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P-glycoprotein Limits the Brain Penetration of Olopatadine Hydrochloride, H1-Receptor Antagonist

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Summary: Olopatadine, a new second-generation antihistamine, is widely used in the treatment of allergic disorders. The low levels of histamine H1 receptor occupancy in human brain by olopatadine, which is related to its minimal sedation, suggest its low penetration into the brain. The present study evaluates the impact of P-glycoprotein (P-gp) on brain penetration and plasma concentration of olopatadine. The uptake amount of olopatadine in human P-gp transfected LLC-PK1 cells (LLC-GA5-COL150) was lower than that in LLC-PK1. The uptake of olopatadine in LLC-GA5-COL150 was increased in the same level as that in LLC-PK1 in the presence of cyclosporine A, a P-gp inhibitor. After intravenous or oral administration of olopatadine to wild type (WT) and mdr1a/1b knockout (KO) mice at a dose of 1 mg/kg, the brain concentration in KO mice was higher than that in WT mice. On the other hand, the plasma concentration of olopatadine after either route of administration was not different between WT and KO mice. These results suggest that olopatadine is a substrate of P-gp, and that P-gp limits the brain penetration but does not affect the plasma concentration of olopatadine.

Keywords: olopatadine; second-generation H1-antagonists; P-glycoprotein; brain distribution; pharmacokinetics; mouse

Introduction

Histamine H1 receptor antagonists (H1-antagonists) are the mainstays of treatment for a number of allergic disorders. H1-antagonists cause sedation or other adverse effects of the central nervous system (CNS) by blockade of H1 receptors in the CNS. They are categorized into the new second-generation H1-antagonists and classical first-generation H1-antagonists according to the frequency of sedation. Differences in sedimenting potential among H1-antagonists are ascribed to differences in H1 receptor occupancy in the CNS.1,2 H1 receptor occupancy in human CNS can be measured by positron emission tomography (PET) with 11C-doxepin as a radioactive tracer that specifically binds to H1 receptors.3–5 Mean cortical H1 receptor occupancy of first-generation H1-antagonists such as d-chlorphenilamine and ketotifen is more than 50%, whereas as for second-generation H1-antagonists such as fexofenadine and terfenadine, the receptors are less occupied (<20%).3–5 Variation in CNS H1 receptor occupancy by H1-antagonists could be explained by the difference not only in an affinity and a selectivity to H1 receptors but also in penetration through the blood-brain barrier (BBB).2 CNS penetration across the BBB is influenced by various factors of compounds, such as lipophilicity, molecular size and number of hydrogen bonds.6 In addition, several efflux transporters including P-glycoprotein (P-gp) play important roles in brain penetration of clinically important drugs.6,7

Abbreviations: P-gp, P-glycoprotein; KO, knockout; WT, wild type; H1-antagonists, histamine H1 receptor antagonists; CNS, central nervous system; BBB, blood brain barrier; BCECs, brain capillary endothelial cells; MDR, multidrug resistance; PET, positron emission tomography; CONGA, The Consensus Group on New Generation Antihistamines; FBS, fetal bovine serum; PBS, Dulbecco's phosphate buffered saline; IS, internal standard; CyA, cyclosporine A; AUC0–t, area under the curve; CL, clearance; Vss, distribution volume at steady state; Cmax, maximum concentration; tmax, time to reach maximum concentration; BSA, bovine serum albumin; PS, the permeability-surface area.
P-gp is encoded by the multidrug resistance gene 1 (MDR1) expressed in brain capillary endothelial cells (BCECs), and also exists in various normal tissues such as intestinal epithelium, liver bile canaliculi and kidney epithelial cells in humans. In rodents, P-gp is encoded by two different genes (mdr1a and mdr1b), and is expressed in the same tissues as in humans. Recently, some in vitro studies using bovine BCECs or cell lines expressing MDR1 have reported that P-gp accepts second-generation H1-antagonists as a substrate, but not first-generation H1-antagonists. Moreover, when some second-generation H1-antagonists were intravenously administered to P-gp knockout (KO) mice, the brain concentrations in KO mice were higher than those in wild type (WT) mice. Therefore, the difference in BBB penetration among H1-antagonists would be associated with efflux transport by P-gp.

