Lansoprazole exacerbates pemetrexed-mediated hematological toxicity by competitive inhibition of renal basolateral human organic anion transporter 3

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- 247 words in the Abstract
- 538 words in the Introduction
- 1490 words in the Discussion

Abbreviations: ALT, aminotransferase; AST, aspartate aminotransferase; C_{max}, maximum plasma concentration; CrCl, creatinine clearance; CTCAE, Common Terminology Criteria for Adverse Events; GFR, glomerular filtration rate; hOAT, human organic anion transporter; Hb, hemoglobin; hOCT, human organic cation transporters; NSAIDs, non-steroidal anti-inflammatory drugs; NSCLC, non-squamous non-small cell lung cancer; PLT, platelet; PPI, proton pump inhibitor; WBC, white blood cell
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, the drug interaction between pemetrexed and PPIs was examined in hOAT3-expressing cultured cells, and the impact of PPIs on the development of hematological toxicity was retrospectively analyzed in 108 patients who received pemetrexed and carboplatin treatment for non-squamous non-small cell lung cancer for the first time between January 2011 and June 2015. We established that pemetrexed was transported via hOAT3 (K_m = 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 higher than those of other PPIs, and the apparent IC_{50} value of lansoprazole against pemetrexed transport via hOAT3 was 0.57 ± 0.17 µM. Inhibitory type of lansoprazole was competitive. In a retrospective study, multivariate analysis revealed that co-administration of lansoprazole, but not other PPIs, with pemetrexed and carboplatin was an independent risk factor significantly contributing to the development of hematological toxicity (odds ratio: 10.004, P = 0.005). These findings demonstrated that co-administration of lansoprazole could exacerbate the hematological toxicity associated with pemetrexed, at least in part, by competitive inhibition of hOAT3. Our results would aid clinicians to make decisions of co-administration drugs to avoid drug interaction-induced side effects for achievement of safe and appropriate chemotherapy with pemetrexed.
Introduction

Pemetrexed, a potent multitargeted antifolate, is a key drug in the treatment of non-squamous non-small cell lung cancer (NSCLC) (Adjei, 2004). It is increasingly used as first-line treatment in combination with platinum compounds (Scagliotti et al., 2008), and as maintenance monotherapy for non-squamous NSCLC (Hanna et al., 2004).

Pemetrexed is mainly eliminated from the kidney and has a tubular secretion rate approximately 2.5-fold higher than the glomerular filtration rate (GFR) in advanced cancer patients with normal renal function (Rinaldi et al., 1999; Mita et al., 2006). In the renal proximal tubules, membrane transport proteins expressed specifically at the basolateral membranes are responsible for the urinary secretion of various drugs. The structures and functions of human organic anion transporters (hOATs) and human organic cation transporters (hOCTs) encoded by SLC22A genes have been characterized (Sweet and Pritchard, 1999; Inui et al., 2000; Sekine et al., 2000). Both hOAT1 (SLC22A6) and hOAT3 (SLC22A8) mediate organic anion/α-ketoglutarate exchange at the basolateral membrane of the proximal tubules (Sekine et al., 1997; Sweet and Pritchard, 1999). Previous studies investigating the characteristics of pemetrexed transport via various solute carrier transporters revealed that pemetrexed was primarily transported by hOAT3 (Kurata et al., 2014; Posada et al., 2015). It has been reported that hOAT3 substrates, such as non-steroidal anti-inflammatory drugs (NSAIDs) and cephalosporin antibiotics, inhibited pemetrexed transport via hOAT3 (Kurata et al., 2014; Posada et al., 2015). Recently, Posada et al. (2015) reported that hOAT4 (SLC22A11) expressed at the brush-border membrane was predominantly involved in the transport of pemetrexed from the renal tubular cells to urine. However, inhibition of...
hOAT4 was shown to have no effect on the pemetrexed plasma clearance in the experiment using physiological based pharmacokinetic models (Posada et al., 2015). Therefore, hOAT3-mediated uptake, but not hOAT4-mediated efflux, should contribute to plasma clearance of pemetrexed. These findings suggested that particular care should be taken during the co-administration of inhibitors and/or substrates of hOAT3 in patients receiving pemetrexed.

