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Determinants of the elimination of methotrexate and 7-hydroxy-methotrexate following high-dose infusional therapy to cancer patients

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Aims
To characterize determinants of the elimination of methotrexate (MTX) and 7-hydroxy-methotrexate (7-OH-MTX) in patients receiving high-dose MTX therapy (HDMTX).

Methods
24 and 48-h blood samples from 76 patients receiving HDMTX (dose range 300 mg m^{-2} to 12 g m^{-2}) were analysed, and concentration-time data were subjected to population pharmacokinetic and covariate analysis using nonlinear mixed-effect modelling (NONMEM).

Results
Treatment-related mortality was 1.3% (one patient with renal failure). Values for MTX clearance (CL_{MTX}) and 7-OH-MTX clearance ($CL_{7\text{-OH-MTX}}$) were estimated at 8.85 and 2 L^{-1}, respectively. Baseline creatinine clearance correlated with CL_{MTX} and $CL_{7\text{-OH-MTX}}$. Concurrent administration of benzimidazoles led to a 27% decrease in CL_{MTX} and a 39% decrease in $CL_{7\text{-OH-MTX}}$. Prior administration of nonsteroidal anti-inflammatory drugs (NSAIDs) resulted in a 16% decrease in CL_{MTX} and a 38% decrease in $CL_{7\text{-OH-MTX}}$. Plasma MTX concentrations were significantly higher in patients also receiving benzimidazoles at 24 h (2.01 μmol L^{-1} *vs.* 0.66 μmol L^{-1}, $P < 10^{-4}$) and at 48 h (0.25 μmol L^{-1} *vs.* 0.12 μmol L^{-1}, $P < 10^{-4}$). 7-OH-MTX plasma concentrations were also significantly higher in patients with concurrent benzimidazoles as compared with patients without benzimidazoles at 24 h (4.47 μmol L^{-1} *vs.* 2.52 μmol L^{-1}, $P = 0.0009$) and at 48 h (1.11 μmol L^{-1} *vs.* 0.72 μmol L^{-1}, $P = 0.031$).

Conclusions
In patients receiving HDMTX, concurrent administration of benzimidazoles was associated with a significant decrease of CL_{MTX} and $CL_{7\text{-OH-MTX}}$, resulting in significantly higher plasma concentrations of MTX and 7-OH-MTX. The data suggest that benzimidazole treatment should be seen as a relative contraindication for HDMTX.

Introduction

Methotrexate (MTX) is a widely used antifolate drug. Antiproliferative activity is achieved by blocking thymidylate synthase, dihydrofolate reductase and *de novo* purine synthesis [1]. 7-Hydroxy-methotrexate (7-OH-MTX) is the main metabolite in serum following high-dose MTX (HDMTX) [1], and it contributes to the activity [2] and toxicity [3, 4] of the drug. 7-OH-MTX concentrations exceed those of the parent compound in plasma shortly after MTX infusion [5]. MTX and 7-OH-MTX both exhibit first-order pharmacokinetics [1, 3, 5–8]. MTX enters the cell through the reduced folate carrier system, and by additional diffusion at higher plasma concentrations (>20 μmol L^{-1}) [3]. Finally, MTX undergoes intracellular activation by polyglutamation [3], which is increased at higher MTX doses and results in enhanced drug activity. MTX is eliminated by renal excretion involving passive glomerular filtration and active tubular reabsorption and secretion. 7-OH-MTX is also renally cleared but more slowly than MTX [5]. The elimination of MTX is prolonged in patients with renal impairment or third space fluid collections, due to a slow redistribution from these extravascular fluid accumulations [1, 3]. MTX is particularly prone to drug–drug interactions. Non-steroidal anti-inflammatory drugs (NSAIDs), salicylates [9, 10], sulphonamides [11], penicillin [12], benzimidazoles [13] and probenecid [14] can increase exposure to MTX, and may result in increased drug toxicity. Concurrent administration of NSAIDs in particular has been associated with increased MTX toxicity and combination is contraindicated [10]. Various compounds, including sulphonamides [15], leucovorin, vincristine [16], L-asparaginase [17] and corticosteroids [18] interact with MTX by altering its cellular uptake.

