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**Title page**

# Effects of proton pump inhibitors and famotidine on elimination of plasma methotrexate: evaluation of drug-drug interactions mediated by organic anion transporter 3

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Conflicts of interests

The authors declare that there are no conflicts of interest.

**Title**

Effects of proton pump inhibitors and famotidine on elimination of plasma methotrexate: evaluation of drug-drug interactions mediated by organic anion transporter 3


**Abstract**

Methotrexate (MTX) is an antifolate agent used in the treatment of numerous types of cancer, and eliminated by active tubular secretion via organic anion transporter 3 (OAT3). Gastric antisecretory drugs, such as proton pump inhibitors (PPIs) and histamine $H_2$ receptor antagonists, are widely used among patients with cancer in clinical practice. The aim of the present study was to analyze the potential drug-drug interactions between MTX and gastric antisecretory drugs in high-dose MTX (HD-MTX) therapy. We retrospectively analyzed the impact of PPIs on the plasma MTX concentration on 73 cycles of HD-MTX therapy performed in 43 patients. We also investigated the involvement of OAT3 in PPI-MTX drug interaction in an *in vitro* study using human OAT3 expressing HEK293 cells. In a retrospective study, patients who received a PPI had significantly higher MTX levels at 48 h (0.38 vs. 0.15 μmol $l^{-1}$, respectively, $P = 0.000018$) and 72 h (0.13 vs. 0.05 μmol $l^{-1}$, respectively, $P = 0.0002$) compared to patients who did not receive a PPI (but received a famotidine). Moreover, *in vitro* experiments demonstrated that PPIs (esomeprazole, lansoprazole, omeprazole, and rabeprazole) inhibited hOAT3-mediated uptake of MTX in a concentration-dependent manner ($IC_{50}$ values of 0.40–5.5 μM), with a rank order of lansoprazole > esomeprazole > rabeprazole > omeprazole. In contrast to PPIs, famotidine

showed little inhibitory effect on hOAT3-mediated MTX uptake. These results demonstrated that co-administration of PPI, but not famotidine, could result in a pharmacokinetic interaction that increases the plasma MTX levels, at least in part, via hOAT3 inhibition.

Keywords: organic anion transporter 3, methotrexate, proton pump inhibitor, famotidine, lansoprazole

## Introduction

Methotrexate (MTX) is an antifolate drug that inhibits dihydrofolate reductase. High-dose MTX (HD-MTX) is widely used in the treatment regimens for various malignancies, including acute lymphoblastic leukemia, lymphoma, and osteosarcoma (Stoller et al., 1977; Abrey et al., 1998). The monitoring of drug concentrations is essential to prevent toxicity from high plasma MTX levels, because delayed elimination can result in serious toxicities, such as myelosuppression, mucositis, and renal impairment (Goh et al., 1979; Widemann and Adamson, 2006). Numerous factors are known to influence MTX elimination, including urinary pH, renal function, and concomitant administration of drugs known to interact with MTX elimination (e.g., nonsteroidal anti-inflammatory drugs (NSAIDs), penicillins, and probenecid) (Aherne et al., 1978; Sand and Jacobsen, 1981; Jolivet et al., 1983; Zarychanski et al., 2006; Levêque et al., 2011).

Many cancer patients have been treated with antisecretory drugs [e.g., proton pump inhibitors (PPIs) and histamine $H_2$ receptor antagonists ($H_2$RAs)] as supportive care for symptoms related to either their cancer and its treatment or a common primary diagnosis of gastroesophageal reflux disease. Antisecretory drugs have relatively few known pharmacokinetic drug-drug interactions. In recent years, several reports have shown an association between PPI co-administration and delayed elimination of MTX (Joerger et al., 2006; Suzuki et al., 2009; Santucci et al., 2010; Bezabeh et al., 2012). Moreover, two case reports described no evidence of delayed MTX elimination when a $H_2$RA, such as ranitidine, was substituted for a PPI (Beorlegui

et al., 2000; Bauters et al., 2008). However, the mechanism of this interaction is not clear, and no evidence that suggests a reduced risk for patients treated with $H_2$RAs in conjunction with HD-MTX.

MTX is predominantly excreted renally, and cytochrome P450 (CYP) enzymes are not involved in MTX metabolism. Although several transporters have been previously reported to be involved in the renal elimination of MTX, organic anion transporter 3 (OAT3) has been shown to be a high-affinity transporter for MTX uptake into renal proximal tubule cells (Takeda et al., 2002; Chen et al., 2002; Uwai et al., 2004; Breedveld et al., 2004). Human OAT3 is localized to the basolateral membrane of proximal tubular epithelial cells and plays an important role in renal drug elimination from the blood to proximal tubules (Cha et al., 2001; Rizwan and Burckhardt, 2007).

Several drugs, such as ketoprofen and probenecid, are known to inhibit the elimination of MTX (Aherne et al., 1978; Thyss et al., 1986). The mechanism underlying these interactions partially relies on the inhibition of the renal elimination of MTX via OAT3 (Takeda et al., 2002; Nozaki et al., 2007). A recent study reported that PPI, such as lansoprazole, inhibited hOAT3-mediated transport of pemetrexed, which has a chemical structure similar to MTX, in a competitive manner (Ikemura et al., 2016). However, there is little information regarding the association between the inhibitory effect of antisecretory drugs on OAT3-mediated MTX transport and delayed elimination of MTX.

In the present study, the impact of antisecretory drugs on plasma MTX elimination was

retrospectively analyzed in hospitalized cancer patients who received HD-MTX therapy. We also assessed the inhibitory effects of four PPIs (esomeprazole, lansoprazole, omeprazole, and rabeprazole) or famotidine on OAT3-mediated transport of MTX in hOAT3-expressing cultured cells.