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 (Smelick 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 hematological 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 effect. Taking these findings into consideration, we hypothesized that co-administration of PPIs may exacerbate the hematological 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 hematological 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 hematological toxicity was retrospectively analyzed in hospitalized patients who
received combination therapy of pemetrexed and carboplatin for the treatment of non-squamous NSCLC.
Material and Methods

Materials

Pemetrexed disodium, pantoprazole, and rabeprazole were obtained from LKT Laboratories, Inc. (St. Paul, MN). Probenecid, lansoprazole, and omeprazole were purchased from WAKO Pure Chemical (Osaka, Japan). Esomeprazole was purchased from Sigma-Aldrich (St. Louis, MO). Vonoprazan fumarate (TAK-438) was obtained from ChemScene, LLC (Monmouth Junction, NJ). All other chemicals 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 kindly gifted by 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, St. Louis, MO) 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 h 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 CaCl$_2$, 0.5 mM MgCl$_2$, 5 mM D-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 min 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 probenecid 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 was 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 Coomassie Brilliant Blue protein assay kit (Nacalai Tesque, Kyoto, Japan) with bovine $\gamma$-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 (Respaud 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 x 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:

\[ V = \frac{V_{\text{max}} \cdot S}{K_m + S}, \]

where \( V \) is the transport velocity, \( S \) is the concentration of pemetrexed, \( V_{\text{max}} \) is the maximal velocity, and \( K_m \) is Michaelis-Menten constant by nonlinear regression analysis. The apparent IC50 values were calculated from the inhibition plots according to the equation:

\[ V = V_{\text{bottom}} + \frac{(V_{\text{top}} - V_{\text{bottom}})}{[1 + (\log \frac{I}{IC50})^n]}, \]

by nonlinear least square regression analysis, where \( V \) is the transport velocity, \( V_{\text{bottom}} \) is the transport velocity at the highest concentration of inhibitor, \( V_{\text{top}} \) is the transport velocity without inhibitor, \( I \) is the concentration of the inhibitor, and \( n \) is the Hill coefficient.

Patients and data collection

A retrospective study was conducted in 116 hospitalized patients who received combination therapy of pemetrexed and carboplatin in the absence or presence of bevacizumab for the treatment of non-squamous NSCLC for the first time at Mie University Hospital between January 2011 and June 2015. Eligible patients received an intravenous infusion of pemetrexed (500 mg/m²) for 10 min, followed by a 60-min intravenous infusion of carboplatin at a dose calculated to produce an area under the concentration-time curve of 5 or 6 mg·min/ml, and an intravenous infusion of bevacizumab at a dose of 15 mg/kg for 90 min. Patients were excluded if they had missing data, creatinine clearance (CrCl) < 45 ml/min as estimated by the
Cockcroft-Gault equation (Cockcroft and Gault, 1976), did not receive vitamin B₁₂ and folic acid supplements for preventing the adverse effects of pemetrexed, showed alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≥ 100 IU/l, had Eastern Cooperative Oncology Group performance status ≥ 2, and hematologic parameters ≥ grade 2 before chemotherapy, including white blood cell (WBC) count, platelet (PLT) count, or hemoglobin (Hb) level, defined as the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE).

Demographic data were extracted from the electronic medical records. In addition to the use of PPIs (lansoprazole 15 or 30 mg/day, omeprazole 10 mg/day, esomeprazole 20 mg/day, and rabeprazole 10 mg/day), periodic co-administration drugs, which may cause potential interactions was identified by Lexicomp® Lexi-Interact™ Online (Lexi-Comp, Inc., Hudson, OH). Hematological toxicity was defined as ≥ grade 3 of WBC, PLT, or Hb by the CTCAE, within 21 days of pemetrexed administration. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Mie University Graduate School of Medicine and Faculty of Medicine.

