ of $d$-[${}^{14}$C]lactic acid, an index of absorbability, was 16.0 ng eq. min/mL, 1.8 times higher than that of the $l$-isomer, while the $AUC_{0-60\text{min}}$ of $d$-[${}^{14}$C]lactic acid was comparable with that of the $l$-isomer.

(2) Absorption Ratio: Figure 3 shows the absorption ratio after a single administration of 1 $\mu$M $l$- and $d$-[${}^{14}$C]lactic acids, and 33 nm [${}^{3}H$]mannitol to the jejunum of male rats. The absorption ratio of $d$-[${}^{14}$C]lactic acid at 15 min after administration was 48.5±0.7%, which was 1.6 times greater than that of $l$-[${}^{14}$C]lactic acid (29.5±2.0%), whereas there was no significant difference in the absorption ratio at 60 min between the $l$- and $d$-isomers, 72.0±2.5% and 72.4±3.6%, respectively. Since the absorption ratio of $l$- and $d$-[${}^{14}$C]lactic acids at all times tested was apparently higher than the paracellular permeability estimated from the [${}^{3}H$]mannitol transport, the transport of $l$- and $d$-lactic acids was presumed to occur mainly through transcellular permeation rather than via the paracellular pathway. The following absorption studies were performed 15 min after administration, because the uptake was reproducible with little deviation, increased almost linearly with time and was significantly inhibited by the agents mentioned below.

(3) Concentration-Dependence: Figures 4A and 4B show the concentration-dependence of the absorption ratio at 15 min after administration and the initial absorption rate of $l$- and $d$-lactic acids at concentrations ranging from $10^{-8}$ M to $10^{-2}$ M. The $d$-isomer exhibited greater absorption than the $l$-isomer, especially at higher concentrations. The absorption ratio of the $d$-isomer, 44 to 50% over the dosage range from $10^{-7}$ to $5\times10^{-5}$ M, and fell steeply at concentrations higher than $5\times10^{-3}$ M to 32.8±2.4% at 4×$10^{-5}$ M. In contrast, the absorption ratio of the $l$-isomer, which was 38.2±8.1% at $10^{-8}$ M, fell slowly with increasing concentration, reaching 11.4±1.4% at $4\times10^{-5}$ M. The results indicate that the absorption of both $l$- and $d$-lactic acids involves both saturable and nonsaturable processes. Kinetic analysis of the concentration-dependent absorption according to Eq. (3), gave $J_{max}$, $K_s$, and $k_e$ values of 21.6±16.4 nmol/min/kg, 3.03±2.63 mm and 7.00±0.74 $\mu$l/min/kg, respectively, for $l$-lactic acid and 825.0±105.7 nmol/min/kg, 34.8±34.8 mm and 10.1±9.25 $\mu$l/min/kg, respectively, for $d$-lactic acid (mean±S.D.). The saturable process for $l$-lactic acid showed a higher affinity and a lower capacity compared with that of the $d$-isomer, whereas the nonsaturable process was comparable for the two isomers.

(4) Inhibitory Effect on $l$-Lactic Acid Transport: Table 1 shows the effects of the pH of the administered solution, metabolic inhibitors and various anionic compounds on the absorption ratio of $l$-[${}^{14}$C]lactic acid and [${}^{3}H$]mannitol at 15 min after administration to the jejunum of rats. The ab-

---

Fig. 2. Plasma Concentrations of Total Radioactivity after a Single Administration of 1 $\mu$M (1 nmol/ml/kg) $l$- (Open Symbols) or $d$- (Closed Symbols) [${}^{14}$C]Lactic Acid into the Jejunum of Male Rats

Plasma samples were obtained from portal (circle) and jugular (square) veins. Each point represents the mean±S.E.M. of three experiments.

Fig. 3. Absorption Ratio after a Single Administration of 1 $\mu$M (1 nmol/ml/kg) $l$- (○) or $d$- (●) [${}^{14}$C]Lactic Acid or 33 nm of [${}^{3}H$]Mannitol (○) into the Jejunum of Male Rats

Each point represents the mean±S.E.M. of three experiments.

Fig. 4. Concentration-Dependence of the Absorption Ratio at 15 min after Administration (Panel A) and the Initial Absorption Rate (Panel B) of $l$/$d$-Lactic Acids at Concentrations from $10^{-8}$ M, $l$-isomer (○), and $10^{-3}$ M, $d$-isomer (●) to $4\times10^{-2}$ M

Each point represents the mean±S.E.M. of three experiments.
Table 1. Inhibitory Effect of Conditions on L-[¹⁴C]Lactic Acid and [³H]Mannitol Absorption in Rats

<table>
<thead>
<tr>
<th>Entry</th>
<th>Condition</th>
<th>Relative Absorption Ratio (% of control)⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-[¹⁴C]Lactic acid</td>
</tr>
<tr>
<td>Control (pH 6.0)⁷</td>
<td>100.0±6.9</td>
<td>100.0±6.9</td>
</tr>
<tr>
<td>1</td>
<td>pH 7.4⁷</td>
<td>78.4±2.5*</td>
</tr>
<tr>
<td>2</td>
<td>+10 mM NaNO₃</td>
<td>75.4±10.3*</td>
</tr>
<tr>
<td>3</td>
<td>+10 mM DNP</td>
<td>47.5±13.5*</td>
</tr>
<tr>
<td>4</td>
<td>+10 mM DIDS</td>
<td>64.0±24.5*</td>
</tr>
<tr>
<td>5</td>
<td>+10 mM L-Lactic acid</td>
<td>54.0±10.3*</td>
</tr>
<tr>
<td>6</td>
<td>+10 mM D-Lactic acid</td>
<td>65.0±17.0*</td>
</tr>
<tr>
<td>7</td>
<td>+10 mM (S)-Mandelic acid</td>
<td>55.0±17.0*</td>
</tr>
<tr>
<td>8</td>
<td>+10 mM (R)-Mandelic acid</td>
<td>110.1±13.0**</td>
</tr>
<tr>
<td>9</td>
<td>+10 mM (S)-2-Phenylproionic acid</td>
<td>70.7±5.7*</td>
</tr>
<tr>
<td>10</td>
<td>+10 mM (R)-2-Phenylproionic acid</td>
<td>65.6±7.1*</td>
</tr>
<tr>
<td>11</td>
<td>+10 mM benzoic acid</td>
<td>72.0±6.7*</td>
</tr>
</tbody>
</table>