## Materials and Methods

### Patients and data collection

A retrospective study was conducted in hospitalized patients who received treatment with HD-MTX at Hokkaido University Hospital (Sapporo, Japan) between April 2010 and March 2014. The study included 73 cycles of HD-MTX therapy involving 43 patients. All the patients received intravenous fluids for hydration and alkalization of urine using sodium bicarbonate to maintain urine pH >7 during treatment. Patients who met any of the following criteria were excluded from the study: (1) below 18 years of age; (2) used NSAIDs during HD-MTX treatment; (3) had no monitored plasma MTX concentration at 48 h and/or 72 h. Patient information and data were extracted from electronic medical records kept in a central database in our hospital. The present study was approved by the Institutional Review Board of the Hokkaido University Hospital and also was conducted in accordance with the Declaration of Helsinki.

Electronic medical records of patients were followed until plasma MTX levels fell below $\leq 0.1$ μmol $l^{-1}$. Baseline characteristics (demographic, clinical, and laboratory data) were collected for all patients. In addition to the use of gastric antisecretory drugs (i.e., those used in our hospital: esomeprazole, lansoprazole, omeprazole, rabeprazole, and famotidine), the use of drugs known to interact with MTX was also noted, including NSAIDs. Plasma MTX levels were determined 48 and 72 h after the start of HD-MTX therapy, and delayed elimination of MTX was defined by plasma concentration over 1.0 μmol $l^{-1}$ at 48 h and/or 0.1 μmol $l^{-1}$ at 72 h. Plasma MTX concentrations was collected until MTX levels were $\leq 0.1$ μmol $l^{-1}$.

Chemicals

[$^3$H]Methotrexate ([$^3$H]MTX; 250 mCi; 9.25 MBq; 32.3 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). 6-Carboxyfluorescein hydrate (6-CF) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Lansoprazole (2-([[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methane]sulfinyl)-1H-1,3-benzodiazole), omeprazole (6-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methane]sulfinyl]-1H-1,3-benzodiazole), and famotidine (3-(2-Guanidinothiazol-4-ylmethylthio)-$N^1$-sulfamoylpropionamide) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Esomeprazole ((*S*)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-3H-benzoimidazole) and rabeprazole (2-([4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl)-1H-benzo[d]imidazole) were purchased from Sigma (St. Louis, MO, USA). All other reagents were of the highest grade available and used without further purification. Lansoprazole, omeprazole, esomeprazole, rabeprazole, and famotidine were dissolved in dimethylsulfoxide organic solvent (DMSO). The concentration of DMSO in the final study medium was limited to 1% in presence or absence of inhibitors.

Cell culture and transfection

HEK293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and incubated at 37°C in 5% $CO_2$. Human OAT3 cloned into the

pcDNA3.1(+) was kindly provided by Prof. Dr. M. Sugawara (Hokkaido University, Sapporo, Japan). HEK293 cells were transfected with hOAT3 using Lipofectamin 2000 reagent (Invitrogen). Stable cell lines were created by culturing transfectants in 500 μg/ml G418 (Invitrogen, Carlsbad, CA) for 3 weeks. A stable transformant transfected with pcDNA3.1(+) (no insert) was used as a control. Expression of hOAT3 in the G418 resistant clones was determined by RT-PCR and functional analyses (Supplemental Figure 1).

Uptake study in hOAT3-expressing cells

hOAT3- and vector-transfected cells were seeded at a cell density of $2.0 \times 10^5$ cells/well on collagen-coated 24-well plastic plates. Two days after the cells had been seeded, the accumulation of [$^3$H]MTX or 6-CF in the cells was examined. After removal of the culture medium, each well was washed and pre-incubated with the incubation buffer Hank's balanced salt saline (HBSS - HEPES (pH 7.4) buffer (25 mM D-glucose, 137 mM NaCl, 5.37 mM KCl, 0.3 mM $Na_2HPO_4$, 0.44 mM $KH_2PO_4$, 1.26 mM $CaCl_2$, 0.8 mM $MgSO_4$, 4.17 mM $NaHCO_3$, and 10 mM HEPES; pH 7.4 adjusted with 1 M Tris). Then, 0.5 ml of incubation buffer containing a substrate in the absence or presence of various inhibitors was added. The monolayers were incubated for the indicated time at 37°C. Each cell monolayer was rapidly washed twice with 0.5 ml ice-cold incubation buffer at the end of the incubation period. To quantify the radioactivity of [$^3$H]MTX, the cells were solubilized in 1% SDS/0.2 N NaOH. The remainder of the sample was mixed with 3 ml of scintillation cocktail (Perkin Elmer, Waltham, MA) to measure the radioactivity. To quantify the 6-CF accumulation, the cells were dissolved in 0.5 ml 0.5 N NaOH

and the 6-CF accumulation was determined in a fluorescence spectrophotometer (Hitachi, Tokyo, Japan) at 490/520 nm (excitation/emission). The protein concentration was determined using a Pierce® BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA), in accordance with the manufacturer's instructions. All uptake values were corrected for protein content.

Statistical analysis

Statistical assessment of patient data was analyzed using IBM SPSS Statistics software, version 23, (SPSS Inc., Chicago, IL, USA). The differences in baseline clinical characteristics were compared between patients receiving PPI and not receiving PPI by the Fisher's exact probability test or Mann–Whitney *U*-test. Differences in plasma MTX levels between the two groups were assessed using the Mann-Whitney *U*- test.

Data of *in vitro* experiments are expressed as the mean ± standard deviation (SD). To determine the $IC_{50}$ value of each inhibitor for the inhibition of MTX uptake, the data points were analyzed using nonlinear regression analysis fitting a 4-parameter logistic equation using SigmaPlot 12.5 (Systat Software, Inc.; San Jose, CA, USA).