Olopatadine hydrochloride (olopatadine, 11-[Z]-3-(dimethylamino)propylidene)-6,11-dihydropibenzo[e]oxepin-2-acetic acid monohydrochloride, Allelock), a new H1-antagonist, was approved in Japan for the oral treatment of allergic rhinitis, chronic urticaria, eczema, dermatitis, prurigo, pruritus cutaneous, psoriasis vulgaris and erythema exudativum multiforme. An ophthalmic solution of olopatadine (Patanol®) was approved in the United States and Japan for the treatment of seasonal allergic conjunctivitis. Olopatadine was rapidly and extensively absorbed after oral administration to healthy volunteers. The plasma concentration of olopatadine reached a maximum at 0.5–2 hours after oral dosing. The urinary excretion of unchanged olopatadine up to 48 hours after oral administration was 58.7–73.4% of the dose, and the urinary excretion of olopatadine metabolites was 0.2–4.6% of the dose. These results indicate that approximately 60% of the administered olopatadine is absorbed at least and that olopatadine is mainly excreted into urine without extensive metabolism in humans. The H1-receptor occupancy of olopatadine in healthy Japanese male volunteers was determined as 15.0% after oral administration of olopatadine at 5 mg by PET with \(^{11}C\)-doxepin. In rats, after oral administration of \(^{14}C\)-olopatadine (1 mg/kg), the \(C_{\text{max}}\) of radioactivity in the brain was about 4% of that in plasma. These results suggest that the penetration of olopatadine to the CNS will be limited. However it was not reported that olopatadine was a substrate of P-gp.

The present study was performed to investigate whether olopatadine was a P-gp substrate, and thereby the CNS exposure to olopatadine was reduced. To clarify olopatadine as a P-gp substrate, the effect of P-gp on the cellular uptake was examined using LLC-GA5-COL150 cells, which overexpress human P-gp on the apical membrane by transfection of human MDR1 in LLC-PK1 cells. The effects of P-gp on the cellular uptake of some other second-generation H1-antagonists were also examined to compare with olopatadine. In addition, the influence of P-gp on brain and plasma concentrations of olopatadine was determined in mdr1a/1b KO mice and their WT mice after intravenous and oral administration of olopatadine.

Materials and methods

Chemicals and animals: Olopatadine was synthesized at our institute of Kyowa Hakko Kogyo (Shizuoka, Japan). Azelastine hydrochloride (azelastine), epinastine hydrochloride (epinastine), bepotastine besilate (bepotastine), cetirizine hydrochloride (cetirizine), fexofenadine hydrochloride (fexofenadine), desloratadine and loratadine were extracted from commercially available formula at our institute. Quindine was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Diazepam, cyclosporine A (CyA), caffeine and colchicine were obtained from Wako Pure Chemical Industries (Osaka, Japan). Bovine serum albumin (BSA) was obtained from Sigma-Aldrich (St. Louis, MO). Medium 199, fetal bovine serum (FBS), Dulbecco’s phosphate buffered saline (PBS), and Trypsin-EDTA (0.25% Trypsin 1 mmol/L EDTA ’4Na) were purchased from Invitrogen (Carlsbad, CA).

Male FVB (WT) mice and mdr1a/1b knockout (KO) mice of 5 weeks of age were obtained from Taconic (Germantown, NY). Both strains of mice were maintained for at least 1 week on a 12-h light/dark cycle in a temperature- and humidity-controlled environment. Food and water were available ad libitum before and during the experiments. The experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, and the experimental protocol used in this study was approved by the Committee for Animal Experiments in Kyowa Hakko Kogyo.

Cell culture: LLC-PK1 and LLC-GA5-COL150 cells were kindly gifted from Dr. Kazumitsu Ueda (Kyoto University, Kyoto, Japan). LLC-GA5-COL150 cells were established by transfection of human MDR1 cDNA into LLC-PK1 cells. LLC-PK1 cells were cultured in Medium 199 containing 3% FBS. For LLC-GA5-COL150 cells, 150 ng/ml colchicine was added to Medium 199 containing 10% FBS. LLC-PK1 and LLC-GA5-COL150 cells were seeded in plastic dishes and grown at 37°C in a 5% CO\(_2\), 95% air atmosphere.