**Statistical analyses**

The *in vitro* experimental data are expressed as the mean ± S.E. Statistical comparisons between the two groups were performed using the unpaired t-test with GraphPad Prism version 6.0. Multivariate logistic regression analysis was performed to identify the impact of co-administration of lansoprazole on the development of hematological toxicity after administration of pemetrexed and carboplatin, with the following variables: age, WBC, PLT, CrCl, Hb, co-administration of lansoprazole, other.
PPIs, or NSAIDs, pemetrexed dose, and carboplatin dose. Statistical analyses of the clinical data were performed with IBM SPSS statistics for windows version 22.0 (Armonk, NY). Significance was established at a $P$-value $< 0.05$. 
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 Figure 1, the uptake of pemetrexed in HEK-hOAT3 cells increased in a time-dependent manner and reached equilibrium state after 5 min. Moreover, the uptake of pemetrexed was significantly higher in HEK-hOAT3 cells than that 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 min 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. From the results in Figure 2, the apparent $K_m$ and $V_{max}$ values of hOAT3-mediated uptake of pemetrexed were $68.3 \pm 11.1$ µM and $157 \pm 9$ pmol/mg protein/min, respectively. Moreover, the Eadie-Hofstee plots were liner (insert of Figure 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 µM) was measured for 2 min in the absence or presence of various concentrations of probenecid, a typical inhibitor of hOATs (Figure 3). Probenecid inhibited hOAT3-mediated uptake of pemetrexed in a
concentration-dependent manner. The apparent IC<sub>50</sub> values for the probenecid were calculated from the inhibition plot (Figure 3). The apparent IC<sub>50</sub> value of probenecid against pemetrexed transport via hOAT3 was 1.97 ± 1.31 µM.

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 µM) was measured for 2 min in the absence or presence of various concentrations of PPIs (Figure 4). All investigated PPIs inhibited hOAT3-mediated uptake of pemetrexed in a concentration-dependent manner. The apparent IC<sub>50</sub> values for the PPIs were calculated from the inhibition plot (Figure 4). Lansoprazole demonstrated potent inhibitory effect (IC<sub>50</sub> = 0.57 ± 0.17 µM) 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 (Figure 5). Cellular uptake of pemetrexed (12.5, 25, and 50 µM) was measured for 2 min in the absence and presence of lansoprazole (0.2, 0.5, and 1.0 µM). 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 ± 0.08 µM.
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 male.

Hematological toxicity ≥ grade 3 after administration of pemetrexed and carboplatin was observed in 22 patients (20%). Among the 22 patients with hematological toxicity, leukopenia (n = 9), thrombocytopenia (n = 20), and anemia (n = 3) were identified. On the other hand, the co-administration 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 co-administrated drugs in 22 patients with hematological toxicity were listed in Supplemental Table 1. Seven out of 15 patients (47%) with lansoprazole developed hematological 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 out of 15 patients (53%) with amlodipine developed hematological toxicity (Supplemental Table 1).

Impact of co-administration of lansoprazole on the development of hematological 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 hematological toxicity in patients who received combination therapy of pemetrexed and carboplatin for the treatment of non-squamous NSCLC (Table 2). The results revealed that co-administration of lansoprazole with pemetrexed and carboplatin was an independent risk factor significantly contributing to the development of hematological 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 co-administration of NSAIDs and other PPIs were not significant risk factors for hematological 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 hematological 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 Figure 2, hOAT3 was confirmed as a high affinity type transporter for pemetrexed. Moreover, the inhibition of hOAT3-mediated transport of pemetrexed was verified with probenecid (Figure 3). 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 similar structure and pharmacokinetics to pemetrexed, was inhibited by PPIs (Chioukh et al., 2014). Several case reports and retrospective studies have demonstrated that co-administration of PPIs delayed the elimination of methotrexate (Suzuki et al., 2009; Santucci et al., 2010; Reeves et al., 2014). However, it remains unclear whether PPIs inhibit pemetrexed transport via hOAT3. As shown in Figures 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 IC$_{50}$ value of lansoprazole against pemetrexed transport via hOAT3 was 0.57 ± 0.17 µM. Moreover, its inhibitory type of lansoprazole was competitive (Figure 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 $C_{\text{max}}$ to IC$_{50}$ value ≥
0.1 indicates recommendation for the evaluation of clinical drug interaction. When 30 mg of lansoprazole was administered, the C_{max} 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 C_{max} of lansoprazole was estimated to be 0.11–0.22 µM. The ratio of unbound C_{max} to IC_{50} 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 C_{max} to IC_{50} values of the PPIs, excluding lansoprazole, were much lower than 0.1 (data not shown). Therefore, these findings suggest that co-administration of lansoprazole and pemetrexed could lead to clinical drug interaction.