## Results

### Patient characteristics

One hundred and six cycles of HD-MTX were initially identified. However, 33 cycles were excluded for the following reasons: 1 was younger than 18, 13 involved co-administration of NSAIDs, and 19 had incomplete medical data. Thus, 43 patients receiving 73 cycles of HD-MTX were included in this analysis. Retrospectively, the 73 cycles of HD-MTX were categorized into two groups: with PPI (n = 26; esomeprazole (n = 7), lansoprazole (n = 3), omeprazole (n = 4), and rabeprazole (n = 12)) and without PPI (n = 47). The baseline patient characteristics are shown in Table 1. Baseline characteristics were well balanced among the 2 groups, except for sex, MTX dose, and MTX infusion time. Male sex and long infusion time were significantly more frequent in the group receiving PPI than in the group not receiving PPI. All patients in the group not receiving PPI were co-administered famotidine, a commonly used $H_2$RA.

### Comparison of the plasma MTX levels

Patients who received a PPI had significantly higher MTX levels at 48 h (0.38 vs. 0.15 μmol $l^{-1}$, respectively, $P = 0.000018$) and 72 h (0.13 vs. 0.05 μmol $l^{-1}$, respectively, $P = 0.0002$) compared with patients who received famotidine instead of a PPI (Figure 1a-b). Moreover, delayed elimination of MTX was significantly more frequent in the PPI group than in the group not receiving PPIs (53.8% vs. 10.6%, $P = 0.00015$) (data not shown).

Inhibition of hOAT3-mediated MTX uptake by antisecretory drugs

To test the inhibitory effects of PPIs on OAT3-mediated MTX uptake *in vitro*, we established a HEK293 cell line stably expressing the human OAT3. We validated the stable cell lines by examining the gene expression levels of hOAT3 and the function of HEK-hOAT3 cells. The RT-PCR analysis demonstrated hOAT3 mRNA expression in the HEK-hOAT3 cells, which was not detected in the vector-transfected HEK293 (mock) cells (Supplemental Figure 1a). The HEK-hOAT3 cells accumulated significantly more 6-CF, a fluorescent substrate of OAT3 (Rödiger et al., 2010), than the mock cells (Supplemental Figure 1b). These results indicate that the stable cell lines expressed functionally active OAT3. Each PPI tested significantly inhibited hOAT3-mediated 6-CF uptake in a concentration-dependent manner (Supplemental Figure 2a-d). On the other hand, the inhibitory effects of famotidine were relatively weak compared to the inhibition by PPIs (Supplemental Figure 2e).

We also examined the inhibitory effects of PPIs and famotidine on [$^3$H]MTX uptake into HEK-hOAT3 cells. Each PPI tested significantly inhibited hOAT3-mediated MTX uptake in a concentration-dependent manner with $IC_{50}$ values of 0.40–5.5 μM, and a rank order of lansoprazole > esomeprazole > rabeprazole > omeprazole (Figure 2a-d). In contrast, famotidine yielded little inhibition on hOAT3-mediated uptake of [$^3$H]MTX, as well as 6-CF, in the concentration range tested (Figure 2e).

## Discussion

A number of retrospective studies have suggested an association between co-administration of PPIs and delayed elimination of MTX, although the mechanism for this interaction is not well understood (Bezabeh et al., 2012). The present study was conducted to provide important information for safe and appropriate chemotherapy with HD-MTX during co-administration with gastric antisecretory drugs. To achieve this goal we retrospectively analyzed the impact of PPIs on plasma MTX elimination, and examined the drug interaction between MTX and antisecretory drugs, including PPIs, using a cell system that stably expresses the human OAT3.

In a retrospective study, the 73 cycles of HD-MTX were categorized into two groups, those which received PPIs and those which did not receive PPIs, and a statistically significant difference was found in the plasma MTX levels between the groups (Table 1, Figure 1). Although patients who received a PPI had significantly higher MTX levels at each measured time point, patients who received a PPI were also more likely to have a long MTX infusion time compared to patients who did not receive a PPI. This finding is consistent with a previous report that showed patients receiving PPIs had significantly longer infusion times along with higher MTX levels (Reeves et al., 2014). Additionally, there were significantly more males in the group receiving PPI than in the that group did not receive PPI. Wiczer et al. reported that male sex may predispose patients to nephrotoxicity associated with HD-MTX clearance (Wiczer et al., 2016). Therefore, a clinically significant increase in plasma MTX levels is also likely present in those with additional

risk factors for delayed elimination. In the group not receiving PPIs, famotidine was co-administered in all patients. Our finding that patients who concomitantly received famotidine (and did not receive a PPI) had lower MTX levels supports previous case reports that no delayed MTX elimination was found when a $H_2$RA was substituted for a PPI (Beorlegui et al., 2000; Bauters et al., 2008). Therefore, substitution of $H_2$RA in place of PPI, or stopping PPI a few days before HD-MTX therapy, would seem prudent until further data have been obtained. However, similar to the other studies describing a possible interaction between MTX and PPIs, the present study is limited by its retrospective nature, and was analyzed per cycle of treatment with HD-MTX, but not per patient (Suzuki et al., 2009; Santucci et al., 2010; Bezabeh et al., 2012). Future studies should include a large-scale prospective study to adjust for the number of HD-MTX cycles per patient and other potential confounders, such as sex and MTX infusion time.

Multiple renal transporters are involved in MTX pharmacokinetics. In the apical membrane of proximal tubular epithelial cells, breast cancer resistance protein (BCRP) and multidrug resistance-associated proteins (MRPs) are considered to be responsible for the active secretion of MTX into the urine. Breedveld et al. reported that pantoprazole inhibited MTX transport via inhibition of BCRP but not MRP2 (Breedveld et al., 2007). Another study has shown that four PPIs (omeprazole, lansoprazole, rabeprazole, and pantoprazole) inhibited BCRP-mediated transport of MTX (Suzuki et al., 2009). The reported $IC_{50}$ values of these PPIs were much higher than unbound plasma levels of PPIs in the therapeutic range. Therefore, the drug-drug interaction between MTX and PPIs is unlikely the result of the inhibitory effect of PPIs

