Regular Article

Characteristics of Pemetrexed Transport by Renal Basolateral Organic Anion Transporter hOAT3

Tomohiko Kurata[1], Takuya Iwamoto[1,2], Yuki Kawahara[1] and Masahiro Okuda[1,2,*]

[1]*Department of Clinical Pharmacy and Biopharmaceutics, Mie University Graduate School of Medicine, Tsu, Japan*
[2]*Department of Pharmacy, Mie University Hospital, Faculty of Medicine, Mie University, Tsu, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Purpose: Pemetrexed transport by human organic anion transporters, hOAT1 (SLC22A6) and hOAT3 (SLC22A8), were characterized in comparison with methotrexate. Methods: Accumulation of pemetrexed and methotrexate in hOAT1- and hOAT3-expressing cells were evaluated. Pemetrexed and methotrexate were determined by HPLC. Kinetic parameters were calculated by Eadie-Hofstee plot. Results: When HEK-hOAT3 and -hOAT1 cells were incubated with $100\,\mu M$ pemetrexed for $30\,min$, pemetrexed was accumulated at 14- and 1.7-fold greater than that in control cells, respectively. Pemetrexed and methotrexate transport by hOAT3 was saturated at high concentrations with apparent K_m values $28.2\,\mu M$ and $76.6\,\mu M$, respectively. In addition, intrinsic activity (V_{max}/K_m) of pemetrexed and methotrexate transport by hOAT3 was 4.82 and $0.42\,\mu l/min/mg$ protein, respectively, suggesting 11-fold higher transport of pemetrexed than methotrexate by hOAT3. Furthermore, loxoprofen, ibuprofen, pravastatin, and cefazolin, transport substrates of hOAT3, inhibited pemetrexed transport by hOAT3 with IC_{50} values, 34.2, 27.9, 76.3 and $650\,\mu M$, respectively. Conclusions: Pemetrexed is a superior substrate to methotrexate for hOAT3. Loxoprofen, ibuprofen, and cefazolin could cause drug-drug interactions when attaining high blood concentrations.

Keywords: pemetrexed; organic anion transporter; methotrexate; renal tubular secretion; drug interaction

Introduction

A multitargeted antifolate, pemetrexed (**Fig. 1A**), is used in the treatment of malignant mesothelioma of pleura and non-small cell lung cancer (NSCLC).[1–3] Pemetrexed is primarily eliminated in the urine, and 70% to 90% of the dose is recovered in the urine as an unchanged form within the first 24 h after administration.[4–6] It is reported that the renal clearance/total clearance ratio of pemetrexed was 0.53 and renal tubular secretion was about 2.5-fold higher than glomerular filtration in advanced cancer patients with normal renal function.[7]

Organic anion transporters OAT1 (SLC22A6) and OAT3 (SLC22A8), expressed in the basolateral membrane of renal proximal tubules, have been shown to be responsible for tubular secretion of organic anions including anionic drugs.[8,9] It is reported that an antifolate drug, methotrexate (**Fig. 1B**), which has a similar structure to pemetrexed, is secreted *via* OAT1 and OAT3 in the renal proximal tubules.[9,10] In addition, methotrexate uptake by Xenopus oocytes expressing hOAT1 or hOAT3 was markedly inhibited by non-steroidal anti-inflammatory drugs (NSAIDs).[11] Moreover, in humans, it is reported that concomitant use of methotrexate with acidic drugs, such as NSAIDs, caused severe side effects.[12,13] However, there is no information on the mechanisms responsible for the tubular secretion of pemetrexed and its involvement in the drug-drug interactions. Furthermore, FDA draft guidance recommended that an investigational drug should be evaluated *in vitro* to determine whether it is a substrate of OAT1/3 or OCT2 when its renal active secretion is important (active secretion by kidney is more than or equal to 25% of total clearance),[14] and pemetrexed obviously meets these criteria.

In the present study, we characterized pemetrexed transport *via* hOAT1 and hOAT3 and assessed the possible involvement of these transporters in the drug-drug interactions between pemetrexed and concomitantly administered anionic drugs.
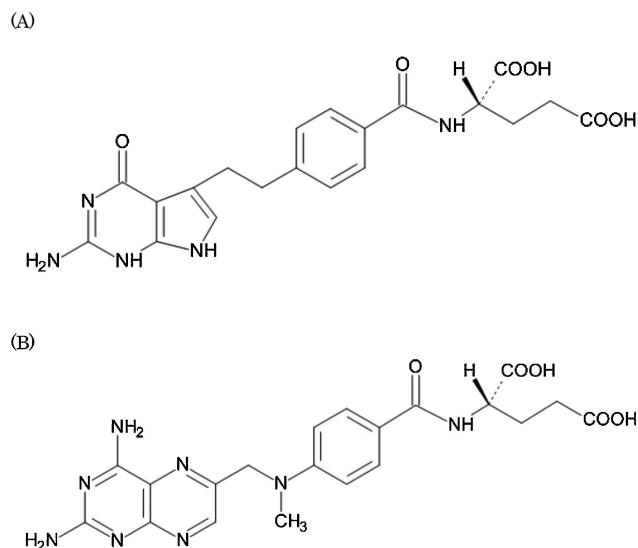
148

(A)

(B)

Fig. 1. Chemical structures of pemetrexed (A) and methotrexate (B)

Materials and Methods

Materials: Pemetrexed disodium was obtained from Chemie Tek (Indianapolis, IN). Methotrexate, loxoprofen sodium, and ibuprofen were purchased from Wako Pure Chemical Industries (Osaka, Japan). Pravastatin sodium was purchased from Sigma-Aldrich (St. Louis, MO). Cefazolin sodium was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). All other chemicals used were of the highest purity available.

