Co-administration of proton pump inhibitors delays elimination of plasma methotrexate in high-dose methotrexate therapy

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Co-administration of proton pump inhibitors (PPIs) increases plasma methotrexate (MTX) concentration in cancer patients receiving high-dose MTX (HDMTX) therapy.
- There is controversy as to whether or not co-administration of PPIs affects plasma MTX elimination in HDMTX therapy.
- Inhibitory activity of PPIs on breast cancer resistance protein (BCRP) is a possible mechanism for the drug interaction between MTX and PPIs.

WHAT THIS STUDY ADDS

- Co-administration of a PPI (omeprazole, lansoprazole, or rabeprazole) was more frequently observed in the delayed MTX elimination group than in the normal MTX elimination group.
- Multiple logistic regression analysis with adjustment for significant covariates revealed that PPI co-administration was a significant risk factor for delayed plasma MTX elimination.
- The half-maximal inhibitory concentration of each PPI in inhibiting BCRP function was much higher than the therapeutic unbound concentration in the plasma.

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AIM

To assess whether or not co-administration of proton pump inhibitors (PPIs) is a risk factor for delayed elimination of plasma methotrexate (MTX) in high-dose MTX (HDMTX) therapy for malignant diseases.

METHODS

To assess the effects of PPI co-administration on elimination of plasma MTX, we examined plasma MTX concentration data on 171 cycles of HDMTX therapy performed in 74 patients. We performed multiple logistic regression analysis to evaluate PPI co-administration as a risk factor. Inhibitory potencies of omeprazole, lansoprazole, rabeprazole and pantoprazole on MTX transport via breast cancer resistance protein (BCRP, ABCG2) were also investigated in an *in vitro* study using membrane vesicles expressing human BCRP.

RESULTS

We identified co-administration of PPIs as a risk factor for delayed elimination (odds ratio 2.65, 95% confidence interval 1.03, 6.82) as well as renal and liver dysfunction. All four PPIs inhibited BCRP-mediated transport of MTX, with half-maximal inhibitory concentrations of 5.5–17.6 μM – considerably higher than the unbound plasma concentrations of the PPIs.

CONCLUSIONS

Our results support previous findings suggesting that PPI co-administration is associated with delayed elimination of plasma MTX in patients with HDMTX therapy. This drug interaction, however, cannot be explained solely by the inhibitory effects of PPIs on BCRP-mediated MTX transport.

Introduction

High doses of methotrexate (MTX), an antifolate drug, are an accepted treatment for lymphoid malignancy, osteogenic sarcoma and acute leukaemia [1]. Therapeutic drug monitoring of MTX is essential to prevent toxicity from high plasma MTX concentrations [2], a problem that commonly requires rescue administration of calcium folinate. Large inter- and intraindividual variations in plasma MTX elimination are associated with co-administration of non-steroidal anti-inflammatory drugs (NSAIDs) [3, 4] and vancomycin [5], as well as renal dysfunction, third space fluid accumulations such as pleural effusion and ascites, and insufficient hydration [1]. A recent report has suggested that co-administration of proton pump inhibitors (PPIs), including omeprazole and lansoprazole, decreased MTX clearance, resulting in delayed plasma MTX elimination [6]. In contrast, one case report showed that omeprazole did not alter MTX clearance [7]. It is therefore controversial as to whether or not co-administration of PPIs affects elimination of plasma MTX in high-dose MTX (HDMTX) therapy. Because membrane transport of MTX is known to be mediated by breast cancer resistance protein (BCRP, ABCG2) and multidrug-resistance-related protein, the inhibitory effects of PPIs on these transporters may be involved in the mechanism of the PPI–MTX interaction [8].

We therefore used multiple logistic regression analysis to examine the impact of PPI co-administration on plasma MTX elimination in patients undergoing HDMTX therapy. We also assessed the inhibitory effects of four PPIs (omeprazole, lansoprazole, rabeprazole and pantoprazole) on BCRP-mediated transport of MTX in an *in vitro* study of membrane vesicles expressing human BCRP.