Cellular uptake study: LLC-PK1 cells and LLC-GA5-COL150 cells were seeded in 24-well culture dishes (Asahi Techno Glass, Chiba, Japan) at a cell density of 2.0 × 10\(^4\) cells/cm\(^2\). Cells were cultured on plates for 7 days and the
medium was refreshed every second day. Five hours before
the uptake study, the whole culture medium was replaced
with fresh colchicine-free culture medium. After removal of
the culture medium from dishes, the cells were washed
three times with incubation buffer composed as follows: 145 mmol/L NaCl, 3 mmol/L KCl, 1 mmol/L CaCl$_2$, 0.5
mmol/L MgCl$_2$, 5 mmol/L d-glucose, 5 mmol/L HEPES (pH 7.4). The cells were incubated at 37°C in a 5% CO$_2$, 95%
air atmosphere for 1 hour in 500 µL of incubation buffer.
The uptake study was initiated by replacing the medium with
500 µL of incubation buffer containing 10 µmol/L test
compound and 4% BSA. To determine the effect of CyA on
the cellular uptake of olopatadine, quinidine and diazepam,
10 µmol/L CyA was added to incubation buffer 1 hour be-
fore and during the uptake study. After incubation for 2
hours, an aliquot of incubation buffer (300 µL) was taken
from each well, and the cells were washed three times with
ice-cold PBS. The cells were lysed by the incubation with
0.1% Triton-X100 for 2 hours at 37°C. The incubation
buffer was diluted by 9 times volume of PBS. Diluted buffer
and cell lysates (50 µL) were mixed with 50 µL of acetonitrile
and 20 µL of internal standard (IS) methanol solution.
After centrifugation, the supernatants (25 µL) were injected
into an LC/MS system described below.

**Pharmacokinetic study of olopatadine in mice:**
Dosage solutions of olopatadine hydrochloride for in-
travenous (0.5 mg/mL, 1.34 mmol/L) and oral (0.2 mg/mL,
0.335 mmol/L) administration were prepared using saline.
A 1 mg/kg (2.67 µmol/kg) dose of olopatadine hydrochloride
was administered intravenously via the tail vein (2 µL/g
of body weight) or orally (5 µL/g of body weight). Blood
and brain samples were obtained at 0.05, 0.25, 0.5, 2 and 4
hours post intravenous dosing (n = 3 mice/time point), or at
0.0833, 0.25, 0.5, 1, 2 and 4 hours post oral dosing (n = 3
mice/time point). Plasma samples were obtained by cen-
trifuging the blood samples. Brain was rinsed with PBS, blotted
dry and weighed. Samples were stored at −20°C until
analysis.

**Plasma and brain analysis:** Calibration standards
(2.5–1000 nmol/L) were prepared by spiking untreated
mouse plasma with appropriate standard solutions. Each
plasma sample (100 µL) was mixed with 20 µL of IS solu-
tion (1 µmol/L azelastine in methanol) and 110 µL of dis-
tilled water. Each mixture was transferred to an OASIS®
HLB cartridge (30 mg/1 mL, Waters, Milford, MA). The car-
tridge had been pre-treated sequentially with 1 mL of
methanol and 1 mL of water. After adding the plasma,
the cartridge was washed with 1 mL of 5% methanol. The com-
pound was eluted with 600 µL of methanol and the eluate
was evaporated to dryness under a nitrogen stream. The brain
was weighed and homogenized in a 1:2 weight-to-
volume ratio of PBS. Calibration standards (0.5–200
nmol/L) were prepared by spiking untreated brain
homogenate with appropriate standard solutions. Brain
homogenate (500 µL) was mixed with 100 µL of IS solution,
50 µL of distilled water and 500 µL of methanol. After cen-
trifugation, the supernatant (850 µL) was mixed with 1 mL
of PBS, and transferred to an OASIS® HLB cartridge (60
mg/3 mL). The cartridge had been pre-treated sequentially
with 2 mL of methanol and 2 mL of water. After adding the
brain homogenate, the cartridge was washed with 2 mL of
5% methanol. The compound was eluted with 1 mL of
methanol and the eluate was evaporated to dryness under a
nitrogen stream. The residue was reconstituted with 100 µL
of 0.05% formic acid/methanol = 50/50 (v/v), and after
filtering the sample through an Ultrafree MS (0.2 µm, Milli-
pore, Billerica, MA), the filtrate was injected into the LC/MS
system.