Cell culture: Stably transfected HEK cells (a generous gift from Prof. Ken-ichi Inui, Department of Pharmacy, Kyoto University Hospital, Japan) were selected by adding G418 (500 μg/ml) (Sigma-Aldrich) to the culture medium. HEK cells expressing hOAT1, hOAT3, and hOCT2 were grown on the bottom of a dish in Medium 199 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 at 37°C.

Uptake study of various compounds by HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells: Uptake studies were performed as described previously with slight modifications.[15] HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells were seeded on 12-well plates at a density of 4×10^5 cells/well for uptake of p-[^{14}C]aminohippurate, [^{3}H]estrone sulfate, and [^{14}C]metformin, respectively. At 48 h after seeding, the uptake of these compounds was examined with monolayer cultures of HEK-hOAT1, HEK-hOAT3 (HEK-pBK as the control), and HEK-hOCT2 (HEK-VEC as the control) cells. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4). Cells were preincubated with 1 ml of incubation medium for 10 min at 37°C. After preincubation, the medium was replaced with 1 ml of incubation medium containing each compound. At the end of the incubation period, the medium was aspirated, and cells were washed three times with ice-cold incubation medium. Cells were lysed in 0.5 ml of 0.2 N NaOH solution, and kept for 6 h at room temperature. After adding 100 μl of 1 N HCl, the radioactivity in aliquots (450 μl) was determined in 3 ml of ACS II (Amersham International, Little Chalfont, UK) by a liquid scintillation counter. The remaining 50 μl of the aliquots of cell lysate was used to determine protein concentrations by the method of Bradford with IgG as a standard.[16]

Uptake study of pemetrexed and methotrexate by HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells: HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells were seeded on 3.5-cm dishes at a density of 12×10^5 cells/dish. At 48 h after seeding, the uptake of pemetrexed and methotrexate by cells was examined with monolayer cultures of HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells. Cells were preincubated with 1 ml of the incubation medium for 10 min at 37°C. After preincubation, the medium was replaced with 1 ml of incubation medium containing pemetrexed or methotrexate. At the end of the incubation period, the medium was aspirated, and cells were washed three times with 2 ml of ice-cold incubation medium. Cells were solubilized with 1 ml of extraction solution (30 mM phosphate buffer (pH 7.0)/methanol = 50/50) for 1 h at room temperature. The extraction solution was centrifuged $10,000 \times g$ for 15 min, and the supernatant was subjected to high-performance liquid chromatography (HPLC).

HPLC conditions: Pemetrexed and methotrexate concentrations in cells were determined according to the methods reported previously with slight modifications.[17,18] An LC-20AD HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a UV spectrophotometric detector (SPD-20A; Shimadzu) was adjusted to 254 and 303 nm for pemetrexed and methotrexate, respectively. The stationary phase: NUCLEOSIL 5C8, 4.6 mm I.D. × 250 (6A) for pemetrexed and 5C18-AR-II, 4.6 mm I.D. × 150 mm for methotrexate. Flow rate was 1.2 and 1.0 ml/min and the column temperature was 50 and 40°C for pemetrexed and methotrexate, respectively. The mobile phase consisted of 0.2% formic acid (pH 3.08 adjusted with 10 N NaOH): acetonitrile = 80:20 for pemetrexed and 50 mM ammonium acetate: acetonitrile = 93:7 for methotrexate, respectively.

Kinetic analysis: Kinetic parameters of pemetrexed and methotrexate for hOAT3 were estimated using the Eadie-Hofstee plot method. Fitting was performed by nonlinear least squares regression analysis using GraphPad Prism, version 5.01 (GraphPad Software, San Diego, CA). Calculated IC_{50} values of pemetrexed and methotrexate for hOAT3 were estimated by nonlinear least-squares regression analysis of the competition curve according to the following equation, $R = 100 \times IC_{50}/(IC_{50} + [I]) + A$, where R is relative uptake amount of pemetrexed without inhibitors, [I] is the concentration of each inhibitor, and A is the non-specific pemetrexed uptake (% of control).

Statistical analysis: Data were analyzed by an unpaired t-test using GraphPad Prism, version 5.01. Differences were considered significant at $p < 0.05$.

