Lansoprazole Exacerbates Pemetrexed-Mediated Hematologic Toxicity by Competitive Inhibition of Renal Basolateral Human Organic Anion Transporter 3

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ABSTRACT

Pemetrexed, a multitargeted antifolate, is eliminated by tubular secretion via human organic anion transporter 3 (hOAT3). Although proton pump inhibitors (PPIs) are frequently used in cancer patients, the drug interaction between PPIs and pemetrexed remains to be clarified. In this study, we examined the drug interaction between pemetrexed and PPIs in hOAT3-expressing cultured cells, and retrospectively analyzed the impact of PPIs on the development of hematologic toxicity in 108 patients who received pemetrexed and carboplatin treatment of nonsquamous non–small cell lung cancer for the first time between January 2011 and June 2015. We established that pemetrexed was transported via hOAT3 (Km = 68.3 ± 11.1 μM). Lansoprazole, rabeprazole, pantoprazole, esomeprazole, omeprazole, and vonoprazan inhibited hOAT3-mediated uptake of pemetrexed in a concentration-dependent manner. The inhibitory effect of lansoprazole was much greater than those of other PPIs and the apparent IC50 value of lansoprazole against pemetrexed transport via hOAT3 was 0.57 ± 0.17 μM. The inhibitory type of lansoprazole was competitive. In a retrospective study, multivariate analysis revealed that coadministration of lansoprazole, but not other PPIs, with pemetrexed and carboplatin was an independent risk factor significantly contributing to the development of hematologic toxicity (odds ratio: 10.004, P = 0.009). These findings demonstrated that coadministration of lansoprazole could exacerbate the hematologic toxicity associated with pemetrexed, at least in part, by competitive inhibition of hOAT3. Our results would aid clinicians to make decisions of coadministration drugs to avoid drug interaction-induced side effects for achievement of safe and appropriate chemotherapy with pemetrexed.

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ABBREVIATIONS: AST, aspartate aminotransferase; CrCl, creatinine clearance; CTCAE, Common Terminology Criteria for Adverse Events; GFR, glomerular filtration rate; Hb, hemoglobin; hOAT, human organic anion transporter; hOCT, human organic cation transporter; NSAIDs, nonsteroidal anti-inflammatory drugs; NSCLC, non–small cell lung cancer; PLT, platelet; PPI, proton pump inhibitor; WBC, white blood cell.
Proton pump inhibitors (PPIs) are the most commonly prescribed drugs for the treatment of gastroesophageal hyperacidity (Targownik et al., 2007). An estimated 20% of cancer patients have been treated with PPIs for alleviating the symptoms of gastroesophageal reflux (Smelck et al., 2013), highlighting the importance of investigating the drug interaction between PPIs and anticancer agents to provide safe and appropriate chemotherapy. Recent studies have reported that PPIs are inhibitors of hOATs and hOCTs (Nies et al., 2011; Chioukh et al., 2014). Since hematologic toxicity as a serious side effect of pemetrexed has been correlated with drug exposure (Rollins and Lindley, 2005), decreased clearance results in greater systemic exposure, which may be associated with increased side effects. Taking these findings into consideration, we hypothesized that coadministration of PPIs may exacerbate the hematologic toxicity of pemetrexed by inhibiting the renal elimination of pemetrexed via hOAT3; however, the drug interaction between pemetrexed and PPIs and the impact of PPIs on the development of hematologic toxicity of pemetrexed remain to be explored in clinical situations.

In the present study, drug interaction between PPIs and pemetrexed was examined in hOAT3-expressing cultured cells, and the impact of PPIs on the development of hematologic toxicity was retrospectively analyzed in hospitalized patients who received combination therapy of pemetrexed and carbolipin for the treatment of nonsquamous NSCLC.

Material and Methods

Materials. Pemetrexed disodium, pantoprazole, and rabeprazole were obtained from KLT Laboratories, Inc. (St. Paul, MN). Probencid, lansoprazole, and omeprazole were purchased from WAKO Pure Chemical (Osaka, Japan). Esomeprazole was purchased from Sigma-Aldrich (St. Louis, MO), and vonoprazan fumarate (TAK-438) was obtained from ChemScience, LLC (Monmouth Junction, NJ). All other chemical used were of the highest purity available.