Based on the findings, we hypothesized that co-administration of lansoprazole may exacerbate the hematological 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 hematological toxicity in patients administered with pemetrexed and carboplatin. As shown in Table 2, the multivariate logistic regression analysis suggested that the co-administration of lansoprazole, but not other PPIs, was an independent risk factor significantly contributing to the development of hematological toxicity after pemetrexed administration (odds ratio: 10.004, \( P = 0.005 \)). Interestingly, among the seven patients who developed hematological toxicity during co-administration of lansoprazole, one patient did not develop hematological toxicity when famotidine (histamine H\(_2\) receptor antagonist) was administered instead of lansoprazole during the next course of chemotherapy with pemetrexed and carboplatin. These findings strongly suggest that co-administration of lansoprazole could exacerbate the hematological 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 Japanese 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 hematological toxicity by pemetrexed during co-administration of lansoprazole.

Carboplatin is primarily eliminated from the kidney, and approximately 90% of the dose is recovered in the urine as unchanged form within 24 h after administration in patients with normal renal function (Go and Adjei, 1999). Unlike other platinum compounds, carboplatin is mainly excreted via glomerular filtration (Sorensen et al., 1992), and is not transported by organic cation transporters including hOCT1 and hOCT2 (Yonezawa et al., 2006). Moreover, 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 hematological toxicity associated
with pemetrexed. However, we demonstrated that the co-administration of other PPIs was not a significant risk factor for hematological 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 hematological toxicity by pemetrexed.

In the present retrospective study, the variables such as CrCl and co-administration of NSAIDs were not significant risk factors for hematological 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. However, it was reported that pemetrexed was well tolerated 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 with 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 hematological toxicity.

A previous retrospective study demonstrated that the co-administration of NSAIDs was not a risk factor for the development of hematological 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 C_{max} to IC_{50} value of
ibuprofen for hOAT3-mediated pemetrexed transport was 0.38. Among the 23 patients who received NSAIDs in our clinical study (Table 1), the majority of patients received loxoprofen (n = 16) and no patients received ibuprofen. A previous study demonstrated that the ratio of unbound C_{max} to IC_{50} value of loxoprofen for hOAT3-mediated pemetrexed transport was much lower than 0.1 (Kurata et al., 2014). These findings suggested that co-administration of NSAIDs excluding ibuprofen was not a risk factor for the development of hematological toxicity after pemetrexed therapy in patients without severe renal dysfunction.

Interestingly, 8 out of 22 patients with hematological toxicity received amlodipine (Supplemental Table 1). Multivariate logistic analysis with variables including co-administration of amlodipine revealed that co-administration of amlodipine was also an independent risk factor significantly contributing to the development of hematological toxicity by pemetrexed (odds ratio: 22.910, P < 0.001) as shown in Supplemental Table 2. Two patients who developed hematological toxicity received both lansoprazole and amlodipine (Supplementary Table 1). However, the severities of hematological toxicity in these two patients with both lansoprazole and amlodipine were not higher than those in patients with either lansoprazole or amlodipine. Moreover, there were no reports regarding the inhibitory effect of amlodipine on the activity of hOAT3 or development of hematological toxicity by amlodipine. Further study is needed to clarify the detailed mechanism.