on renal apical membrane-mediated MTX transport. Different OATs (OAT1 and OAT3) localize to the basolateral membranes of the renal proximal tubule and have been shown to transport MTX (Hosoyamada et al., 1999; Cha et al., 2001; Uwai et al., 2004). The affinity of MTX for hOAT3 ($K_m$: 17 μM) obtained from previously published data was much higher than that for hOAT1 ($K_m$: 724 μM) (Uwai et al., 2004). Nozaki et al. demonstrated that MTX uptake by human kidney slices was saturable with $K_m$ values of 44.6 ± 23.4 μM. Considering that the $K_m$ value determined in that study is similar to the value for hOAT3, it is likely that hOAT3 contributes more significantly to renal MTX clearance and affects the pharmacokinetics of MTX (Nozaki et al., 2007). Chioukh et al. reported that three PPIs (omeprazole, lansoprazole, and pantoprazole) can significantly inhibit hOAT3-mediated uptake of MTX (Chioukh et al., 2014). However, little is known about the effect of famotidine and other PPIs on hOAT3-mediated MTX transport. Based on these findings, we hypothesized that the underlying mechanisms whereby PPIs, but not famotidine, may affect elimination of plasma MTX is partially dependent on the inhibition of the renal elimination via hOAT3. We first examined the inhibitory effects of these drugs on hOAT3-mediated uptake of 6-CF; among the PPIs tested, lansoprazole exhibited the most potent inhibitory effect (Supplemental Figure 2). Additionally, each PPI tested also inhibited hOAT3-mediated uptake of MTX in a concentration-dependent manner (Figure 2). The observed $IC_{50}$ values of each PPI for the inhibition of MTX uptake were in the micromolar range (1.2, 0.40, 5.5, and 4.8 μM for esomeprazole, lansoprazole, omeprazole, and rabeprazole, respectively). The $IC_{50}$ value of lansoprazole for the inhibition of hOAT3-mediated MTX uptake

is nearly consistent with that of the previous reports (Chioukh et al., 2014). In contrast to PPIs, famotidine showed little inhibitory effect on hOAT3-mediated uptake of neither MTX nor 6-CF. Although famotidine is a known substrate of hOAT3 ($K_m$: 124 μM), the uptake of E3S, a substrate of hOAT3, is not inhibited by famotidine at 10 μM (Motohashi et al., 2004; Tahara et al., 2005). After a single oral dose of 40 mg, the maximum plasma concentration for famotidine is much less than 1 μM (Lin et al., 1987). These results suggest that a relevant interaction of hOAT3 with famotidine under a clinically used concentration is unlikely. According to the recommendation of the International Transporter Consortium (Giacomini et al., 2010), if the ratio of unbound $C_{max}$ to $IC_{50}$ value ($C_{max,u}$ / $IC_{50}$) is greater than or equal to 0.1 then a clinical drug-drug interaction study should be performed. As shown in Supplemental Table 1 (Yasuda et al., 1994; Lind et al., 2000; Andersson et al., 2001; Freston et al., 2003; Tahara et al., 2005; McCallum et al., 2014 ), the ratio of unbound $C_{max}$ to $IC_{50}$ value of lansoprazole was 0.33 whereas those of the other PPIs were lower than 0.1. These findings suggest that co-administration of PPIs, especially lansoprazole, and MTX could lead to a clinical drug interaction.

To the best of our knowledge, there are no reports correlating the inhibitory effect of antisecretory drugs on hOAT3-mediated MTX transport with variations in plasma disposition of MTX in humans. To confirm the significant effects of PPI, the incidence of delayed plasma MTX elimination was compared between HD-MTX therapy with (n = 26) and without (n = 47) PPI co-administration. Delayed elimination of MTX was significantly more frequent in the PPI group

than in the group not receiving PPIs (53.8% vs. 10.6%). The frequency of delayed MTX elimination in patients administered esomeprazole, lansoprazole, omeprazole, or rabeprazole was 71.4% (5/7), 100% (3/3), 25.0% (1/4), and 41.7% (5/12), respectively. Additionally, all patients in the group not receiving PPI were co-administered famotidine. Considering the rank order of inhibitory potency of gastric antisecretory drugs for hOAT3-mediated MTX uptake, the differential inhibitory effects of these drugs on hOAT3-mediated MTX transport *in vitro* may explain the frequency of delayed MTX excretion. Interestingly, our retrospective data revealed that delayed elimination of MTX was observed in every patient receiving lansoprazole. Lansoprazole exhibited the most potent inhibitory effect against hOAT3-mediated MTX uptake *in vitro*, and this interaction may be, at least in part, a contributing factor to delay the elimination of MTX. However, delayed elimination was similarly observed in patients receiving the other PPI or famotidine, and other factors are also likely to contribute to the delayed elimination of MTX. PPIs are metabolized by multiple CYP enzymes, including CYP2C19. Approximately 20% of the Japanese population has been identified as poor metabolizers of CYP2C19 (Kubota et al., 1996). A previous report described the $C_{max}$ of esomeprazole in CYP2C19-poor (7.8 μM) and -extensive (5.4 μM) metabolizers, after a single oral dose of 40 mg (Andersson et al., 2001). In poor metabolizers of CYP2C19, the ratio of unbound $C_{max}$ to esomeprazole $IC_{50}$ values, especially at the 40-mg dose, was greater than 0.1. Therefore, CYP2C19 metabolizer status, which provides higher plasma levels of PPIs, may significantly influence the magnitude of the PPI-MTX interaction. Another genetic risk factor for delayed MTX elimination may involve

polymorphisms in the organic anion-transporting polypeptide 1B1 (OATP1B1, encoded by the SLCO1B1 gene). OATP1B1 is human hepatic uptake transporter localized on the sinusoidal membrane of hepatocytes, where it mediates the uptake of a wide variety of drugs from blood into liver (Abe et al., 1999; König et al., 2000). OATP1B1 is able to transport MTX *in vitro*, and mediates the uptake of MTX into the liver (Abe et al., 2001; van de Steeg et al., 2009). Several reports have shown that single-nucleotide polymorphisms (SNPs) in SLCO1B1 associated with decreased transport activity were linked to decreased MTX clearance in patients treated with HD-MTX, suggesting the importance of hepatic uptake pathways in MTX pharmacokinetics (Tirona et al., 2001; Treviño et al., 2009; Lopez-Lopez et al., 2011). Therefore, the difference in genetic background, particularly involving CYP2C19 and OATP1B1 activity, may partially explain the discrepancy in the association between co-administration of PPIs and delayed MTX excretion. Additionally, it may be possible that OATP1B1 is involved in the PPI-MTX drug interaction, although the inhibitory effect of PPIs on OATP1B1-mediated MTX transport remain to be elucidated.