Cell Culture. The hOAT3-expressing human embryonic kidney cell line HEK293 (HEK-hOAT3) and mock-transfectants obtained by transfecting pBK-CMV vector into HEK293 cells (HEK-pBK), were kind gifts from Prof. Ken-ichi Inui (Department of Pharmacy, Kyoto University Hospital, Japan). HEK293 cells were cultured in Medium 199 (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum containing G418 (0.5 mg/mL) (Sigma-Aldrich) and were used between passage numbers 88 and 107. These cells were maintained at 37°C under 5% CO₂ in a humidified atmosphere. For the uptake study, cells (12 × 10⁵ cells/dish) were seeded in 3.5-cm dishes with culture medium in the absence of G418. The cell monolayers were used for the uptake study after 48 hours of culture.

Uptake Experiments of Pemetrexed. Cellular uptake of pemetrexed was measured with monolayer cultures of HEK-hOAT3 and HEK-pBK cells. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM glucose, and 5 mM HEPES (pH 7.4). After the culture medium was removed, the cells were washed once with incubation medium and preincubated with 1 mL of incubation medium for 10 minutes at 37°C. After preincubation, the medium was replaced with 1 mL of incubation medium containing pemetrexed in the absence or presence of various concentrations of probencid or PPI. The medium was aspirated at the end of the incubation, and the monolayers were rapidly rinsed three times with ice-cold incubation medium. To evaluate the accumulation of pemetrexed into the cells, pemetrexed was eluted with 0.5 mL of extraction solution (30 mM phosphate buffer, pH 7.0: methanol = 50:50) and then subjected to high-performance liquid chromatography (HPLC). The cells were solubilized in 1 M NaOH, and the protein contents of the cells were measured using the Bradford method (Bradford, 1976) by using a Coomasie Brilliant Blue protein assay kit (Nacalai Tesque, Kyoto, Japan) with bovine γ-globulin as a standard.

Determination of Pemetrexed in Cells. The concentrations of pemetrexed in cells were determined by HPLC according to the method in previous studies (Resped et al., 2011; Kurata et al., 2014) with slight modifications. HPLC analysis was performed using a Waters Alliance 2695 HPLC system (Waters, Milford, MA) connected to a TSKgel ODS-80Tm 5-μm column (150 × 4.6 mm i.d.; Tosho, Tokyo, Japan) and a Waters 2996 photodiode array detector (Waters, Milford, MA). The column temperature was set at 40°C. Pemetrexed was eluted with 0.2% formic acid (pH 3.08 adjusted with 1 M NaOH): acetonitrile = 20: 80 at 1 mL/min. The detection wavelength was 254 nm.

Kinetic Analyses. Kinetic analyses were performed with GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA). The apparent Michaelis-Menten constant (Kₘ) and maximal velocity (Vₘₐₓ) values were calculated using the Michaelis-Menten equation: V = Vₘₐₓ × S/(Kₘ + S), where V is the transport velocity, S is the concentration of pemetrexed, Vₘₐₓ is the maximal velocity, and Kₘ is the Michaelis-Menten constant by nonlinear regression analysis. The apparent IC₅₀ values were calculated from the inhibition plots according to the equation: V = Vₘₐₓ × (1 + (IC₅₀/Vₘₐₓ))⁻¹ by nonlinear least square regression analysis, where V is the transport velocity. Vₘₐₓ is the transport velocity at the highest concentration of inhibitor, Vₘₐₓ is the transport velocity without inhibitor, I is the concentration of the inhibitor, and n is the Hill coefficient.

Results

Time Course of Pemetrexed Uptake by HEK293 Cells Expressing hOAT3. The uptake of pemetrexed (100 μM) by HEK293 cells transfected with hOAT3 and pBK was evaluated. As shown in Fig. 1, the uptake of pemetrexed in HEK-hOAT3 cells increased in a time-dependent manner and reached equilibrium state after 5 minutes. Moreover, the uptake of pemetrexed was significantly higher in HEK-hOAT3 cells than in HEK-pBK cells, the corresponding controls, at all time points.