Results

Uptake of reference substrates by HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells: At the beginning, transport of p-aminohippurate (PAH), estrone sulfate (ES), and metformin were assessed to confirm the functionality of HEK293 cells expressing hOAT1, hOAT3, and hOCT2, respectively. Uptake of PAH, ES, and metformin in HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells were about 34, 8.4, and 12 times larger than those in corresponding control cells, respectively (data not shown).

Uptake of pemetrexed by HEK-hOAT1, HEK-hOAT3, and HEK-hOCT2 cells: Next, we evaluated the activity of pemetrexed transport by hOAT1, hOAT3, and hOCT2 by measuring accumulation of pemetrexed in HEK-hOAT1, HEK-hOAT3,
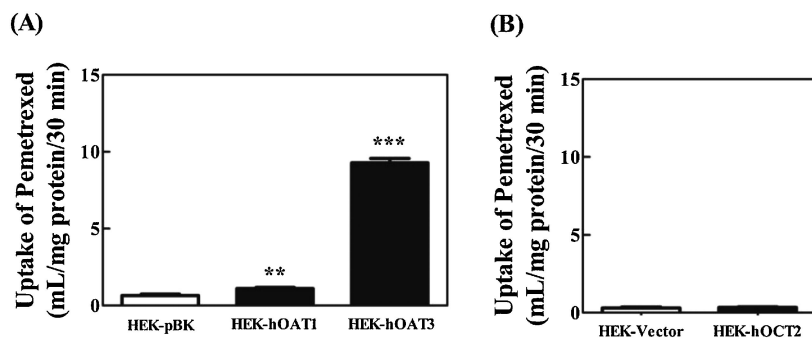
(A)

(B)

Fig. 2. Uptake of pemetrexed by HEK-hOAT1, HEK-hOAT3 (A), and HEK-hOCT2 (B)
Pemetrexed (100 μM) was incubated for 30 min at 37°C. After incubation, accumulation of pemetrexed in the cells was extracted and then determined by HPLC. Each column represents mean ± S.E. ($n = 3$). **p < 0.05, ***p < 0.001: significantly different from control.
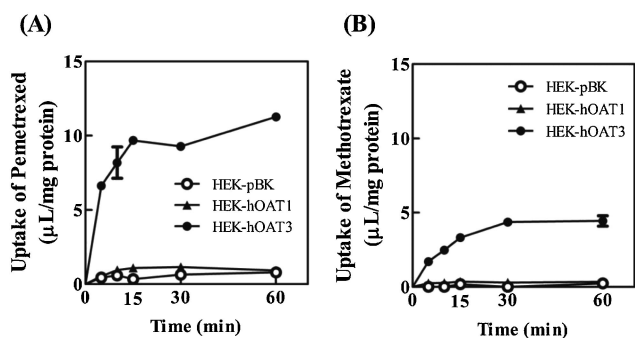
(A)

(B)

Fig. 3. Time-profiles of pemetrexed (A) and methotrexate (B) uptake by HEK-hOAT1, HEK-hOAT3, and HEK-pBK cells
HEK-hOAT1 (▲), HEK-hOAT3 (●), and HEK-pBK (○) cells were incubated with 100 μM pemetrexed or 100 μM methotrexate at 37°C for up to 60 min. After incubation, accumulation of pemetrexed and methotrexate in the cells was extracted and then determined by HPLC. Each point represents the mean ± S.E. ($n = 3$).

and HEK-hOCT2 cells by incubation of the cells with 100 μM pemetrexed for 30 min. As shown in **Figure 2**, accumulations of pemetrexed in HEK-hOAT1 and HEK-hOAT3 cells were about 1.7-fold and 14-fold higher than that in HEK-pBK cells, the corresponding controls, respectively. In contrast, there was no difference in pemetrexed uptake between HEK-hOCT2 and HEK-VEC cells, the corresponding controls.

Time-profile of pand methotrexate uptake by HEK-hOAT1 and HEK-hOAT3 cells: We examined further the time-profiles of pemetrexed and methotrexate uptake by HEK-hOAT1 and HEK-hOAT3 cells (**Fig. 3**). The accumulation of pemetrexed increased time-dependently, and was saturated after 15 min. Accumulations of pemetrexed in HEK-hOAT1 and HEK-hOAT3 cells at 1 h incubation were approximately 1.2-fold and 14-fold higher than that in the control cells, respectively. On the other hand, accumulation of methotrexate in HEK-hOAT1 and HEK-hOAT3 cells was approximately 1.5-fold and 19-fold higher than that in the control cells, respectively.

Concentration-dependence of pemetrexed uptake by HEK-hOAT3 cells: To estimate the kinetic parameters of pemetrexed transport by hOAT3, the concentration-dependence of pemetrexed uptake was investigated. When the cells were incubated with various concentrations of pemetrexed; i.e. 2, 5, 10, 20, 50, 100, 200, and 500 μM, for 5 min, pemetrexed uptake by HEK-hOAT3

cells was curvilinear and thereby saturated at high concentrations (**Fig. 4A**). Using an Eadie-Hofstee plot, apparent K_m value for the uptake of pemetrexed for HEK-hOAT3 was calculated as 28.2 ± 2.6 μM. On the other hand, apparent K_m value for the uptake of methotrexate was 76.6 ± 10.1 μM, when the methotrexate uptake was evaluated at 2, 5, 10, 20, 50, 100, and 200 μM concentrations for 15 min. The V_{max} values of pemetrexed for HEK-hOAT3 (136 ± 7 pmol/mg protein/min) were about 4-fold higher than those of methotrexate (32 ± 3 pmol/mg protein/min). Therefore, the intrinsic transport activity (V_{max}/K_m) of pemetrexed for hOAT3 (4.8 ± 0.7 μl/mg protein/min) was about 11-fold higher than that of methotrexate (0.42 ± 0.05 μl/mg protein/min) (**Table 1**).