**LC/MS analysis:** Drug concentrations were quantified
by LC/MS. LC was conducted on an Alliance2790 (Waters).
Chromatography was performed at 25°C on an XTerra™
MS C18 (2.1 × 150 mm, 5-µm, Waters) for uptake study
and a Symmetry C18 (2.1 × 150 mm, 5-µm, Waters) for
pharmacokinetic study. Elution was performed with an ap-
propriate gradient of 0.05% formic acid/methanol at a flow
rate of 0.2 mL/min. For fexofenadine, acetonitrile was used
instead of methanol. A Waters ZQ2000 single quadrupole
mass spectrometer was used for MS system. The ionization
mode was electrospray positive. Single ion recording was
used for quantification. The capillary voltage was set at 3.30
kV. The source and desolvation temperatures were set at
100°C and 350°C, respectively. The desolvation and cone
gas flow rates were set at 310 L/h and 50 L/h, respectively.
Monitoring ion was set at molecular ion ([M + H$^+$]) of each
compound, and cone voltage was set for each compound.
The peak areas of the analyte and IS were obtained using
MassLynx 4.0 (Waters).

**Data analysis:** Cellular uptake of the test compound
was described with C/B ratio calculated by following equa-
tion:

First, from the result of LC/MS analysis, peak area ratio of
test compound to IS was calculated in cell lysates sample
(Ratio in cell) and in buffer sample (Ratio in buffer). Then,
the compound amount in cell lysates or buffer is calculated
by equation (1) and (2):

**Test compound in cell lysates (nmol/well)**

$= \frac{\text{Ratio in cell}}{\frac{\text{volume of cell lysates}}{\text{injection volume}}}$

(1)

**Test compound in buffer (nmol/well)**

$= \text{Ratio in buffer} \times \frac{\text{injection volume}}{\text{dilution ratio} \times \frac{\text{volume of buffer}}{\text{injection volume}}}$

(2)

Here, a volume of cell lysates and a volume of buffer
were 200 µL and 500 µL, respectively. An injection volume
was 25 µL. A dilution ratio was 10.

C/B ratio was calculated by equation (3):

$= \frac{\text{C (cell)} - \text{B (buffer)}}{\text{B (buffer)}}$

(3)
Brain penetration of olopatadine is limited by P-gp

C/B ratio = \frac{\text{Test compound in cell lysates (nmol/well)}}{\text{Test compound in buffer (nmol/well)}}

(3)

The effect of P-gp on the cellular uptake was performed with PK1/GA5 ratio calculated by equation (4):

\[
\frac{\text{PK1/GA5 ratio}}{=\frac{\text{C/B ratio in LLC-PK1 cells}}{\text{C/B ratio in LLC-GA5-COL150 cells}}}
\]

(4)

The quantification of olopatadine in mouse plasma and brain was achieved by comparison of the IS ratio of the sample with that of the standard curve. The concentration at each time point was shown as mean ± standard deviation (SD) of three animals. Pharmacokinetic parameters of plasma clearance (CL), distribution volume at steady state (Vss), maximum concentration of plasma (Cmax), time to reach for Cmax (tmax) and area under the curve of plasma or brain concentration (AUC0–4) were calculated from the mean concentration by WinNonlin Professional ver. 4.1 or 5.0.1 (Phar- sight, Mountain View, CA). Significant difference in the cellular uptake between LLC-GA5-COL150 and LLC-PK1 cells and that in the brain and plasma concentrations between KO and WT mice were evaluated by the Student’s t test (for homogeneous data) or the Aspin-Welch’s t test (for heterogeneous data) after the homogeneity of variance was estimated by the F-test. The correlation of between PK1/GA5 ratio and transport ratio was calculated by simple linear regression and correlation. Statistical analysis was performed with SAS System version 9.1.3 (SAS Institute, Cary, NC). The 5% level of probability was considered to be significant.