Concentration-Dependent Uptake of Pemetrexed by HEK293 Cells Expressing hOAT3. To examine the characteristics of pemetrexed transport via hOAT3, concentration-dependent uptake studies for 2 minutes
were conducted. Figure 2 shows the concentration-dependent uptake of pemetrexed via hOAT3 by subtracting the uptake in HEK-pBK cells from that in HEK-hOAT3 cells. The uptake of pemetrexed mediated by hOAT3 was saturated at high concentrations. Figure 2 shows the results: the apparent $K_m$ and $V_{\text{max}}$ values of hOAT3-mediated uptake of pemetrexed were $68.3 \pm 11.1 \mu M$ and $157 \pm 9 \text{pmol/mg protein/min}$, respectively. Moreover, the Eadie-Hofstee plots were linear (insert, Fig. 2).

Inhibition of hOAT3-Mediated Transport of Pemetrexed by Probenecid. To verify whether pemetrexed is specifically transported by hOAT3, the cellular uptake of pemetrexed (25 $\mu M$) was measured for 2 minutes in the absence or presence of various concentrations of probenecid, a typical inhibitor of hOATs (Fig. 3). Probenecid inhibited hOAT3-mediated uptake of pemetrexed in a concentration-dependent manner. The apparent IC$_{50}$ values for the probenecid were calculated from the inhibition plot (Fig. 3). The apparent IC$_{50}$ value of probenecid against pemetrexed transport via hOAT3 was $1.97 \pm 1.31 \mu M$.

Inhibition of hOAT3-Mediated Transport of Pemetrexed by PPIs. To assess whether PPIs inhibit hOAT3-mediated transport of pemetrexed, the cellular uptake of pemetrexed (25 $\mu M$) was measured for 2 minutes in the absence or presence of various concentrations of PPIs (Fig. 4). All investigated PPIs inhibited hOAT3-mediated uptake of pemetrexed in a concentration-dependent manner. The apparent IC$_{50}$ values for the PPIs were calculated from the inhibition plot (Fig. 4). Lansoprazole demonstrated potent inhibitory effect (IC$_{50} = 0.57 \pm 0.17 \mu M$) against pemetrexed transport via hOAT3. The rank order of inhibitory effect on hOAT3-mediated transport of pemetrexed was as follows: lansoprazole > rabeprazole > pantoprazole > esomeprazole > omeprazole > vonoprazan.

Dixon Plot of the Inhibitory Effect of Lansoprazole against hOAT3-Mediated Transport of Pemetrexed. A Dixon plot was constructed to clarify the type of inhibition of lansoprazole against hOAT3-mediated transport of pemetrexed (Fig. 5). Cellular uptake of pemetrexed (12.5, 25, and 50 $\mu M$) was measured for 2 minutes in the absence and presence of lansoprazole (0.2, 0.5, and 1.0 $\mu M$). The Dixon plot clearly indicated that the inhibitory type of lansoprazole against hOAT3-mediated transport of pemetrexed was competitive; the inhibitory constant value was $0.42 \pm 0.08 \mu M$.

Patients’ Characteristics. According to the exclusion criteria, 108 of 116 patients were enrolled in the retrospective study. Patients’ characteristics are summarized in Table 1. The median age of patients was 68 (range, 39–82 years). Seventy-nine patients (73%) were men. Hematologic toxicity ≥grade 3 after administration of pemetrexed and carboplatin was observed in 22 patients (20%). Among the 22 patients with hematologic toxicity, leukopenia ($n = 9$), thrombocytopenia
(n = 20), and anemia (n = 3) were identified. On the other hand, the coadministration of PPIs and pemetrexed was reported in 26 patients (24%). These patients received lansoprazole 15 mg/day (n = 13) or 30 mg/day (n = 2), esomeprazole 20 mg/day (n = 6), rabeprazole 10 mg/day (n = 4), and omeprazole 10 mg/day (n = 1), respectively. The coadministered drugs in 22 patients with hematologic toxicity were listed in Supplemental Table 1. Seven of 15 patients (47%) with lansoprazole developed hematologic toxicity. Furthermore, potential drug interactions of pemetrexed were verified using Lexi-Interact for all patients. Drug interaction between pemetrexed and NSAIDs was identified in 23 patients (21%). These patients received celecoxib 200 mg/day (n = 3) or 400 mg/day (n = 1), diclofenac 75 mg/day (n = 1), loxoprofen 180 mg/day (n = 16), meloxicam 10 mg/day (n = 1), and naproxen 300 mg/day (n = 1), respectively. In addition, 15 patients (14%) received amlodipine 2.5 mg/day (n = 4) or 5 mg/day (n = 11). Interestingly, 8 of 15 patients (53%) who received amlodipine developed hematologic toxicity (Supplemental Table 1).