OAT1 can also transport MTX but with much lower affinity than OAT3, as mentioned above. Zarychanski et al. reported that the peak serum levels of MTX were higher than 100 μM following high-dose infusional therapy (Zarychanski et al., 2006). Our preliminary data show slight inhibitory effects of PPIs on the uptake of 6-CF via hOAT1 (data not shown). These findings imply that inhibitory effects of PPIs on both hOAT3 and hOAT1 may also be attributable to PPI-MTX drug interaction. However, the contribution of their transporters to this

interaction remains unclear and requires further investigation. Other transporters have been identified in the kidney. OATP4C1 is also localized at the basolateral membranes of proximal tubule cells, and has been shown to transport MTX (Mikkaichi et al., 2004). A recent study found that lansoprazole (100 μM) and rabeprazole (100 μM) caused no inhibition of OATP4C1-mediated transport of triiodothyronine (Sato et al., 2017). These findings suggest that OATP4C1 is not responsible for the PPI-MTX interaction. Studies measuring the contribution of other transporters to PPI-MTX drug interaction, as well as the impact of genetic variants on drug metabolism and transport, are required to clarify the clinical significance of PPI-MTX drug interaction.

## Conclusion

We found that patients who received a PPI had significantly higher MTX levels following HD-MTX treatment than patients who received famotidine. Additionally, our *in vitro* data indicate that PPIs, but not famotidine, inhibit hOAT3-mediated MTX transport under clinical concentrations, which likely explains, at least in part, the drug-drug interactions observed between MTX and PPIs.

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**Figure legends**

**Figure 1: Comparison of plasma MTX levels at 48 h (a) and 72 h (b) after the start of HD-MTX therapy between the group receiving PPI and the group not receiving PPI.** Graphical analysis was done using an SPSS box and whiskers plot. The box spans data between two quartiles (IQR), with the median represented as a bold horizontal line. The upper and lower lines outside the boxes represent minimum and maximum values that are not outliers. Open circles are outliers (1.5 to 3 box lengths from the upper line of the box) and stars are extremes (>3 box lengths from the upper line of the box).

**Figure 2: Concentration-dependent inhibition of hOAT3-mediated [$^3$H]MTX uptake by gastric antisecretory drugs.** The uptake of [$^3$H]MTX (20 nM) by hOAT3-expressing HEK293 cells and mock cells was performed at 37°C, pH 7.4 for 5 min in the absence or presence of various concentrations of esomeprazole (a), lansoprazole (b), omeprazole (c), rabeprazole (d), and famotidine (e). The specific uptake of MTX by hOAT3 was estimated by subtracting its uptake by mock cells from that by hOAT3-expressing HEK293 cells. Each point represents the mean ± SD (n = 3-4). The data are presented as the uptake values relative to the uptake in the absence of inhibitor (%).

**Supplemental Figure 1: Characterization of newly established hOAT3-expressing HEK293**

**cells.** (a) Expression of hOAT3 was assessed by RT-PCR. Total RNA was prepared from HEK293 cells using an ISOGEN (Nippon Gene, Tokyo). RNA was reverse-transcribed using ReverTra Ace (TOYOBO). PCR was performed using the specific primers for human OAT3 (forward: 5'-TGT CCA TTC CCT TCT TCG TC-3' and reverse: 5'- GCT GAG CCT TTC TCC CTC TT-3') and human GAPDH (forward: 5'-AAG GTC ATC CCT GAG CTG AA-3' and reverse: 5'-TTC TAG ACG GCA GGT CAG GT-3'). The primers specific to hOAT3 and hGAPDH were designed on the basis of sequences in the GenBank™ database (accession no.: NM_004254 and NM_002046, respectively). (b) Uptake of 6-CF by hOAT3-expressing HEK293 cells. hOAT3-expressing HEK293 cells and mock cells were exposed to 5 μM 6-CF at pH 7.4 for 3 min. Each column represents the mean with SD (n = 4).

**Supplemental Figure 2: Gastric antisecretory drugs inhibit hOAT3-mediated 6-CF uptake in a concentration-dependent manner.** The uptake of 6-CF (5 μM) by hOAT3-expressing HEK293 cells and mock cells was performed at 37°C, pH 7.4 for 3 min in the absence or presence of various concentrations of esomeprazole (a), lansoprazole (b), omeprazole (c), rabeprazole (d), and famotidine (e). The specific uptake of 6-CF by hOAT3 was estimated by subtracting its uptake by mock cells from that by hOAT3-expressing HEK293 cells. Each point represents the mean ± SD (n = 4). The data are presented as the uptake values relative to the uptake in the absence of inhibitor (%).

## Tables

### Table 1

**Baseline characteristics of the group receiving PPI and the group not receiving PPI**

| | without PPI (n = 47) | | with PPI (n = 26) | | *P*-value |
|---|---|---|---|---|---|
| Sex, male (n) | 17 | (36.2) | 18 | (69.2) | 0.008 |
| Age (years) | 68 | (18-76) | 52 | (18-72) | 0.056 |
| Body surface area ($m^2$) | 1.51 | (1.32-2.07) | 1.67 | (1.40-1.91) | 0.062 |
| Laboratory data | | | | | |
| Serum creatinine (mg $dl^{-1}$) | 0.67 | (0.40-1.10) | 0.71 | (0.30-1.09) | 0.252 |
| Aspartate aminotransferase (units $l^{-1}$) | 19 | (8-53) | 19 | (8-68) | 0.940 |
| Alanine aminotransferase (units $l^{-1}$) | 25 | (7-120) | 27 | (6-102) | 0.649 |
| MTX dose (mg $m^{-2}$) | 3481 | (992-3590) | 2057 | (495-3563) | 0.001 |
| Long infusion time, 24 h (n) | 7 | (14.9) | 15 | (57.7) | < 0.001 |

Data are presented as number (%) or median (range). n, number of cycles.