Results

Contribution of P-gp to the cellular uptake of olopatadine: To evaluate whether olopatadine is a P-gp substrate or not, we investigated the effect of P-gp on the cellular uptake of olopatadine using LLC-PK1 and a P-gp overexpressing cell line, LLC-GA5-COL150 cells. In the present study, quinidine and diazepam were used as control substrates. Quinidine was known to be a substrate of P-gp, and diazepam to be a non P-gp substrate.22 The C/B ratios and PK1/GA5 ratios are shown in Table 1. The C/B ratio of quinidine in LLC-GA5-COL150 cells (0.0288) was significantly lower than that in LLC-PK1 cells (0.635, p < 0.001). The C/B ratio of diazepam in LLC-GA5-COL150 cells was almost the same as that in LLC-PK1 cells (0.0575 in LLC-PK1 cells vs 0.0521 in LLC-GA5-COL150 cells). The C/B ratio of olopatadine in LLC-GA5-COL150 cells (0.00783) was significantly lower than that in LLC-PK1 cells (0.0446, p < 0.05). The PK1/GA5 ratios of diazepam, olopatadine and quinidine were 1.10, 5.70 and 22.0, respectively. The C/B ratios of quinidine and olopatadine in LLC-GA5-COL150 cells were increased in the presence of CyA, a potent P-gp inhibitor.23 As a result in the presence of CyA, the PK1/GA5 ratios of olopatadine and quinidine were 0.823 and 1.05, respectively. The PK1/GA5 ratio of diazepam in the presence of CyA was 1.27, the same level as the ratio in the absence of CyA. These results indicate that olopatadine is a substrate of P-gp.

Cellular uptake of other second-generation H1-antagonists: Six second-generation H1-antagonists were examined by cellular uptake study, which were known as substrates of P-gp.11,13–16 PK1/GA5 ratios examined in the present study were listed in the ascending order: loratadine (PK1/GA5 ratio = 1.31) < fexofenadine (1.94) < bepotastine (3.17) < epinastine (3.55) < cetirizine (6.73) < desloratadine (14.2). Comparison with the other H1-antagonists, PK1/GA5 ratio of olopatadine (5.70) was almost similar with epinastine, bepotastine and cetirizine (Table 1).

Brain and plasma concentrations of olopatadine in KO and WT mice: The plasma and brain concentration-time profiles of olopatadine after intravenous administration at a dose of 1 mg/kg are shown in Figure 2. The plasma concentrations of olopatadine at 0.05 hours after intravenous administration to WT and KO mice were 3620 ± 300 and 4070 ± 580 nmol/L, respectively (Fig. 2A). Plasma olopatadine levels after intravenous administration were decreased bi-exponentially and the plasma concentration-time profile was almost similar between WT and KO mice (Fig. 2A). Pharmacokinetic parameters of olopatadine in plasma and brain are shown in Table 2. The CI, Vss and AUC0–4 in plasma were almost similar between WT and KO mice. In contrast to plasma exposure to olopatadine, the brain concentration of olopatadine in KO mice was higher than that in WT mice at all time points after intravenous administration (Fig. 2B). The AUC0–4 value in brain in KO mice (301 nmol·h/kg brain) was 3.34-fold higher than that.

Table 1. Cellular uptake into LLC-PK1 and LLC-GA5-COL150 cells

<table>
<thead>
<tr>
<th>Test compound</th>
<th>C/B ratio in LLC-PK1</th>
<th>C/B ratio in LLC-GA5-COL150</th>
<th>PK1/GA5 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olopatadine</td>
<td>0.0446 ± 0.0075</td>
<td>0.00783 ± 0.0014*</td>
<td>5.70</td>
</tr>
<tr>
<td>Olopatadine +</td>
<td>0.0417 ± 0.0082</td>
<td>0.0506 ± 0.0024</td>
<td>0.823</td>
</tr>
<tr>
<td>Quinidine</td>
<td>0.635 ± 0.044</td>
<td>0.0288 ± 0.0039**</td>
<td>22.0</td>
</tr>
<tr>
<td>Quinidine +</td>
<td>0.423 ± 0.078</td>
<td>0.404 ± 0.040</td>
<td>1.05</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.0575 ± 0.0111</td>
<td>0.0521 ± 0.0089</td>
<td>1.10</td>
</tr>
<tr>
<td>Diazepam +</td>
<td>0.0529 ± 0.0066</td>
<td>0.0417 ± 0.0114</td>
<td>1.27</td>
</tr>
</tbody>
</table>