Impact of Coadministration of Lansoprazole on the Development of Hematologic Toxicity after Pemetrexed and Carboplatin Administration. Considering the results of the in vitro studies, a multivariate logistic regression analysis was conducted to investigate the impact of lansoprazole on the development of hematologic toxicity in patients who received combination therapy of pemetrexed and carboplatin for the treatment of nonsquamous NSCLC (Table 2). The results revealed that coadministration of lansoprazole with pemetrexed and carboplatin was an independent risk factor that significantly contributed to the development of hematologic toxicity (odds ratio: 10.004, \( P = 0.005 \)). On the other hand, variables such as age, dose of pemetrexed and carboplatin, CrCl, WBC count, PLT count, Hb level, and coadministration of NSAIDs and other PPIs were not significant risk factors for hematologic toxicity after pemetrexed and carboplatin administration.

Discussion

The drug interaction between PPIs and pemetrexed remains to be clarified. To our knowledge, this was the first study to report the effect of lansoprazole on the development of hematologic toxicity associated with pemetrexed by inhibition of pemetrexed transport via hOAT3. Although some transporters have been previously reported to be involved in renal elimination of pemetrexed (Li et al., 2013; Posada et al., 2015), only hOAT3 has been identified as an active transporter of pemetrexed uptake from blood to renal tubular cells. As shown in Fig. 2, hOAT3 was confirmed as a high-affinity type transporter for pemetrexed. Moreover, the inhibition of hOAT3-mediated transport of
Lansoprazole Inhibits hOAT3-Mediated Pemetrexed Transport

Fig. 5. Dixon plot of the inhibition of pemetrexed uptake by lansoprazole in HEK-hOAT3 cells. HEK-hOAT3 cells were incubated at 37°C for 2 minutes with 12.5 μM (closed squares), 25 μM (open circles), and 50 μM (closed circles) of pemetrexed (pH 7.4) in the absence or presence of lansoprazole (0.2, 0.5, and 1.0 μM). Each point represents mean ± S.E. of three separate experiments using three monolayers. When the standard errors of the means are small, they are contained within the symbols. V is the uptake velocity (pmol/mg protein/min).

pemetrexed was verified with probenecid (Fig. 3). Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed was verified with probenecid (Fig. 3). Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed. Therefore, hOAT3 plays an important role in the first step of renal tubular secretion of pemetrexed.

A recent study reported that hOAT3-mediated transport of methotrexate, which has a structure and pharmacokinetics similar to pemetrexed, was inhibited by PPIs (Chioukh et al., 2014). Several case reports and retrospective studies have demonstrated that coadministration of PPIs delayed the elimination of methotrexate (Suzuki et al., 2009; Santucci et al., 2010; Reeves et al., 2014). It remains unclear, however, whether PPIs inhibit pemetrexed transport via hOAT3. As shown in Figs. 3 and 4, the inhibitory effect of lansoprazole was comparable to that of probenecid and was much higher than those of other PPIs. The apparent IC50 value of lansoprazole against pemetrexed transport via hOAT3 was 0.57 ± 0.17 μM. Moreover, its inhibitory type of lansoprazole was competitive (Fig. 5).