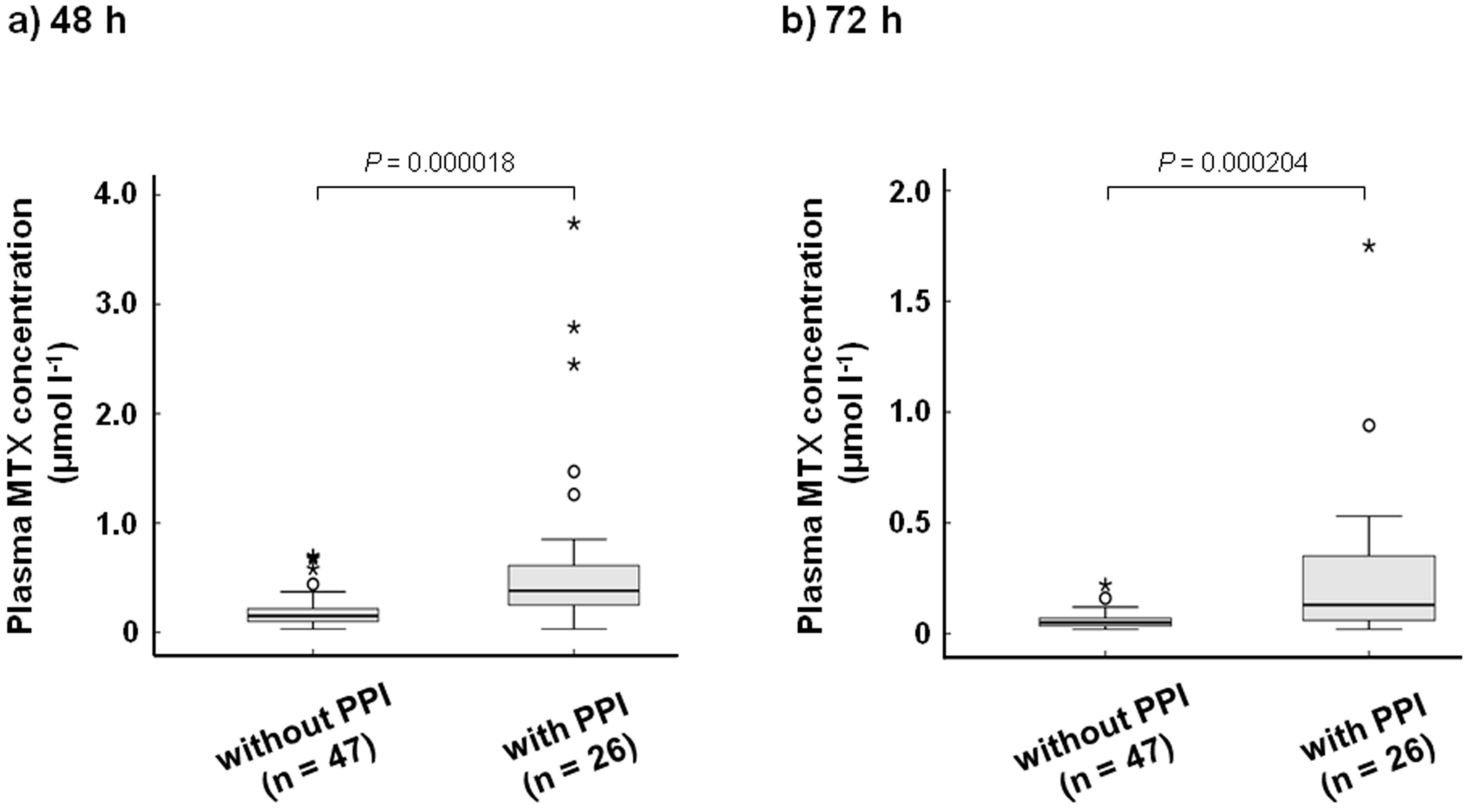
**Supplemental Table 1**

**Comparison of unbound $C_{max}$ / $IC_{50}$ ratio for PPIs as inhibitors of hOAT3**

| PPI | Dose (mg) | $C_{max}$ (μmol $l^{-1}$) | $f_u$ (%) | $C_{max,u}$ (μmol $l^{-1}$) | References | $IC_{50}$ on OAT3 inhibition (μmol $l^{-1}$) | $C_{max,u}$ / $IC_{50}$ |
|---|---|---|---|---|---|---|---|
| **Esomeprazole** | 20 | 2.4 | 3.0 | 0.07 | Lind et al., 2000; Andersson et al., 2001 | 1.2 | 0.058 |
| **Lansoprazole** | 30 | 2.9 | 4.5 | 0.13 | Freston et al., 2003; McCallum et al., 2014 | 0.40 | 0.33 |
| **Omeprazole** | 20 | 1.4 | 5.0 | 0.07 | Lind et al., 2000; McCallum et al., 2014 | 5.5 | 0.013 |
| **Rabeprazole** | 20 | 1.2 | 3.7 | 0.04 | Yasuda et al., 1994 | 4.8 | 0.0083 |