The cellular uptake of olopatadine, quinidine and diazepam into LLC-PK1 and LLC-GA5-COL150 cells in the presence or absence of cyclosporine A after adding the drugs was calculated as shown in Materials and methods. The concentrations of the test compounds and the inhibitor were set at 10 μmol/L. The C/B ratio represents the mean ± S.D. of three samples. Statistical differences were determined in the C/B ratio between LLC-PK1 and LLC-GA5-COL150 cells by the method described in Materials and methods (*p < 0.05, **p < 0.01, ***p < 0.001).
Fig. 2. Plasma and brain concentration-time profiles of olopatadine after a single intravenous administration of olopatadine hydrochloride to WT and KO mice at a dose of 1 mg/kg (2.67 μmol/kg). Plasma (A) and brain (B) concentrations after intravenous administration are presented as mean ± SD (n = 3). The data on WT mice are presented by triangles and those on KO mice are presented by circles. Statistical differences in the plasma and brain concentrations are determined between KO and WT mice by the method described in Materials and methods (*p < 0.05, **p < 0.01).

Table 2. Pharmacokinetic parameters of olopatadine after intravenous administration of olopatadine hydrochloride to WT and KO mice at a dose of 1 mg/kg (2.67 μmol/kg).

<table>
<thead>
<tr>
<th>Mice strain</th>
<th>Plasma</th>
<th>Brain</th>
<th>Brain/plasma AUC&lt;sub&gt;0-4&lt;/sub&gt; ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V&lt;sub&gt;s&lt;/sub&gt; (mL/kg)</td>
<td>CL (mL/h/kg)</td>
<td>AUC&lt;sub&gt;0-4&lt;/sub&gt; (nmol·h/L)</td>
</tr>
<tr>
<td>WT</td>
<td>725</td>
<td>2070</td>
<td>1245</td>
</tr>
<tr>
<td>KO</td>
<td>563</td>
<td>1650</td>
<td>1570</td>
</tr>
<tr>
<td>Ratio of KO to WT</td>
<td>0.777</td>
<td>0.797</td>
<td>1.26</td>
</tr>
</tbody>
</table>

Table 3. Pharmacokinetic parameters of olopatadine after oral administration of olopatadine hydrochloride to WT and KO mice at a dose of 1 mg/kg (2.67 μmol/kg).

<table>
<thead>
<tr>
<th>Mice strain</th>
<th>Plasma</th>
<th>Brain</th>
<th>Brain/plasma AUC&lt;sub&gt;0-4&lt;/sub&gt; ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (nmol/L)</td>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>AUC&lt;sub&gt;0-4&lt;/sub&gt; (nmol·h/L)</td>
</tr>
<tr>
<td>WT</td>
<td>425</td>
<td>0.5</td>
<td>760</td>
</tr>
<tr>
<td>KO</td>
<td>464</td>
<td>0.5</td>
<td>793</td>
</tr>
<tr>
<td>Ratio of KO to WT</td>
<td>1.09</td>
<td>1.0</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Discussion

Differences in sedating potential among H<sub>1</sub>-antagonists are ascribed to the differences in H<sub>1</sub> receptor occupancy in the CNS. H<sub>1</sub> receptor occupancy in the CNS varies among H<sub>1</sub>-antagonists because of the difference in brain penetration and affinity and/or selectivity to H<sub>1</sub> receptors. In vitro receptor binding studies have demonstrated the high affinity of olopatadine to the H<sub>1</sub> receptors (K<sub>d</sub> = 2.5 ± 0.12 nmol/L). The mean histamine H<sub>1</sub> receptor occupancy of olopatadine in the CNS was low, which was 15.0% after oral administration of 5 mg of olopatadine (the highest clinically recommended dose of Allelock<sup>5</sup>). The present study using both MDR1 expressed cells and mdr1a/1b knockout mice provided the first evidence that P-gp played an important role for low brain penetration of olopatadine.

In vitro study showed that the cellular uptake of olopatadine in MDR1-expressed cells (LLC-GA5-COL150 cells) was lower than that in control cells (LLC-PK1 cells). In addition, the cellular uptake of olopatadine in LLC-GA5-COL150 cells was increased to the same level of LLC-PK1 cells.
Plasma and brain concentration-time profiles of olopatadine after a single oral administration of olopatadine hydrochloride to WT and KO mice at a dose of 1 mg/kg (2.67 μmol/kg). Plasma (A) and brain (B) concentrations after oral administration are presented as mean ± SD (n = 3). In WT mice, the brain concentration at 0.083 hour is not calculated as the mean concentration because the concentrations of 2 in 3 samples were under the lower limit of quantification (0.75 nmol/kg brain). Therefore the concentration of this time point is not presented in this figure. The data on WT mice are presented by triangles and those on KO mice are by circles. Statistical differences in the plasma and brain concentrations were determined between KO and WT mice by the method described in Materials and methods (*p < 0.05, **p < 0.01, ***p < 0.001).