The decision tree defined by the U.S. Food and Drug Administration’s 2012 draft guidance on drug-drug interaction concludes that a ratio of unbound Cmax to IC50 value ≥0.1 indicates recommendation for the evaluation of clinical drug interaction. When 30 mg of lansoprazole was administered, the Cmax of lansoprazole was approximately 2.5–4.9 μM (Ieiri et al., 2001). Since the protein binding of lansoprazole is 95.5% (McCallum et al., 2014), the unbound Cmax of lansoprazole was estimated to be 0.11–0.22 μM. The ratio of unbound Cmax to IC50 value of lansoprazole was 0.2–0.4 (≥0.1), indicating that clinical trial regarding drug interaction between pemetrexed and lansoprazole should be performed; however, the ratios of unbound Cmax to IC50 Values of the PPIs, excluding lansoprazole, were much lower than 0.1 (data not shown). Therefore, these findings suggest that coadministration of lansoprazole and pemetrexed could lead to clinical drug interaction.

Based on these findings, we hypothesized that coadministration of lansoprazole may exacerbate the hematologic toxicity of pemetrexed by inhibiting the tubular secretion of pemetrexed via hOAT3. The retrospective analysis of clinical data confirmed the impact of lansoprazole on the development of hematologic toxicity in patients administered with pemetrexed and carboplatin. As shown in Table 2, the multivariate logistic regression analysis suggested that the coadministration of lansoprazole, but not other PPIs, was an independent risk factor that significantly contributes to the development of hematologic toxicity after pemetrexed administration (odds ratio: 10.004, P = 0.005).

Interestingly, among the seven patients who developed hematologic toxicity during coadministration of lansoprazole, one patient did not develop hematologic toxicity when famotidine (histamine H2 receptor antagonist) was administered instead of lansoprazole during the next course of chemotherapy with pemetrexed and carboplatin. These findings strongly suggest that coadministration of lansoprazole could exacerbate the hematologic toxicity of pemetrexed.

Lansoprazole is metabolized by the cytochrome P450 2C19 (CYP2C19). It is well known that genetic polymorphism exists for this enzyme, and the pharmacokinetics of lansoprazole differs between extensive and poor metabolizers of CYP2C19 (Katsuki et al., 1997; Sohn et al., 1997). The frequency of poor metabolizers of CYP2C19 in

### TABLE 1

Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n = 108)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>68 (39–82)</td>
</tr>
<tr>
<td>Male</td>
<td>79 (73)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.4 (143.6–188.1)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>59.5 (32.4–92.7)</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.63 (1.24–2.19)</td>
</tr>
<tr>
<td>Clinical disease stage (II/IV)</td>
<td>20 (19)/88 (81)</td>
</tr>
<tr>
<td>History of platinum-based chemotherapy</td>
<td>12 (11)</td>
</tr>
<tr>
<td>Pemetrexed dose (mg/m²)</td>
<td>497 (427–552)</td>
</tr>
<tr>
<td>Carboplatin dose (mg/mgprotein/min)</td>
<td>5.21 (4.21–6.60)</td>
</tr>
<tr>
<td>Combination of bevacizumab</td>
<td>48 (44)</td>
</tr>
<tr>
<td>Baseline biologic parameters</td>
<td></td>
</tr>
<tr>
<td>WBC (×10⁹/liter)</td>
<td>6.87 (3.37–23.85)</td>
</tr>
<tr>
<td>PLT (×10⁹/liter)</td>
<td>247 (75–508)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.2 (8.8–18)</td>
</tr>
<tr>
<td>CrCl (ml/min)</td>
<td>73.4 (45.1–156.8)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.9 (3.0–4.7)</td>
</tr>
</tbody>
</table>

### TABLE 2

Multivariate analysis of the development of hematologic toxicity after pemetrexed and carboplatin administration