The absorption ratio of radioactivity was measured at 15 min after the administration of L-[¹⁴C]lactic acid (1 μg) and [³H]mannitol (53 μg) to the jejunum of rat. a) Each value represents the mean±S.E.M. of three experiments, and is expressed as percentage of the control. * The pH value of the administrated solution. + Significantly different from the control value by Student's t-test (p<0.05). ** Significant difference between optical isomers by Student's t-test (p<0.05).

Table 2. Absorption Ratio of Radioactivity after Administration of L/D-[¹⁴C]Lactic Acids to Male and Female Rats

<table>
<thead>
<tr>
<th>Administered isomer</th>
<th>Absorption Ratio (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ</td>
</tr>
<tr>
<td>L</td>
<td>29.4±2.0</td>
</tr>
<tr>
<td>D</td>
<td>48.5±0.7</td>
</tr>
</tbody>
</table>

The absorption ratio of radioactivity was measured at 15 min after administration of L- and D-[¹⁴C]lactic acid (1 μg) to the jejunum of rats. a) Each value represents the mean±S.E.M. of three experiments.

The absorption ratio of L-[¹⁴C]lactic acid was significantly reduced by changing the pH from 6.0 to 7.4, as well as by DNP (1 mM), sodium azide (10 mM) and DIDS (2 mM). L/D-Lactic acids and (S)/(R)-mandelic acids significantly reduced the absorption ratio of L-[¹⁴C]lactic acid in a stereoselective manner, whereas no such stereoselectivity was observed in the inhibitory effects of (S) and (R)-2-phenylpropionic acids. The absorption ratio of [³H]mannitol was unchanged in the presence of DNP, sodium azide or DIDS.

(5) Sex Differences in Absorption Ratio: Table 2 shows the sex difference in L- and D-[¹⁴C]lactic acid absorption at 15 min after administration to the jejunum of male and female rats. No significant difference in absorption ratio between the sexes was observed when L- and D-[¹⁴C]lactic acids were used as substrates.

DISCUSSION

We have previously demonstrated that L- and D-lactic acids are transported across Caco-2 cell monolayers in a stereoselective manner via a specific carrier-mediated transport mechanism.⁷ Moreover, we and others have reported the stereoselective transport of L- and D-lactic acids via MCT1, a proton/mono-carboxylic acid cotransporter MCT1. In addition, an anion exchange inhibitor, DIDS, reduced the absorption ratio of l-lactic acid (Table 1). We have previously suggested the participation of the anion exchange transporter, AE2, in the transport of monocarboxylic acids across intestinal apical membranes and this could account in part for the pH-dependent intestinal absorption of monocarboxylic acids, in addition to the effect of the proton-coupled transporter MCT1.⁸,¹⁰,¹⁷ The results obtained here suggest that specific transporters are mainly involved in the intestinal absorption process for lactic acid, rather than passive diffusion.

The absorption ratio of L-[¹⁴C]lactic acid was significantly reduced in a stereoselective manner by 2-hydroxymonocarboxylic acids such as (S)/(R)-mandelic acids, as well as L/D-lactic acids, whereas there was no significant stereoselectivity in the inhibitory effect of (S)/(R)-2-phenylpropionic acids.
acids. We have observed using Caco-2 cells that the transport of L-[^14]C]lactic acid was inhibited by 2-hydroxy- and 2-alkoxymonocarboxylic acids in a stereoselective manner, but not by other monocarboxylic acids with C-2 chirality. According, the presence and position of hydroxylation might be important for the stereoselective transport of monocarboxylic acids across the intestinal membrane.

Since Tanaka et al. suggested that there is a sex difference in the anion exchange transporter in rat kidneys, we examined whether there is a sex difference in the carrier-mediated absorption of L- and D-lactic acids in rats. At 15 min after the administration of L- and D-lactic acids to the rat jejunum, no sex-related differences were observed. It is thought that there is no sex difference in the absorption of monocarboxylic acids in rats, and probably also in humans, since sex differences are observed in rats more frequently than in humans.

In conclusion, it was demonstrated that the intestinal absorption of lactic acid showed stereoselectivity ascribed to the participation of transporters on the epithelial cell membrane both in vitro and in vivo. Moreover, several monocarboxylic acids appear to share a common transporter(s) with lactic acid. Intestinal absorption of acidic drugs could be accounted for by the involvement of specific pH-dependent transporters rather than passive diffusion. These observations also suggest that there is a carrier-mediated monocarboxylic acid transport system(s) for basolateral-to-apical transport as well as apical-to-basolateral transport and that absorptive and secretory transport predominantly determine the apparent permeation of monocarboxylic acids in intestinal absorption in vivo.

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, and a grant from the Japan Health Sciences Foundation, Drug Innovation Project.

REFERENCES