Methods

Patients

Plasma MTX data on 171 cycles of HDMTX therapy were evaluated in 74 patients (45 male and 29 female) treated at Tsukuba University Hospital (Ibaraki, Japan). Twenty-five HDMTX treatment protocols with a median MTX dose of 3500 mg m⁻² (range 1000–5000 mg m⁻²) for malignant lymphoma, leukaemia, chronic active Epstein–Barr virus infection or plasmacytoma were included. Median HDMTX infusion duration was 6 h (range 2.9–29.0 h). The patients were well hydrated to keep the urine pH > 7 during treatment. Intravenous or oral calcium folinate rescue was conducted when the plasma MTX concentration was high, according to each protocol guideline. Any suspected presence of pleural effusion and ascites was confirmed by radiography or computed tomography. Laboratory data such as serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum creatinine (SCr) and blood urea nitrogen levels for the assessment of liver and kidney function were determined at baseline. Cut-off values for

judging liver and kidney dysfunction were estimated in accordance with the corresponding normal laboratory data ranges used at our hospital.

The study was approved by the ethics committee of the University of Tsukuba. Informed consent was obtained from the patients.

Determination of MTX concentration for group categorization

Plasma MTX concentrations were determined 24, 48 and/or 72 h after the start of HDMTX therapy. A TDx Abbott analyser (Abbott Japan, Tokyo, Japan) with a detection limit of 0.02 µmol l⁻¹ was used for MTX assay. The 171 cycles of HDMTX therapy were categorized into delayed (*n* = 41) and normal (*n* = 130) elimination groups according to the following definitions of toxic MTX concentrations at each measurement time. Patients with plasma MTX concentrations ≥ 10 µmol l⁻¹ at 24 h after the start of HDMTX therapy, ≥ 1 µmol l⁻¹ at 48 h or ≥ 0.1 µmol l⁻¹ at 72 h were classed in the delayed elimination group [9, 10]. In those patients in whom the duration of infusion exceeded 24 h we did not use the plasma MTX concentration 24 h after the start of HDMTX in our assessment of delayed elimination.

Statistical analysis

Differences in patient characteristics and MTX dosing regimen were compared between the delayed and normal elimination groups by the χ^2 test, Fisher's exact probability test or Mann–Whitney *U*-test. Multiple logistic regression analysis was performed to identify possible risk factors for delayed elimination of plasma MTX. Six variables for which there were significant differences between the normal and delayed elimination groups (Table 1) were subjected to stepwise logistic analysis to establish the best predictive model. The odds ratio (OR) and its 95% confidence interval (CI) were calculated by StatView 5.0 (SAS Institute, Cary, NC, USA). A *P*-value < 0.05 was considered to be statistically significant.

Inhibitory effects of PPIs on BCRP

BCRP-expressing membrane vesicles, which express human BCRP on their membranes in an 'inside-out' manner, were purchased from SOLVO Biotechnology (Budapest, Hungary). ³H-MTX [3',5',7'-³H(N)] was purchased from Moravsek Biochemicals, Inc. (Brea, CA, USA). Uptake of ³H-MTX into the membrane vesicles was measured by a rapid filtration technique. The membrane vesicles were incubated for 2 min at 37°C in uptake buffer (pH 7.0) containing 100 µmol l⁻¹ MTX and PPI (5, 20, or 100 µmol l⁻¹). Adenosine triphosphate (ATP)-dependent uptake of MTX was determined by subtracting the uptake in the absence from that in the presence of ATP.

Results

We evaluated 311 measurements of plasma MTX in 171 cycles of HDMTX therapy. Plasma MTX concentrations in

Table 1

The comparison of patient characteristics and methotrexate dosing regimen between delayed and normal methotrexate elimination group

	Delayed elimination	Normal elimination	P-value
Number of cycles	41	130	
Gender (male/female) (n)	24/17	85/45	NS
Age (years)	57 (16–79)	58 (15–74)	NS
Weight (kg)	51.0 (39.0–83.4)	54.0 (36.5–82.5)	NS
Laboratory data*			
Serum creatinine >1.1 (mg dL ⁻¹)	8 (19.5)	7 (5.4)	<0.05
Blood urea nitrogen >20 (mg dL ⁻¹)	7 (17.1)	13 (10.0)	NS
Aspartate aminotransferase >40 (IU L ⁻¹)	13 (31.7)	11 (8.5)	<0.05
Alanine aminotransferase >40 (IU L ⁻¹)	17 (41.5)	33 (25.4)	<0.05
Methotrexate dose (mg m ⁻²)	2000 (600–5000)	1000 (500–5000)	NS
Infusion time (h)	7.3 (3.0–26.0)	6.0 (2.9–29.0)	<0.05
Number of calcium folinate rescue	24 (8–146)	12 (5–28)	<0.05
Plasma methotrexate concentrations (μmol L ⁻¹)			
24 h	11.50 (1.78–48.68)	1.10 (0.14–32.26)	<0.05
48 h	0.87 (0.08–13.38)	0.11 (0.01–0.82)	<0.05
72 h	0.23 (0.10–3.45)	0.05 (0.01–0.09)	<0.05
Pleural effusion and ascites (n)	12 (29.3)	14 (10.8)	<0.05
Co-administration drug (n)			
Proton pump inhibitors	13 (31.7)	18 (13.8)	<0.05
Nonsteroidal anti-inflammatory drugs	3 (7.3)	8 (6.2)	NS
Vancomycin	3 (7.3)	4 (3.1)	NS