Effect of various anionic drugs on pemetrexed uptake by HEK-hOAT3 cells: The effects of various anionic drugs on pemetrexed uptake by hOAT3 were investigated. The accumulation of pemetrexed was measured by incubation of the cells with 100 μM pemetrexed in the presence or absence of 1 mM anionic drugs including NSAIDs, and cephalosporin antibiotics or tetraethylammonium (TEA), an inhibitor of organic cation transporter, for 20 min. As shown in **Table 2**, pemetrexed uptake was markedly inhibited by estrone sulfate, loxoprofen, ibuprofen, salicylate, flufenamate, indomethacin, and pravastatin. Among cephalosporin antibiotics, cefotiam, cefazolin, and cefotaxime, but not ceftibuten or ceftriaxone, significantly inhibited pemetrexed uptake by HEK-hOAT3 cells. Moreover, apparent 50% inhibitory concentration (IC_{50}) values of loxoprofen, ibuprofen, pravastatin, and cefazolin on pemetrexed transport by hOAT3 were estimated by pemetrexed uptake in the presence of various concentrations of these drugs. The IC_{50} values were estimated as 34.2 ± 4.2, 27.9 ± 7.7, 76.3 ± 3.5, and 650 ± 6 μM for loxoprofen, ibuprofen, pravastatin, and cefazolin, respectively (**Fig. 5** and **Table 3**).

Discussion

In the present study, we clarified for the first time that pemetrexed is a substrate of hOAT3. In addition, it is suggested that the intrinsic activity of pemetrexed transport by hOAT3 was markedly higher than that for methotrexate. Furthermore, pemetrexed transport was inhibited by some clinically relevant anionic drugs at high concentrations.

OAT1 and OAT3 play important roles in renal uptake of anionic drugs.[19,20] It was also suggested that structural similarities could be recognized among substrates of hOAT1 and hOAT3.[21,22] On the other hand, even a minor difference in the structures of antifolates between aminopterin and methotrexate led to quite different
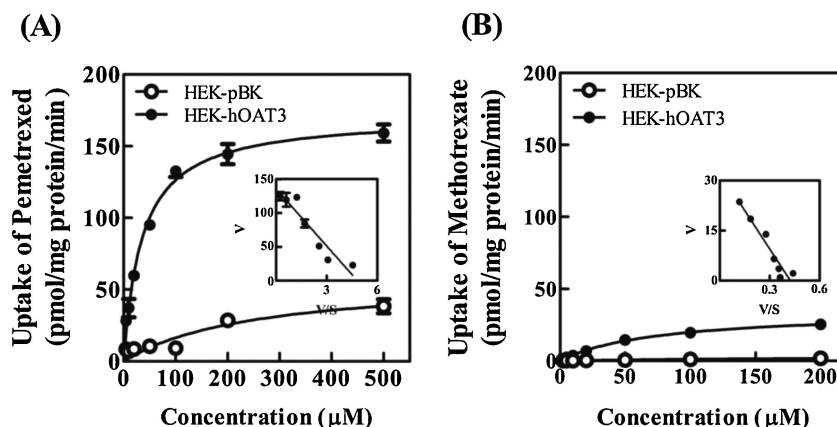
Fig. 4. Concentration dependence of pemetrexed (A) and methotrexate (B) uptake by HEK-hOAT3 (●) and HEK-pBK (○) cells
Cells were incubated with various concentrations of pemetrexed and methotrexate at 37°C for 5 min and 15 min, respectively. After incubation, accumulation of pemetrexed and methotrexate in HEK cells was extracted and then determined by HPLC. Inset: Eadie-Hofstee plots of the data; V is the uptake velocity (pmol/mg protein/min), S is the concentration of pemetrexed or methotrexate (μM). Each point represents the mean $\pm$ S.E. ($n = 3$).

Table 1. Kinetic parameters of the uptake of pemetrexed and methotrexate by HEK-hOAT3

	Pemetrexed	Methotrexate
K_m (μM)	28.2 ± 2.6*	76.6 ± 10.1
V_{max} (pmol/mg protein/min)	136 ± 7**	32 ± 3
V_{max}/K_m (μl/mg protein/min)	4.8 ± 0.7**	0.42 ± 0.05

The apparent K_m and V_{max} were determined from Eadie-Hofstee plots. Data are calculated from **Figure 4**. Each value represents the mean $\pm$ S.E. ($n = 3$). *$p < 0.05$, **$p < 0.01$: significantly different from that of methotrexate.