Table 4. The comparison of between PK1/GA5 ratio and transport ratio in second-generation H1-antagonists

<table>
<thead>
<tr>
<th>H1-antagonists</th>
<th>PK1/GA5 ratio</th>
<th>Transport ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fexofenadine</td>
<td>1.94</td>
<td>2.3</td>
<td>13</td>
</tr>
<tr>
<td>Loratadine</td>
<td>1.31</td>
<td>1.9</td>
<td>11</td>
</tr>
<tr>
<td>Desloratadine</td>
<td>14.2</td>
<td>9.1</td>
<td>11</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>6.73</td>
<td>5.5</td>
<td>11, 16</td>
</tr>
<tr>
<td>Epinastine</td>
<td>3.55</td>
<td>6.0</td>
<td>14</td>
</tr>
<tr>
<td>Bepotastine</td>
<td>3.17</td>
<td>4.0</td>
<td>15</td>
</tr>
</tbody>
</table>

The transport ratios were obtained from reference. Transport ratios of fexofenadine and epinastine were calculated from figures in the each reference. Transport ratio of cetirizine was determined by mean of two references.

The presence of CyA, a P-gp inhibitor,23) these results clearly demonstrate that olopatadine is a substrate of human P-gp (Table 1). Recent reports demonstrated that some second-generation H1-antagonists were substrates of P-gp such as olopatadine.11–16) In many cases, MDR1 transfected monolayer cells were used for transcellular transport study. The effect of P-gp on the monolayer transport was represented by a transport ratio, which is ratio of the translocation rate of the basal-to-apical to apical-to-basal. In the present study, the effect of P-gp on the cellular uptake was represented by a PK1/GA5 ratio. In the examination using 6 second-generation H1-antagonists, which were already known as substrates of P-gp,11–16) PK1/GA5 ratios of those compounds were shown in Table 4. We compared those PK1/GA5 ratios with transport ratios using human MDR1 transfected monolayer cells, which were previously reported.11,13–16) In these studies, the results of controls (such as quinidine or digoxin) or marker of paracellular transport (such as inulin or mannitol) were in good agreement with each other. Cellular uptake study could be comparable with the result of transcellular transport study, because PK1/GA5 ratios in this study were well correlated with the transport ratios (r = 0.914, p < 0.05). Therefore, the uptake study is thought to be useful for classifying compounds as a P-gp substrate.

The second-generation H1-antagonists (cetirizine, bepotastine, epinastine, etc.) have demonstrated that the brain concentrations in KO mice were higher than those in WT mice, whereas the plasma concentrations were not different between KO and WT mice after intravenous administration.11,14–16) It was reported that the plasma as well as brain concentrations of fexofenadine in KO mice were higher than those in WT mice after intravenous and oral administration,13,17) because P-gp was thought to influence the pharmacokinetics of fexofenadine such as brain penetration and intestinal absorption including re-absorption after excretion into bile.17) To determine the effect of P-gp on the brain distribution and plasma concentration, olopatadine was intravenously or orally administered to WT and KO mice. When olopatadine was intravenously administered to WT and KO mice at a dose of 1 mg/kg, brain AUC0-4 of olopatadine in KO mice was 3.34-fold higher than that in WT mice (Table 2). Moreover, when olopatadine was orally administered to WT and KO mice at a dose of 1 mg/kg, brain AUC0-4 of olopatadine in KO mice was 5.57-fold higher than that in WT mice (Table 3). These data indicated that P-gp limited the brain penetration of olopatadine. The brain AUC0-4 ratio of KO to WT after oral administration (5.57) was higher than that after intravenous administration (3.34). In KO mice, the brain to plasma AUC0-4 ratio was almost same value (0.192 and 0.208) in both administration routes.
while in WT mice the ratio after intravenously administration was 0.0724 and was 2-fold higher than that after oral administration (0.0389, Table 2 and 3). Therefore, it was speculated that P-gp mediated transport at BBB in WT after intravenous administration was lower than that after oral administration, because of saturation of P-gp mediated transport at BBB.