*Laboratory data were determined at baseline. Data are presented as number (%) or median (range). Delayed: cycles that plasma methotrexate concentration were $\geq 10 \mu\text{mol L}^{-1}$ at 24 h, $\geq 1 \mu\text{mol L}^{-1}$ at 48 h or $\geq 0.1 \mu\text{mol L}^{-1}$ at 72 h after administration of high-dose methotrexate. n, number of cycles; NS, not significant.

the delayed elimination group were significantly higher than those in the normal elimination group (median 11.50 vs. 1.10 $\mu\text{mol L}^{-1}$ at 24 h after the start of HDMTX therapy; 0.87 vs. 0.11 $\mu\text{mol L}^{-1}$ at 48 h; 0.23 vs. 0.05 $\mu\text{mol L}^{-1}$ at 72 h, $P < 0.05$, Table 1), resulting in the need for frequent calcium folinate rescue (median 24 vs. 12 rescues, $P < 0.05$).

Patient characteristics and MTX dosing regimen were compared between the delayed elimination group and the normal elimination group (Table 1). Gender, age, weight and MTX dose did not differ between the groups. Abnormally high SCr and serum AST and ALT concentrations were significantly more frequent in the delayed elimination group than in the normal elimination group (19.5% vs. 5.4%, 31.7% vs. 8.5%, and 41.5% vs. 25.4%, respectively, $P < 0.05$). Infusion time for HDMTX was significantly longer in the delayed elimination group than in the normal elimination group (median 7.3 vs. 6 h, $P < 0.05$). Pleural effusion and ascites were significantly more frequent in the delayed elimination group than in the normal elimination group (29.3% vs. 10.8%, $P < 0.05$). Co-administration of PPIs, including omeprazole, lansoprazole and rabeprazole, was also significantly more frequent in the delayed elimination group than in the normal elimination group (31.7% vs. 13.8%, $P < 0.05$). No difference was found in NSAID or vancomycin co-administration between the groups.

Multiple logistic regression analysis was performed to identify the significant risk factors for delayed plasma MTX elimination (Figure 1). After adjustment for six variables – PPI co-administration, pleural effusion and ascites, MTX infusion time, and SCr, AST and ALT levels – PPI

co-administration was still a significant risk factor for delayed elimination (adjusted OR 2.65; 95% CI 1.03, 6.82), as well as SCr elevation (adjusted OR 4.60; 95% CI 1.31, 16.16) and AST elevation (adjusted OR 4.12; 95% CI 1.19, 14.28).

To confirm the significant effects of PPI on MTX elimination, the incidence of delayed plasma MTX elimination was also compared between HDMTX therapy with ($n = 31$) and without ($n = 140$) PPI co-administration. Delayed elimination of plasma MTX was more frequent in HDMTX therapy with PPI co-administration than without PPI co-administration (41.9% vs. 20.0%, $P < 0.05$).

All four PPIs inhibited the ATP-dependent uptake of MTX into BCRP-expressing membrane vesicles in a concentration-dependent manner, with half-maximal inhibitory concentrations (IC_{50} values) of 17.6, 14.4, 8.5 and 5.5 $\mu\text{mol L}^{-1}$ for omeprazole, lansoprazole, rabeprazole and pantoprazole, respectively (Figure 2). Inhibitory potencies, as shown by the IC_{50} values and pharmacokinetic parameters, were almost the same among omeprazole, lansoprazole and rabeprazole, which are the three PPIs used in Japan (Table 2).

Discussion

Multiple logistic regression analysis revealed that PPI co-administration was a risk factor for delayed elimination of MTX in HDMTX therapy and for kidney and liver dysfunction. PPI co-administration increased the risk of delayed MTX elimination by 2.65 times (Figure 1). These

Figure 1

Adjusted odds ratios for delayed methotrexate elimination

Figure 2

Inhibitory effects of omeprazole (open circle), lansoprazole (closed circle), rabeprazole (open square) and pantoprazole (closed square) on the breast cancer resistance protein (BCRP)-mediated uptake of 100 μM methotrexate (MTX) into the membrane vesicles expressing human BCRP. Each point represents the mean ± SD. (n = 3)

results support the previous finding that concurrent administration of PPIs causes higher plasma MTX concentration in HDMTX therapy [6].