Table 2. Inhibitory effect of various compounds on pemetrexed uptake by HEK-hOAT3

	Inhibitors (1 mM)	Pemetrexed uptake (% of control)
	Control	100 ± 6
	Estrone sulfate	5.38 ± 0.12***
	TEA	97.8 ± 3.8
NSAIDs	Loxoprofen	22.5 ± 1.0***
	Ibuprofen	14.1 ± 0.7***
	Salicylate	22.1 ± 0.8***
	Flufenamate	9.75 ± 0.67***
	Indomethacin	17.5 ± 0.8***
HMG-CoA reductase inhibitor	Pravastatin	20.4 ± 0.8***
Cephalosporin antibiotics	Cefotiam	85.6 ± 1.5**
	Ceftibuten	87.6 ± 3.0
	Cefazolin	41.8 ± 0.3***
	Cefotaxime	75.2 ± 0.9**
	Ceftriaxone	101 ± 1

HEK-hOAT3 was incubated with 100 μM pemetrexed in the absence (control) or presence of each substrate at 1 mM for 20 min. The uptake amounts of pemetrexed in each cell were determined. **$p < 0.01$, ***$p < 0.001$: significantly different from control values. Each value represents the mean $\pm$ S.E. of pemetrexed uptake as a percentage of control ($n = 3$).

Table 3. Calculated IC_{50} values of loxoprofen, ibuprofen, pravastatin, and cefazolin for the uptake of pemetrexed by hOAT3

	IC_{50} (μM)
Loxoprofen	34.2 ± 4.2
Ibuprofen	27.9 ± 7.7
Pravastatin	76.3 ± 3.5
Cefazolin	650 ± 6

IC_{50} values are calculated from **Figure 5**. IC_{50} values represent the mean $\pm$ S.E. ($n = 3$).
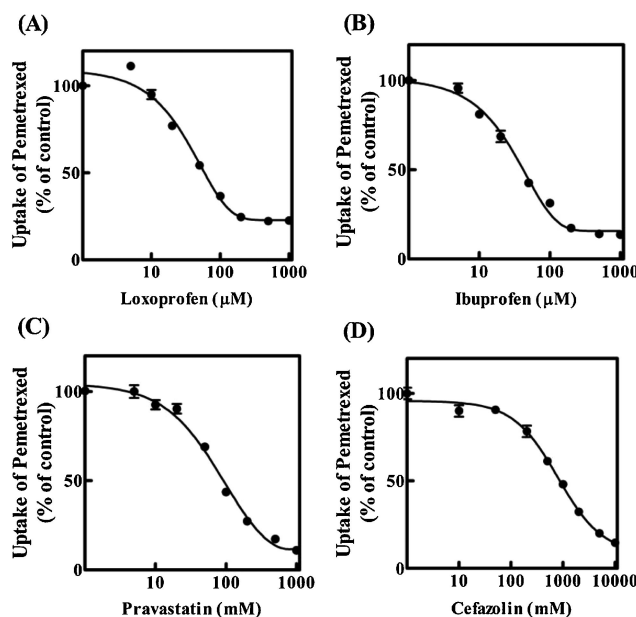

Fig. 5. Inhibition of pemetrexed uptake by loxoprofen, ibuprofen, pravastatin and cefazolin in HEK-hOAT3 cells
The HEK-hOAT3 cells were incubated with 100 μM pemetrexed for 20 min in the presence or absence of various concentrations of loxoprofen (A), ibuprofen (B), pravastatin (C), and cefazolin (D). Accumulation of pemetrexed (percentage of control) in each cell was extracted and determined by HPLC. Each point represents the mean $\pm$ S.E. ($n = 3$).

transport characteristics between them.[19] It was reported that hOAT1 shows lower affinity for methotrexate transport ($K_m = 724.0\,\mu$M) than hOAT3 ($K_m = 17.2\,\mu$M) based on the experiments using hOAT-expressing oocytes.[11] In the present study, apparent K_m values for methotrexate and pemetrexed transport by HEK-hOAT3 cells were 28.2 ± 2.6 and $76.6 \pm 10.1\,\mu$M, respectively. Although the kinetics of methotrexate transport by OAT1 was not determined in the present study because of lower methotrexate uptake activity by HEK-hOAT1 cells, it is likely that hOAT3 is a high-affinity transporter for both methotrexate and pemetrexed with K_m values comparable to respective clinical concentrations.

In the present study, HEK-hOAT3 cells showed 3-fold higher affinity and 4-fold higher capacity for pemetrexed than methotrexate transport as evidenced by the K_m (28.2 ± 2.6 *vs.* $76.6 \pm 10.1\,\mu M$ for pemetrexed and methotrexate, respectively) and V_{max} (136 ± 7 *vs.* $32 \pm 3\,pmol/mg$ protein/min for pemetrexed and methotrexate, respectively) (**Fig. 4** and **Table 1**). Moreover, the intrinsic transport activity (V_{max}/K_m) of pemetrexed by HEK-hOAT3 cells was about 11-fold higher than that of methotrexate. In contrast to hOAT3, the time-course of pemetrexed uptake by HEK-hOAT1 cells was only slightly higher than that of control HEK-pBK cells. It is also reported that mRNA levels of hOAT3 are approximately 3-fold higher than those of hOAT1 in the human kidney.[20] Therefore, it is likely that hOAT3 plays a much more important role in the tubular secretion of pemetrexed than hOAT1.