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lansoprazole</td>
<td>10.004</td>
<td>2.033–49.234</td>
<td>0.005</td>
</tr>
<tr>
<td>Other PPIs</td>
<td>1.959</td>
<td>0.310–12.382</td>
<td>0.475</td>
</tr>
<tr>
<td>Age</td>
<td>1.056</td>
<td>0.951–1.173</td>
<td>0.305</td>
</tr>
<tr>
<td>Pemetrexed dose (mg/m²)</td>
<td>1.049</td>
<td>0.993–1.107</td>
<td>0.087</td>
</tr>
<tr>
<td>Carboplatin dose (mg-min/ml)</td>
<td>0.993</td>
<td>0.983–1.004</td>
<td>0.238</td>
</tr>
<tr>
<td>CrCl (ml/min)</td>
<td>0.992</td>
<td>0.944–1.042</td>
<td>0.739</td>
</tr>
<tr>
<td>WBC (×10⁹/liter)</td>
<td>0.796</td>
<td>0.598–1.060</td>
<td>0.118</td>
</tr>
<tr>
<td>PLT (×10⁹/liter)</td>
<td>0.997</td>
<td>0.989–1.004</td>
<td>0.406</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>0.808</td>
<td>0.539–1.212</td>
<td>0.303</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>2.015</td>
<td>0.432–9.407</td>
<td>0.373</td>
</tr>
</tbody>
</table>

Cl, confidential interval; CrCl, creatinine clearance; Hb, hemoglobin; NSAIDs, nonsteroidal anti-inflammatory drugs; PLT, platelet; PPI, proton pump inhibitor; WBC, white blood cell.

**Notes:**
- Lansoprazole is metabolized by the cytochrome P450 2C19 (CYP2C19).
- Lansoprazole inhibits hOAT3-mediated pemetrexed transport.
- Lansoprazole may exacerbate the hematologic toxicity of pemetrexed.
- Lansoprazole is a competitive inhibitor of hOAT3.
- Lansoprazole inhibits hOAT3-mediated pemetrexed transport.
- Lansoprazole is metabolized by the cytochrome P450 2C19 (CYP2C19).
- Lansoprazole is metabolized by the cytochrome P450 2C19 (CYP2C19).
- Lansoprazole is metabolized by the cytochrome P450 2C19 (CYP2C19).
- Lansoprazole is metabolized by the cytochrome P450 2C19 (CYP2C19).
the Japanese population is approximately 20% (Kimura et al., 1998). Although CYP2C19 polymorphism was not determined in our present study, it is probable that CYP2C19 polymorphism contributed to the development of hematologic toxicity by pemetrexed during coadministration of lansoprazole.

Carboplatin is eliminated primarily from the kidney, and approximately 90% of the dose is recovered in the urine as unchanged form within 24 hours after administration in patients with normal renal function (Go and Adjei, 1999). Unlike other platinum compounds, carboplatin is excreted mainly via glomerular filtration (Sorensen et al., 1992) and is not transported by organic cation transporters including hOCT1 and hOCT2 (Yonezawa et al., 2006). Moreover, a previous study reported that the accumulation of p-aminohippurate (a typical substrate of hOATs) was not inhibited by carboplatin in the experiment using rat renal cortical slices (Kanou et al., 2004). Thus, it is unlikely that lansoprazole inhibits tubular secretion of carboplatin.

To prevent the occurrence of adverse events after administration of pemetrexed, folic acid and vitamin B12 supplements are recommended during pemetrexed therapy (Molina and Adjei, 2003). Although human proton-coupled folate transporter plays a key role in the intestinal absorption of folic acid (Visentin et al., 2014), Urquhart et al. (2010) reported that human proton-coupled folate transporter mRNA levels decreased in patients receiving PPIs. Thus, it is likely that the decreased oral absorption of folic acid caused by PPIs contributed to the development of hematologic toxicity associated with pemetrexed; however, we demonstrated that the coadministration of other PPIs was not a significant risk factor for hematologic toxicity associated with pemetrexed (Table 2). This finding suggests that decreased renal clearance of pemetrexed rather than decreased oral absorption of folic acid was primarily accountable for the increased hematologic toxicity by pemetrexed.