Intravenous HDMTX is used to treat high-grade lymphoma, osteogenic sarcoma and acute leukaemia. It is typically administered at doses of 500 mg m^{-2} or higher over 6–24 h [19]. Intravenous HDMTX requires pharmacokinetic monitoring to identify patients at high risk for developing significant toxicity, especially those with renal dysfunction [20]. In general, plasma drug concentrations >0.1 μmol L^{-1} at 48 h after administration, and/or any plasma concentration >10 μmol L^{-1} require intensive leucovorin rescue [21, 22]. A number of nomograms have been developed for monitoring MTX, using varying plasma drug concentration-time data [21, 23]. The development of HDMTX-induced acute renal dysfunction, which is mediated by the precipitation of MTX and 7-OH-MTX in the kidney tubules, is a potentially life-threatening complication and occurs in 1.8% of patients receiving HDMTX. Elderly patients and those on concurrent nephrotoxic agents are at particular risk of HDMTX-induced renal failure [24]. The introduction of aggressive hydration, urine alkalinization and pharmacokinetically guided leucovorin rescue has been shown to decrease the morbidity rate in patients receiving HDMTX [25], but severe morbidity and mortality secondary to HDMTX-induced renal dysfunction are still major concerns [26].

The aims of this study were to (1) develop a population pharmacokinetic model of MTX and 7-OH-MTX (2), analyse the influence of various anthropometric and biochemical covariates as well as comedication on MTX elimination, and (3) provide guidance on how to increase the safety of HDMTX schedules.

Methods

Patient population and study protocol

Patients with solid tumours receiving intravenous HDMTX either as single agent or in combination with other cytotoxic drugs treated at the Netherlands Cancer Institute were included in the analysis. Some patients participated in a Phase II study that used fixed-dose MTX (3000 mg over a 3-h infusion) as single agent or in combination with fixed-dose intravenous doxorubicin (40 mg every 2 weeks) for the treatment of malignant pleural mesothelioma. The other patients received MTX within standard schedules for the treatment of NHL, acute lymphocytic leukaemia, head and neck cancer and osteosarcoma. The dose of MTX given for head and neck cancer (400 mg m^{-2} MTX) and choriocarcinoma (300 mg m^{-2} MTX) was slightly below usual HDMTX schedules as defined previously [19]. Those patients were retained in the study group for data analysis. For HDMTX treatment, patients had to have sufficient blood cell counts (leucocytes = 4.0×10^9 L^{-1}, granulocytes = 2.0×10^9 L^{-1}, platelets = 100×10^9 L^{-1}), normal renal (serum creatinine <120 μmol L^{-1}) and liver function (serum bilirubin <25 μmol L^{-1}) and a performance status of 0–2 according to WHO criteria. All patients within the pleural mesothelioma study and those with extended pharmacokinetic sampling (see below) were treated within an institutional review board (The Netherlands Cancer Institute) approved study and provided written informed consent.

Supportive therapy was identical within the group studied and included aggressive prehydration and urine alkalinization with oral and intravenous sodium bicarbonate. MTX was only given after the urine was alkalinized to a pH of =7. MTX was dissolved in 1 L sodium chloride solution (0.9%). After MTX was given, a continuous infusion of sodium chloride (0.45%) and glucose (2.5%) was maintained over 22 h. Hydration and