In brain concentration-time profile, a half-life of elimination from brain in WT mice (0.98 hours) was shorter than that in KO mice (1.36 hours) after intravenous administration (Fig. 2B), and the ratio of KO to WT is 1.39. The ratio of elimination half life in both strains is smaller than that of the brain/plasma AUC0–4 ratio (2.65). According to the report of Adachi et al.,22) schematic diagram illustrating the permeability-surface area (PS) products for the penetration of drugs across the blood-brain barrier is shown below.

![Diagram](image)

PS product for the unidirectional influx across the BBB from blood into brain and that for the opposite direction is given by

\[
PS_{\text{plasma-to-brain}} = \frac{PS_{\text{l, inf}}}{PS_{\text{l, inf}} + PS_{\text{l, eff}}} 
\]

\[
PS_{\text{brain-to-plasma}} = \frac{PS_{\text{l, inf}}}{PS_{\text{l, inf}} + PS_{\text{l, eff}}} 
\]

or

\[
PS_{\text{plasma-to-brain}} = \frac{PS_{\text{l, inf}}}{PS_{\text{l, inf}} + PS_{\text{p-gp}} + PS_{\text{l, eff}}} 
\]

\[
PS_{\text{brain-to-plasma}} = \frac{PS_{\text{l, inf}}}{PS_{\text{l, inf}} + PS_{\text{p-gp}} + PS_{\text{l, eff}}} 
\]

where \(PS_{\text{l, inf}}\) and \(PS_{\text{l, eff}}\) represent the PS product for the influx and efflux across the luminal membrane, and \(PS_{\text{l, inf}}\) and \(PS_{\text{l, eff}}\) represent the PS product for the influx and efflux across the antiluminal membrane of the cerebral endothelial cells, respectively. \(PS_{\text{p-gp}}\) represents the PS product mediated by P-gp.22) PS products in KO mice are given by equation (5) and (6), and those in WT mice are given by equation (7) and (8).

Because \(K_{\text{p,brain}}\) is given by \(PS_{\text{blood-to-brain}}/PS_{\text{brain-to-blood}}\), \(K_{\text{p,brain}}\) in KO or WT is given by following equation (9) or (10), respectively:

\[
K_{\text{p,brain}} = \frac{PS_{\text{l, inf}} \times PS_{\text{l, eff}}}{PS_{\text{l, inf}} \times PS_{\text{l, eff}}} 
\]

\[
K_{\text{p,brain}} = \frac{PS_{\text{l, inf}} \times PS_{\text{l, eff}}}{PS_{\text{l, eff}} \times (PS_{\text{l, eff}} + PS_{\text{p-gp}})} 
\]

By taking the ratio of \(K_{\text{p,brain}}\) in KO to that in WT, \(K_{\text{p,brain}}\) ratio is given by following equation:

\[
K_{\text{p,brain}} \text{ ratio} = 1 + \frac{PS_{\text{p-gp}}}{PS_{\text{l, eff}}} 
\]
drug.29) Therefore, olopatadine is thought to be less influenced by P-gp during the absorption due to its high membrane permeability at the upper region of small intestine. In fact, bioavailabilities of olopatadine after oral administration were 60.8, 74.2, 83.3 and 102% in rats, guinea pigs, dogs and monkeys, respectively.26) In a clinical study, olopatadine reached tmax within 1 hour after oral administration at 5 mg and was efficiently excreted into urine (60–70%), indicating the good intestinal absorption of olopatadine.19)

In conclusion, we demonstrate that olopatadine is a P-gp substrate by the cellular uptake using P-gp transfected cells. Moreover, the brain penetration of olopatadine is limited by P-gp at the BBB but plasma concentration of olopatadine is hardly influenced by intestinal P-gp. P-gp is thought to be one of the important factors about the H1-receptor occupancy of olopatadine in the CNS, which is 15.0% in humans.25) This H1-receptor occupancy is lower than the recommended criterion for the lack of CNS side effect by H1-antagonists proposed by the Consensus Group on New Generation Antihistamines (CONGA), which specifies a maximum of about 20% at the highest recommended dose.25) Olopatadine is useful for the treatment of the various allergic disorders after oral administration, because olopatadine produces rapid inhibitory effect against allergic disorders and exhibits low H1-receptor occupancy in the CNS.

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