Various NSAIDs, cephalosporins, and pravastatin are known as substrates and/or inhibitors of OAT3.[11,22,23] Previous reports showed that coadministration of NSAIDs induced a marked elevation in blood methotrexate concentrations and consequent severe toxicity.[12,13] The NSAID-induced reductions in tubular secretion of methotrexate by inhibition of hOAT1 and hOAT3[11] should be involved in the inhibition of tubular secretion of methotrexate in addition to the decreased renal blood flow *via* inhibition of prostaglandin synthesis by NSAIDs.[24,25] In the case of pemetrexed, it is reported that ibuprofen influenced the pharmacokinetics of pemetrexed with a 20% increase in the area under the plasma concentration versus time curve (AUC) of pemetrexed over that with pemetrexed alone in a clinical study.[4] In the present study, $100\,\mu M$ of pemetrexed was employed, which is comparable to the maximum plasma concentration of pemetrexed ($194\,\mu M$) when administered at the dose of $500\,mg/m^2$.[5] In addition, concomitant incubation of the cells with various NSAIDs, pravastatin, and some cephalosporin antibiotics decreased pemetrexed ($100\,\mu M$) uptake by HEK-hOAT3 cells. Therefore, it is rational to suppose that concomitant administrations of some anionic drugs could cause drug-drug interactions at hOAT3.

FDA draft guidance proposed that drugs exhibiting [I] (unbound $C_{max})/IC_{50} \geq 0.1$ should be evaluated by means of an *in vivo* drug-drug interaction study with a sensitive substrate, although the likelihood of the interaction cannot be predicted by the criteria.[14] In the present study, various NSAIDs inhibited the uptake of pemetrexed by HEK-hOAT3 (**Table 2**). Focusing on ibuprofen and loxoprofen, when 400 mg of ibuprofen was administered to the patients with normal renal function, maximum plasma concentrations (C_{max}) of ibuprofen reached about $160\,\mu M$ and when 60 mg of loxoprofen was administered, maximum plasma concentration reached about $25\,\mu M$.[26,27] On the other hand, it has been reported that protein binding of ibuprofen and loxoprofen is greater than 90%. Therefore, the IC_{50} value of ibuprofen for hOAT3 (27.9 ± 7.7 μM) obtained in the present study should be within the therapeutic range, suggesting that the incidence of interaction between pemetrexed and ibuprofen in patients with renal failure would occur. Moreover, considering our results that IC_{20} (20% inhibitory concentration) was about $10\,\mu M$, ibuprofen at a clinical dose could increase pemetrexed AUC by the direct inhibition of pemetrexed transport *via* hOAT3 by ibuprofen (**Fig. 4B**).[4] Furthermore, the IC_{50} value of loxoprofen for hOAT3 determined in this study was about ten times higher than the plasma protein-free concentration of loxoprofen, suggesting that tubular secretion of pemetrexed *via* hOAT3 could be inhibited when the blood concentration of loxoprofen is increased in patients with renal dysfunction or

overdosing of loxoprofen. However, the inhibition of tubular secretion of pemetrexed may be moderate compared with methotrexate, because pemetrexed binds to hOAT3 with higher affinity than methotrexate (**Fig. 4**, and **Table 1**).

In the present study, the IC_{50} value of cefazolin for hOAT3 was $650\,\mu M$. It is reported that C_{max} of cefazolin reached about 440 μM when 1,000 mg of the drug was injected intravenously.[28] Considering that the protein binding of cefazolin is about 80%, IC_{50} value of cefazolin for hOAT3 determined in this study was comparable with the FDA draft guideline proposed equation. On the other hand, the IC_{50} value of pravastatin determined in this study was considerably higher than its therapeutic concentrations, suggesting that drug-drug interaction between pemetrexed and pravastatin *via* hOAT3 is unlikely.

Renal tubular secretion involves uptake from blood *via* the basolateral membrane followed by excretion into the lumen through the brush-border membrane of the proximal tubules. Recent study showed that basolateral localization of mouse reduced folate carrier (RFC-1) in the kidney is responsible for the uptake of methotrexate,[29] suggesting that RFC-1 could also be involved in the renal uptake of pemetrexed. On the other hand, it has been reported that ATP binding cassette transporters such as breast cancer resistance protein (BCRP), multidrug resistance-associated protein (MRP) 2, and MRP4 are also involved in excretion of organic anion compounds into the lumen through the brush-border membrane of proximal tubules.[30–32] Although ATP-dependent uptake of methotrexate by MRP4-expressing membrane vesicles was most potently inhibited by NSAIDs among these transporters, the drug-drug interaction between methotrexate and ibuprofen *via* MRP-4 seems to be unlikely at clinical concentrations.[33] However, because there are no reports regarding drug-drug interactions between pemetrexed and anionic drugs focusing on these transporters, the role of these transporters should be clarified in subsequent studies.