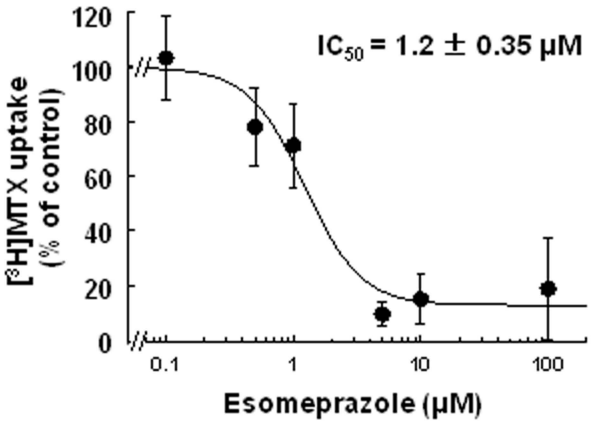
$C_{max}$, maximum plasma concentration; $f_u$, unbound plasma fraction; $C_{max,u}$, $C_{max}$ of unbound drug; $IC_{50}$, experimentally determined concentration for half-maximal inhibition of hOAT3-mediated MTX uptake. $C_{max}$ values and the unbound fraction were obtained from the literature.

**Figure 1**

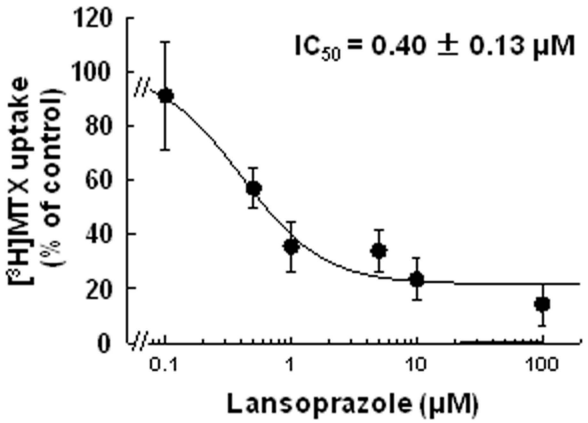

a) 48 h
P = 0.000018
Plasma MTX concentration (µmol l-1)
4.0
3.0
2.0
1.0
0
without PPI (n = 47)
with PPI (n = 26)
b) 72 h
P = 0.000204
Plasma MTX concentration (µmol l-1)
2.0
1.5
1.0
0.5
0
without PPI (n = 47)
with PPI (n = 26)