The present study had some limitations that need to be considered. 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 cofounders in the
retrospective study. Therefore, a prospective study should be conducted to determine the pharmacokinetics of pemetrexed when co-administered with lansoprazole or other PPIs and to assess the toxicities of pemetrexed during co-administration.

In conclusion, our study was the first to demonstrate that co-administration of lansoprazole could exacerbate the hematological 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, dose of pemetrexed could be reduced during co-administration of lansoprazole. The present findings provide important information for safe and appropriate chemotherapy with pemetrexed.
Authorship Contributions

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

Conducted experiments: Ikemura, Hamada, and Kaya.


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

Figure 1  Time course of pemetrexed uptake by HEK-hOAT3 cells. HEK-hOAT3 (closed circles) or pBK (open circles) cells were incubated for specified durations (2, 3, 5, and 15 min) at 37°C with 100 µM pemetrexed (pH 7.4). Each point represents mean ± S.E. of three separate experiments using three monolayers. **: P < 0.01, ***: P < 0.001 compared with HEK-pBK cells. When the standard errors of the means are small, they are contained within the symbols.

Figure 2  Concentration dependent uptake of pemetrexed transport mediated by hOAT3. HEK293 cells were incubated at 37°C for 2 min with various concentrations (5, 10, 20, 50, 100, 200, and 500 µM) of pemetrexed (pH 7.4). The uptake mediated by hOAT3 was obtained by subtracting the uptake in HEK-pBK cells from that in HEK-hOAT3 cells. 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. Insert: Eadie-Hofstee plot of pemetrexed uptake after subtraction of non-saturable components. V is the uptake velocity (pmol/mg protein/min) and S is the concentration of pemetrexed (µM).

Figure 3  Inhibition of pemetrexed uptake by probenecid in HEK-hOAT3 cells. HEK293-hOAT3 cells were incubated at 37°C for 2 min with 25 µM pemetrexed (pH 7.4) in the absence or presence of various concentrations of probenecid. 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. The apparent IC\textsubscript{50} values were calculated by fitting the data to a sigmoidal dose-response regression curve.

**Figure 4** Inhibition of pemetrexed uptake by PPIs in HEK-hOAT3 cells. HEK-hOAT3 cells were incubated at 37°C for 2 min with 25 µM pemetrexed (pH 7.4) in the absence or presence of various concentrations of lansoprazole (A), rabeprazole (B), pantoprazole (C), esomeprazole (D), omeprazole (E), and vonoprazan (F). 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. The apparent IC\textsubscript{50} values were calculated by fitting the data to a sigmoidal dose-response regression curve.

**Figure 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 min 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).
Table 1. Patients’ characteristics

<table>
<thead>
<tr>
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<th>Patients (n = 108)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>68 [39–82]</td>
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<tr>
<td>Male</td>
<td>79 (73)</td>
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<tr>
<td>Height (cm)</td>
<td>164.4 [143.6–188.1]</td>
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<tr>
<td>Body weight (kg)</td>
<td>59.5 [32.4–92.7]</td>
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<tr>
<td>Body surface area (m²)</td>
<td>1.63 [1.24–2.19]</td>
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<tr>
<td>Clinical disease Stage (III/IV)</td>
<td>20 (19)/88 (81)</td>
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<tr>
<td>History of platinum based chemotherapy</td>
<td>12 (11)</td>
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<tr>
<td>Pemetrexed dose (mg/m²)</td>
<td>497 [427–552]</td>
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<tr>
<td>Carboplatin dose (mg·min/ml)</td>
<td>5.21 [4.21–6.60]</td>
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<tr>
<td>Combination of bevacizumab</td>
<td>48 (44)</td>
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<tr>
<td>Baseline biological parameters</td>
<td></td>
</tr>
<tr>
<td>WBC (×10⁹/l)</td>
<td>6.87 [3.37–23.85]</td>
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<tr>
<td>PLT (×10⁹/l)</td>
<td>247 [75–508]</td>
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<tr>
<td>Hb (g/dl)</td>
<td>13.2 [8.8–18]</td>
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<tr>
<td>CrCl (ml/min)</td>
<td>73.4 [45.1–156.8]</td>
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<tr>
<td>Albumin (g/dl)</td>
<td>3.9 [3.0–4.7]</td>
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<td>Co-administration drugs</td>
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<tr>
<td>Lansoprazole</td>
<td>15 (14)</td>
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<tr>
<td>Other PPIs (Esomeprazole/Rabeprazole/Omeprazole)</td>
<td>6 (6)/4 (4)/1 (1)</td>
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<tr>
<td>NSAIDs</td>
<td>23 (21)</td>
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<tr>
<td>Amlodipine</td>
<td>15 (14)</td>
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<tr>
<td>Hematological toxicity</td>
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<tr>
<td>Leukopenia</td>
<td>22 (20)</td>
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<tr>
<td>Thrombocytopenia</td>
<td>9 (8)</td>
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<tr>
<td>Anemia</td>
<td>20 (19)</td>
</tr>
</tbody>
</table>