In the present retrospective study, variables such as CrCl and coadministration of NSAIDs were not significant risk factors for hematologic toxicity of pemetrexed (Table 2). Mita et al. (2006) reported that pemetrexed plasma clearance positively correlated with GFR, resulting in increased drug exposure in patients with impaired renal function. Pemetrexed was reported to be well tolerated, however, at a dose of 500 mg/m² in combination with vitamin supplements in patients with GFR ≥ 40 ml/min (Mita et al., 2006). In the present study, all patients showed CrCl ≥ 45 ml/min and were administered pemetrexed at a dose of approximately 500 mg/m² in combination with folic acid and vitamin B12 supplements, implying that the enrolled patients could tolerate pemetrexed therapy well. Therefore, it was not relevant to evaluate the influence of CrCl on pemetrexed-mediated hematologic toxicity.

A previous retrospective study demonstrated that the coadministration of NSAIDs was not a risk factor for the development of hematologic toxicity by pemetrexed (Sakata et al., 2013); however, NSAIDs are known as substrates and/or inhibitors of hOAT3 (Uwai et al., 2004; Nozaki et al., 2007). A clinical study has reported that a 20% increase in the area under the plasma concentration curve of pemetrexed was observed when 400 mg of ibuprofen was administered orally every 6 hours (Sweeney et al., 2006). Moreover, Posada et al. (2015) reported that ibuprofen was the most likely to cause drug interaction with pemetrexed because the ratio of unbound to the IC50 value of ibuprofen for hOAT3-mediated pemetrexed transport was 0.38. Among the 23 patients who received NSAIDs in our clinical study (Table 1), most patients received loxoprofen (n = 16) and none received ibuprofen. A previous study demonstrated that the ratio of unbound to the IC50 value of loxoprofen for hOAT3-mediated pemetrexed transport was much lower than 0.1 (Kurata et al., 2014). These findings suggested that coadministration of NSAIDs, excluding ibuprofen, was not a risk factor for the development of hematologic toxicity after pemetrexed therapy in patients without severe renal dysfunction.

Interestingly, 8 of 22 patients with hematologic toxicity received amiodipine (Supplemental Table 1). Multivariate logistic analysis with variables, including coadministration of amiodipine, revealed that the ratio of unbound to the IC50 value of amiodipine was also an independent risk factor that significantly contributes to the development of hematologic toxicity by pemetrexed (odds ratio: 22.910, P < 0.001) as shown in Supplemental Table 2. Two patients who developed hematologic toxicity received both lansoprazole and amiodipine (Supplemental Table 1); however, the severity of hematologic toxicity in these two patients with both lansoprazole and amiodipine was not greater than those in patients with either lansoprazole or amiodipine. Moreover, there were no reports regarding the inhibitory effect of amiodipine on the activity of hOAT3 or the development of hematologic toxicity by amiodipine. Further study is needed to clarify the detailed mechanism.

The present study had some limitations. First, the delayed elimination of pemetrexed caused by lansoprazole was not evaluated because the plasma concentration of pemetrexed could not be determined. Second, it was difficult to exclude the potential effects of other unknown confounders in the retrospective study. Therefore, a prospective study needs to be conducted to determine the pharmacokinetics of pemetrexed when coadministered with lansoprazole or other PPIs and to assess the toxicities of pemetrexed during coadministration.

In conclusion, our study was the first to demonstrate that coadministration of lansoprazole could exacerbate the hematologic toxicity of pemetrexed, at least in part, by competitive inhibition of renal hOAT3. Therefore, lansoprazole should be discontinued or switched to other PPIs during chemotherapy with pemetrexed. Alternatively, the dose of pemetrexed could be reduced during coadministration of lansoprazole. The present findings provide important information for safe and appropriate chemotherapy with pemetrexed.

**Authorship Contributions**

**Participated in research design:** Ikemura, Iwamoto, Okuda.

**Conducted experiments:** Ikemura, Hamada, Kaya.

**Performed data analysis:** Ikemura, Hamada, Kaya, Enokiya, Nakahara, Fujimoto, Kobayashi.

**Wrote or contributed to the writing of the manuscript:** Ikemura, Enokiya, Muraki, Iwamoto, Okuda.

**References**


Lansoprazole Inhibits hOAT3-Mediated Pemetrexed Transport


The multispecific organic anion transporter (OAT) family.

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