In conclusion, our results demonstrated for the first time that pemetrexed is transported by hOAT3 with superior affinity and capacity than methotrexate. Some clinical relevant anionic drugs could cause drug-drug interactions with tubular secretion of pemetrexed *via* hOAT3 at high concentrations. Our findings provide useful information for the appropriate use and safe management of pemetrexed.

References

1) Shih, C., Habeck, L. L., Mendelsohn, L. G., Chen, V. J. and Schultz, R. M.: Multiple folate enzyme inhibition: mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). *Adv. Enzyme Regul.*, **38**: 135–152 (1998).

2) Adjei, A. A.: Pemetrexed (ALIMTA), a novel multitargeted antineoplastic agent. *Clin. Cancer Res.*, **10**: 4276s–4280s (2004).

3) Hanauske, A. R., Chen, V., Paoletti, P. and Niyikiza, C.: Pemetrexed disodium: a novel antifolate clinically active against multiple solid tumors. *Oncologist*, **6**: 363–373 (2001).

4) Sweeney, C. J., Takimoto, C. H., Latz, J. E., Baker, S. D., Murry, D. J., Krull, J. H., Fife, K., Battiato, L., Cleverly, A., Chaudhary, A. K., Chaudhuri, T., Sandler, A., Mita, A. C. and Rowinsky, E. K.: Two drug interaction studies evaluating the pharmacokinetics and toxicity of pemetrexed when coadministered with aspirin or ibuprofen in patients with advanced cancer. *Clin. Cancer Res.*, **12**: 536–542 (2006).

5) Nakagawa, K., Kudoh, S., Matsui, K., Negoro, S., Yamamoto, N., Latz, J. E., Adachi, S. and Fukuoka, M.: A phase I study of pemetrexed (LY231514) supplemented with folate and vitamin B12 in Japanese patients with solid tumours. *Br. J. Cancer*, **95**: 677–682 (2006).

6) Rinaldi, D. A., Kuhn, J. G., Burris, H. A., Dorr, F. A., Rodriguez, G., Eckhardt, S. G., Jones, S., Woodworth, J. R., Baker, S., Langley, C., Mascorro, D., Abrahams, T. and Von, Hoff, D.D.: A phase I evaluation of multitargeted antifolate (MTA, LY231514), administered every 21 days,

utilizing the modified continual reassessment method for dose escalation. *Cancer Chemother. Pharmacol.*, **44**: 372–380 (1999).

7) Mita, A. C., Sweeney, C. J., Baker, S. D., Goetz, A., Hammond, L. A., Patnail, A., Tolcher, A. W., Villalona-Calero, M., Sandler, A., Chaudhuri, T., Molpus, K., Latz, J. E., Simms, L., Chaudhary, A. K., Johnson, R. D., Rowinsky, E. K. and Takimoto, C. H.: Phase 1 and pharmacokinetic study of pemetrexed administered every 3 weeks to advanced cancer patients with normal and impaired function. *J. Clin. Oncol.*, **24**: 552–562 (2006).

8) Hosoyamada, M., Sekine, T., Kanai, Y. and Endou, H.: Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am. J. Physiol.*, **276**: F122–F128 (1999).

9) Cha, S. H., Sekine, T., Fukushima, J. I., Kanai, Y., Kobayashi, Y., Goya, T. and Endou, H.: Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol. Pharmacol.*, **59**: 1277–1286 (2001).

10) Uwai, Y., Okuda, M., Takami, K., Hashimoto, Y. and Inui, K.: Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. *FEBS Lett.*, **438**: 321–324 (1998).

11) Uwai, Y., Taniguchi, R., Motohashi, H., Saito, H., Okuda, M. and Inui, K.: Methotrexate-loxoprofen interaction: involvement of human organic anion transporters hOAT1 and hOAT3. *Drug Metab. Pharmacokinet.*, **19**: 369–374 (2004).

12) Maiche, A. G.: Acute renal failure due to concomitant action of methotrexate and indomethacin. *Lancet*, **1**: 1390 (1986).

13) Thyss, A., Milano, G., Kubar, J., Namer, M. and Schneider, M.: Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. *Lancet*, **327**: 256–258 (1986).

14) Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations, DRAFT GUIDANCE, US Department of Health and Human Services, FDA, and Center for Drug Evaluation and Research (CDER), Feb. 2012. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf (accessed 15 Jul 2013).

15) Kimura, N., Okuda, M. and Inui, K.: Metformin transport by renal basolateral organic cation transporter hOCT2. *Pharm. Res.*, **22**: 255–259 (2005).

16) Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248–254 (1976).

17) Li, H., Luo, W., Zeng, Q., Lin, Z., Luo, H. and Zhang, Y.: Method for the determination of blood methotrexate by high performance liquid chromatography with online post-column electrochemical oxidation and fluorescence detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **845**: 164–168 (2007).