# Figure 2

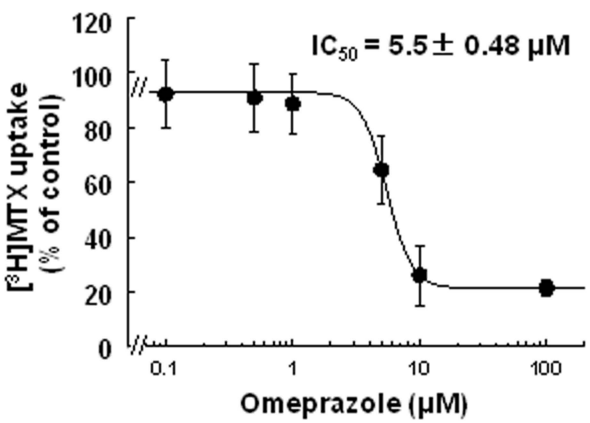
## a) Esomeprazole



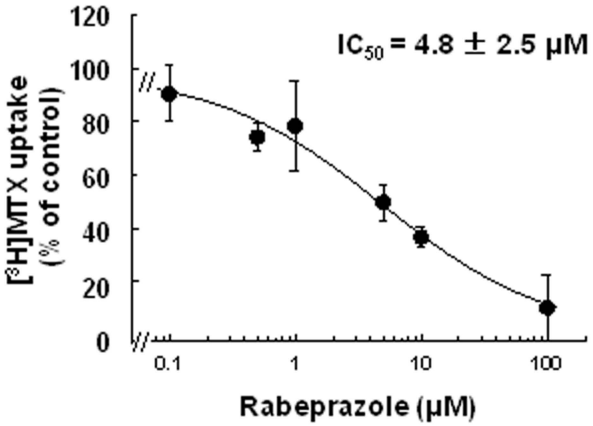

## b) Lansoprazole



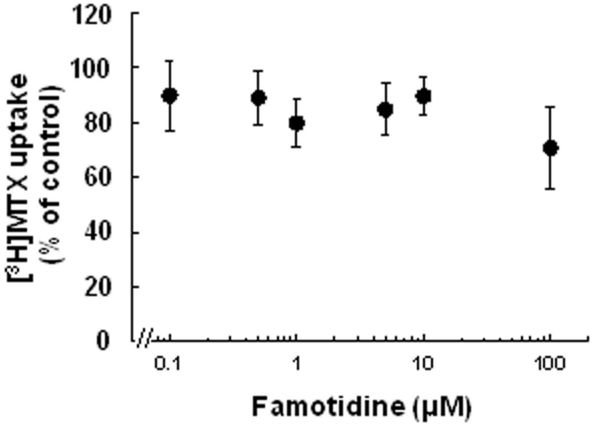

## c) Omeprazole




## d) Rabeprazole

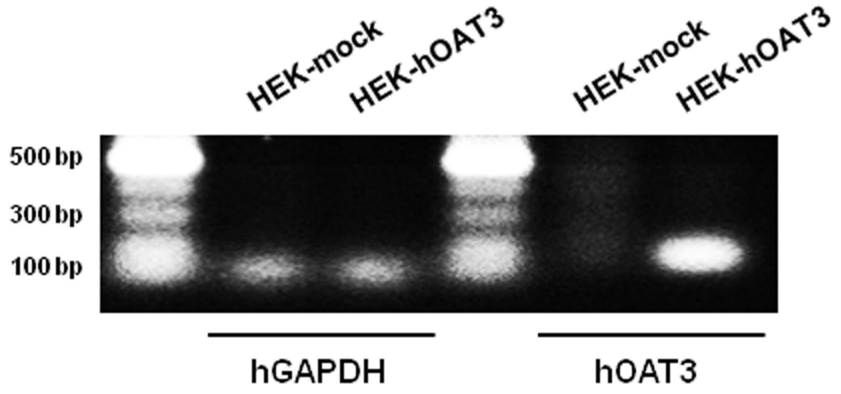



## e) Famotidine

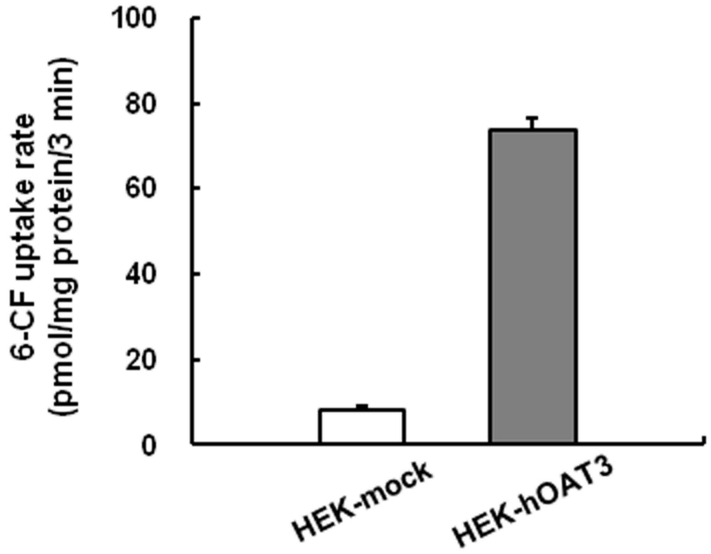


# Supplemental Figure 1

(a)

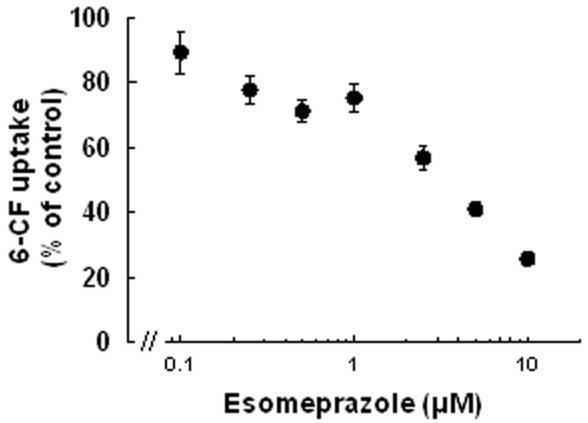



(b)

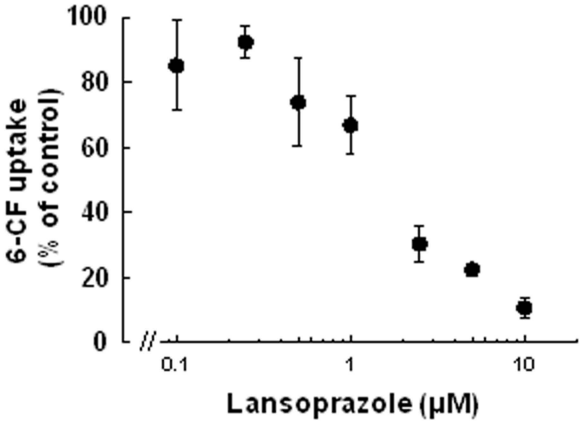


# Supplemental Figure 2

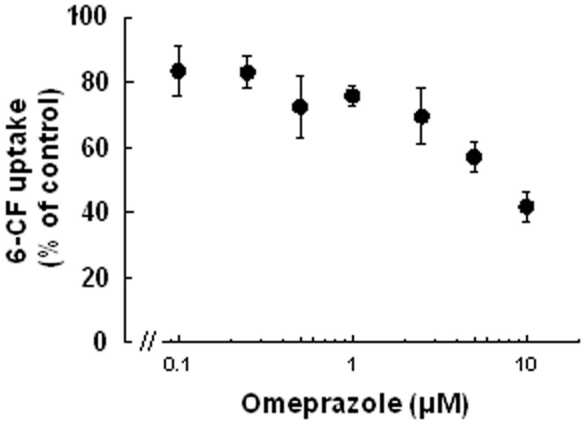
## a) Esomeprazole



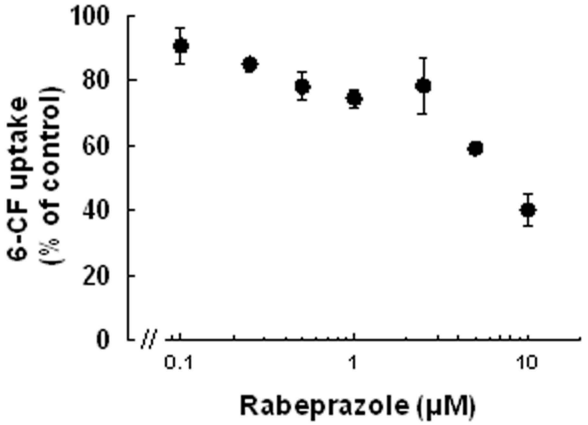

## b) Lansoprazole

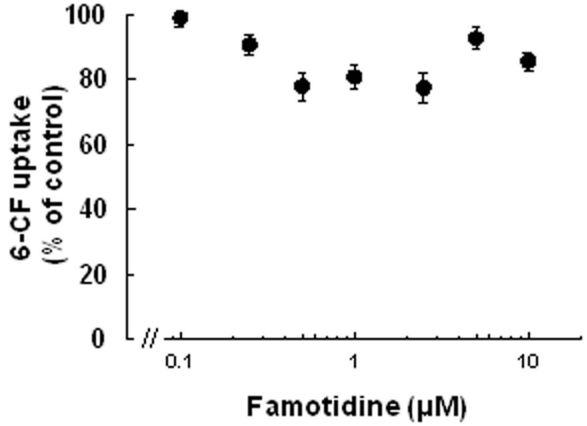



## c) Omeprazole




## d) Rabeprazole




## e) Famotidine