Although other variables, such as co-administration of NSAIDs or vancomycin and pleural effusion and ascites, have been associated with delayed MTX elimination in HDMTX therapy, their contribution to MTX elimination was not statistically significant here. NSAIDs are considered to suppress renal MTX excretion by inhibiting renal prostaglandin synthesis, and vancomycin acts by inducing neph-

rotoxicity [3, 5]. However, unlike PPI co-administration, these effects were not factors strongly affecting MTX elimination. Joerger *et al.* reported that PPI co-administration decreased MTX clearance by 27% – greater than that induced by NSAID co-administration (16%) [6]. Compatible with these observations, our results also show that the contribution of PPI co-administration to delayed plasma MTX elimination was greater than that of NSAIDs.

HDMTX therapy is commonly avoided in patients with third space effusions, pleural effusions and ascites [1]. However, 26 cycles of HDMTX were given to 19 patients with third space effusions because they had no other therapeutic options to control their malignancies. The presence of third space effusions did not significantly affect the elimination of MTX, a result similar to those of a previous report [6].

BCRP is expressed on the apical membranes of renal epithelial cells and is associated with ATP-driven efflux of MTX [8]. All four PPIs showed *in vitro* inhibition of MTX transport via BCRP. However, their IC₅₀ values were 50–200 times their therapeutic unbound plasma concentrations in human subjects (Table 2), suggesting that the interaction between MTX and PPIs cannot be explained solely by the inhibitory effects of PPIs on renal BCRP. It may be possible that other transporters responsible for the renal excretion of MTX, such as organic anion transporter 3, multidrug resistance-associated protein (MRP) 2 and MRP4 [16], are involved in this drug interaction, although the inhibitory effects of PPIs on these transporters remain to be investigated.

It is considered that BCRP is responsible for MTX secretion by the kidneys during the process of renal excretion of MTX. We estimated the renal clearance for MTX secretion to be 64.5 ml min⁻¹ by subtracting the glomerular filtration clearance of MTX (59.5 ml min⁻¹), which is calculated

Table 2

Half-maximal inhibitory concentration (IC_{50}) values for breast cancer resistance protein-mediated uptake of methotrexate and unbound concentrations of proton pump inhibitors in plasma

	Dose (mg)	C_{max} ($\mu\text{mol l}^{-1}$)	f_u	$f_u \cdot C_{max}$ ($\mu\text{mol l}^{-1}$)	IC_{50} ($\mu\text{mol l}^{-1}$)	$\frac{f_u \cdot C_{max}}{IC_{50}}$
Omeprazole	20	1.8 [11]	0.050 [15]	0.090	17.6	0.0051
Lansoprazole	30	2.7 [12]	0.030 [15]	0.081	14.4	0.0056
Rabeprazole	20	1.2 [13]	0.037 [13]	0.045	8.5	0.0053
Pantoprazole	40	5.5 [14]	0.020 [15]	0.109	5.5	0.0200

f_u , plasma unbound fraction.

from the glomerular filtration rate (120 ml min^{-1}) and the plasma unbound fraction (f_u ; 49.6%) [17], from the renal clearance of MTX (124 ml min^{-1}) [6, 18]. Assuming that renal reabsorption of MTX is negligible, the maximum contribution of MTX secretion to MTX renal clearance would be 52.0%. However, because transporters other than BCRP may also be involved in the renal secretion of MTX, the contribution of BCRP itself may be <52.0%. In any case, renal clearance of MTX may not be totally impaired even if renal BCRP function is abolished by PPI co-administration.

Delayed elimination of plasma MTX was not observed in every patient receiving PPIs; the reason is unclear. Polymorphism of CYP2C19, a principal enzyme involved in the metabolism of PPIs [19], can be considered a potential explanation if the drug interaction is plasma PPI concentration-dependent. Subjects with CYP2C19*2 and *3 mutated alleles, both of which provide higher plasma concentrations of PPIs [20], may be susceptible to the drug interaction. Another genetic risk factor for this drug interaction may involve polymorphism of BCRP [21].

In conclusion, by using multiple logistic regression analysis we have confirmed that PPI co-administration was a possible risk factor for delayed plasma MTX elimination. The mechanisms of this delayed elimination, including the genetic factors and the contribution of other MTX transporters, remain to be clarified in further investigations.

Competing interests

None declared.

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