18) Respaud, R., Tournamille, J. F., Croix, C., Laborie, H., Elfakir, C. and Viaud-Massuard, M. C.: Development of an ion-pairing reversed-phase liquid chromatography method using a double detection analysis (UV and evaporative light scattering detection) to monitor the stability of Alimta(®)-pemetrexed preparations: identification and quantification of L-glutamic acid as a potential degradation product. *J. Pharm. Biomed. Anal.*, **54**: 411–416 (2011).

19) Uwai, Y. and Iwamoto, K.: Transport of aminopterin by human organic anion transporters hOAT1 and hOAT3: Comparison with methotrexate. *Drug Metab. Pharmacokinet.*, **25**: 163–169 (2010).

20) Motohashi, H., Sakurai, Y., Saito, H., Masuda, S., Urakami, Y., Goto, M., Fukatsu, A., Ogawa, O. and Inui, K.: Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J. Am. Soc. Nephrol.*, **13**: 866–874 (2002).

21) Uwai, Y., Ida, H., Tsuji, Y., Katsura, T. and Inui, K.: Renal transport of adefovir, cidofovir, and tenofovir by SLC22A family members (hOAT1, hOAT3, and hOCT2). *Pharm. Res.*, **24**: 811–815 (2007).

22) Ueo, H., Motohashi, H., Katsura, T. and Inui, K.: Human organic anion transporter hOAT3 is a potent transporter of cephalosporin antibiotics, in comparison with hOAT1. *Biochem. Pharmacol.*, **70**: 1104–1113 (2005).

23) Takeda, M., Noshiro, R., Onozato, M. L., Tojo, A., Hasannejad, H., Huang, X. L., Narikawa, S. and Endou, H.: Evidence for a role of human organic anion transporters in the muscular side effects of HMG-CoA reductase inhibitors. *Eur. J. Pharmacol.*, **483**: 133–138 (2004).

24) Tracy, T. S., Krohn, K., Jones, D. R., Bradley, J. D., Hall, S. D. and Brater, D. C.: The effects of a salicylate, ibuprofen, and naproxen on the disposition of methotrexate in patients with rheumatoid arthritis. *Eur. J. Clin. Pharmacol.*, **42**: 121–125 (1992).

25) Kremer, J. M. and Hamilton, R. A.: The effects of nonsteroidal anti-inflammatory drugs on methotrexate (MTX) pharmacokinetics: impairment of renal clearance of MTX at weekly maintenance doses but not at 7.5 mg. *J. Rheumatol.*, **22**: 2072–2077 (1995).

26) Dewland, P. M., Reader, S. and Berry, P.: Bioavailability of ibuprofen following oral administration of standard ibuprofen, sodium ibuprofen or ibuprofen acid incorporating poloxamer in healthy volunteers. *BMC Clin. Pharmacol.*, **9**: 19–28 (2009).

27) Kim, I. W., Chung, S. J. and Shim, C. K.: Altered metabolism of orally administered loxoprofen in human subjects after an oral administration of loxoprofen for three consecutive days followed by a seven-day washout. *J. Pharm. Sci.*, **91**: 973–979 (2002).

28) Vella-Brincat, J. W., Begg, E. J., Kirkpatrick, C. M., Zhang, M., Chambers, S. T. and Gallagher, K.: Protein binding of cefazolin is saturable in vivo both between and within patients. *Br. J. Clin. Pharmacol.*, **63**: 753–757 (2007).

29) Nozaki, Y., Kusuhara, H., Endou, H. and Sugiyama, Y.: Quantitative evaluation of the drug-drug interactions between methotrexate and nonsteroidal anti-inflammatory drugs in the renal uptake process based on the contribution of organic anion transporters and reduced folate carrier. *J. Pharmacol. Exp. Ther.*, **309**: 226–234 (2004).

30) Bakos, E., Evers, R., Sinko, E., Varadi, A., Borst, P. and Sarkadi, B.: Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. *Mol. Pharmacol.*, **57**: 760–768 (2000).

31) Mitomo, H., Kato, R., Ito, A., Kasamatsu, S., Ikegami, Y., Kii, I., Kudo, A., Kobatake, E., Sumino, Y. and Ishikawa, T.: A functional study on polymorphism of the ATP-binding cassette transporter ABCG2: critical role of arginine-482 in methotrexate transport. *Biochem. J.*, **373**: 767–774 (2003).

32) Volk, E. L. and Schneider, E.: Wild-type breast cancer resistance protein (BCRP/ ABCG2) is a methotrexate polyglutamate transporter. *Cancer Res.*, **63**: 5538–5543 (2003).

33) Nozaki, Y., Kusuhara, H., Kondo, T., Iwaki, M., Shiroyanagi, Y., Nakayama, H., Horita, S., Nakazawa, H., Okano, T. and Sugiyama, Y.: Species difference in the inhibitory effect of nonsteroidal anti-inflammatory drugs on the uptake of methotrexate by human kidney slices. *J. Pharmacol. Exp. Ther.*, **322**: 1162–1170 (2007).