alkalinization were continued for three days with 1000 mg oral sodium bicarbonate being given every 6 h. Oral leucovorin rescue (15 mg) every 6 h was begun 24 h after the start of MTX. Routine 24- and 48-h blood samples were collected in all patients. If plasma MTX concentrations at 48 h were ≤ 0.04 μmol L^{-1}, leucovorin was discontinued, but if they were >0.04 and <0.1 μmol L^{-1}, leucovorin was continued for another 24 h. If plasma MTX concentrations were ≥ 0.1 μmol L^{-1} 48 h after starting MTX, leucovorin rescue was intensified (continuous intravenous infusion of 1200 mg every 24 h) until concentrations were below 0.1 μmol L^{-1}. Twenty-one patients underwent additional blood sampling (one cycle in eight patients and two cycles in 13 patients) at the following time-points: at the end of the MTX infusion and 3, 6 and 8 h later. Laboratory assessment, including haemoglobin, leucocyte, absolute neutrophil and blood platelet counts, liver function tests and serum creatinine, was usually repeated daily until day 3, and weekly thereafter. Clinical assessment was performed on a daily basis.

Blood samples were collected in glass tubes containing heparin or EDTA and plasma was obtained by centrifugation (2000 *g*) immediately after sampling. Ascorbic acid (1 mg mL^{-1}) was added and samples were stored at −20 °C until analysis. MTX and 7-OH-MTX plasma concentrations were measured using a validated reversed-phase high-pressure liquid chromatography (HPLC) method as published previously [27]. The lower detection limit of the HPLC assay was 0.04 μmol L^{-1} for both MTX and 7-OH-MTX, and the within and between-day coefficients of variation were ≤ 7.0%.

Population pharmacokinetic analysis

Population pharmacokinetic analysis was performed using the nonlinear mixed-effect modelling program (NONMEM) version V (double precision, level 1.1) [28]. NONMEM uses a maximum likelihood criterion to simultaneously estimate population values of fixed-effects parameters (e.g. drug clearance (CL) and anthropometric or biochemical covariates that modify these values in the population) and values of the random-effects parameters (e.g. interindividual, interoccasion and residual variability). Log-transformed plasma drug concentrations were used together with the first-order (FO) estimation method throughout. Standard errors for all parameters were calculated using the COVARIANCE option of NONMEM and individual Bayesian pharmacokinetic parameters were obtained with the POSTHOC option [28]. The S-plus (MathSoft Inc, Seattle, USA) based model building aid Xpose 3.0 was used for graphical processing [29]. In the first step, a basic pharmacokinetic model was developed for MTX and 7-OH-MTX concentration-time data using the NONMEM subroutine ADVAN5. In the second step, an extended covariate analysis was performed on the basic population pharmacokinetic model to study the associations between anthropometric, biochemical covariates as well as comedication on the pharmacokinetic parameters for MTX and 7-OH-MTX. In general, model selection was based on the minimum value of objective function (OFV), as calculated by NONMEM, the precision of parameter estimates (according to the standard error values of the parameter estimates), and the fit of the model to the data.

Interindividual and interoccasion variability were estimated using a proportional error model. For example, interindividual and interoccasion variability in MTX clearance (CL_{MTX}) was defined as follows:

$$CL_{MTX\,i} = CL_{MTX\,POP} \times (1 + \eta_i^{\,CLMTX} + \kappa i^{\,CLMTX})$$

Where $CL_{MTX\,i}$ represents the clearance of MTX of the ith individual, $CL_{MTX\,POP}$ is the typical population value of CL_{MTX}, $\eta_i^{\,CLMTX}$ is the interindividual random effect with mean zero and variance ω^2, and $\kappa_i^{\,CLMTX}$ is the interoccasion random effect with mean zero and variance π^2. Residual variability was modelled as $\log(C_{ij}) = \log(_{Cij}) + \varepsilon_{ij}$, in which C_{ij} and $_{Cij}$ are the j^{th} measured and model predicted drug concentration of the i^{th} individual, respectively, and ε_{ij} is the residual random error with mean zero and variance σ^2.