Values are presented as median [range] or number (%). CrCl: creatinine clearance, Hb: hemoglobin, NSAIDs: non-steroidal anti-inflammatory drugs, PLT: platelet, PPI: proton pump inhibitor, WBC: white blood cell, CrCl was estimated by the Cockcroft-Gault equation.
Table 2. Multivariate analysis of the development of hematological toxicity after pemetrexed and carboplatin administration

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Lansoprazole</td>
<td>10.004</td>
<td>2.033–49.234</td>
<td>0.005</td>
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<tr>
<td>Other PPIs</td>
<td>1.959</td>
<td>0.310–12.382</td>
<td>0.475</td>
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<tr>
<td>Age</td>
<td>1.056</td>
<td>0.951–1.173</td>
<td>0.305</td>
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<tr>
<td>Pemetrexed dose (mg/m²)</td>
<td>1.049</td>
<td>0.993–1.107</td>
<td>0.087</td>
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<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>
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<tr>
<td>WBC (×10⁹/l)</td>
<td>0.796</td>
<td>0.598–1.060</td>
<td>0.118</td>
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<tr>
<td>PLT (×10⁹/l)</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>

CI: confidential interval, CrCl: creatinine clearance, Hb: hemoglobin, NSAIDs: non-steroidal anti-inflammatory drugs, PLT: Platelet, PPI: proton pump inhibitor, WBC: white blood cell
Figure 2

$h\text{OAT3}-\text{mediated uptake of Pemetrexed (pmol/mg protein/min)}$

$K_m = 68.3 \pm 11.1 \mu M$

$V_{\text{max}} = 157 \pm 9 \text{ (pmol/mg protein/min)}$
Figure 3

Uptake of Pemetrexed (% of Control)

\[ IC_{50} = 1.97 \pm 1.31 \, \mu\text{M} \]

Probenecid concentration (\(\mu\text{M}\))
Figure 4

(A) Uptake of Pemetrexed (% of Control) vs. Lansoprazole concentration (μM)

IC$_{50}$ = 0.57 ± 0.17 μM

(B) Uptake of Pemetrexed (% of Control) vs. Rabeprazole concentration (μM)

IC$_{50}$ = 5.42 ± 1.10 μM

(C) Uptake of Pemetrexed (% of Control) vs. Pantoprazole concentration (μM)

IC$_{50}$ = 6.86 ± 1.52 μM

(D) Uptake of Pemetrexed (% of Control) vs. Esomeprazole concentration (μM)

IC$_{50}$ = 12.78 ± 2.57 μM

(E) Uptake of Pemetrexed (% of Control) vs. Omeprazole concentration (μM)

IC$_{50}$ = 17.77 ± 5.19 μM

(F) Uptake of Pemetrexed (% of Control) vs. Vonoprazan concentration (μM)

IC$_{50}$ > 100 μM
Figure 5

![Graph showing the relationship between $1/N$ and Lansoprazole concentration (μM).](image-url)