The following covariates were subsequently tested with respect to their correlation with PK parameters such as CL_{MTX} and 7-OH-MTX clearance ($CL_{7\text{-}OH\text{-}MTX}$): Creatinine clearance (CL_{CREA} according to the Cockroft-Gault formula, and with values >140 mL min^{-1} truncated at this value) before the start of each HDMTX cycle, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) as surrogate markers for liver function, patient age, gender, weight and body-surface area (BSA), third-space fluid collections, comedication with benzimidazoles, NSAIDs, corticosteroids, vinca alkaloids, 5-fluorouracil and L-asparaginase. Additionally, patient weight, age and BSA were tested as potential covariates on the volume of distribution of MTX and 7-OH-MTX. Covariates were entered individually into the basic population pharmacokinetic model by forward inclusion. Continuous covariates such as patient weight were centred to their median values. For example, the relationship between CL_{MTX} and body weight was described as follows:

$$CL_{MTX} = \theta_1 + \theta_2 \times (WT - 70)$$

Where θ_1 represents CL_{MTX} of a (median) patient with a body weight of 70 kg, and θ_2 is the increase or decrease in CL_{MTX} per kg difference in body weight. Dichotomous covariates such as patient gender were modelled as follows:

$$CL_{MTX} = \theta_1 + \theta_2 \times (GEN)$$

Where θ_1 represents the CL_{MTX} value in females (GEN = 0), and θ_2 is the change in CL_{MTX} in males (GEN = 1). Forward selection and backward elimination were used for the purpose of covariate testing, with OFV as the main discriminator between different models. The OFV is equal to minus twice the log likelihood of the data and the difference in OFV between hierarchical models approximates to a chi-squared distribution with one degree of freedom. The difference in OFV was evaluated after the introduction of a covariate into the model (forward inclusion), and the significance level was set at $P < 0.005$, which corresponds to a decrease of OFV of >7.83. All significant covariates were included in an intermediate multivariate model followed by a stepwise backward elimination procedure. Covariates remained in the model when elimination of the covariate caused an OFV increase of >10.8 that is associated with a significance level of $P < 0.001$.

Finally, plasma concentrations of MTX and 7-OH-MTX at 24 and 48 h were compared in patient subgroups who were or were not taking benzimidazoles or prior NSAIDs. To properly account for multiple treatment cycles per patient, repeated measurement analysis of variance (RM-ANOVA) was used [30]. To improve normality of the residuals and constancy of the variation, the analysis was based on the logarithmically transformed concentrations. The correlation between concentrations in the same patient was modelled by assuming a random baseline concentration and first-order autoregressive correlations (i.e. declining according to a power law with increasing distance between cycles). This choice was based on a preliminary analysis of the MTX concentrations at 24 h. RM-ANOVA also allows adjustment of the benzimidazole effect for confounding by the NSAID effect and *vice versa*. Both effects were additionally adjusted for confounding by a possible cycle effect. The level of significance was set at 0.05.

Results

In total, 76 patients who received 304 cylces of HDMTX were included in the study. The dataset consisted of two subpopulations, one group with intensive blood sampling ($n = 21$; 34 courses) and one group with routine 24- and 48- h concentration-time data ($n = 55$; 270 courses). Clinical and laboratory parameters of the study group are further detailed in Table 1. The MTX dose ranged from 300 mg m^{-2} to 12 g m^{-2} given over an infusion period of 1–24 h. Sixty-one of the 76 patients

Table 1
Patient characteristics (total number of patients = 76)

Parameter	*n*	Normal range	Median value	Range
Age (years)			51.1	17.1–77.0
Males/females	62/14			
WHO performance status				
0	10			
1	58			
2	8			
Body surface area (m^2)			1.94	1.56–2.45
Serum albumin (g L^{-1})		35–50	40.0	16–71
Aspartate aminotransferase (U L^{-1})		≤40	17.0	4–234
Alanine aminotransferase (U L^{-1})		≤45	20.0	3–368
Total bilirubin (μmol L^{-1})		<16	6.0	2–60
Alkaline phosphatase (U L^{-1})		40–120	92.0	38–519
Gammaglutamyl transpeptidase (U L^{-1})		≤50	43.0	7–675
Lactic dehydrogenase (U L^{-1})		≤450	273.0	51–3370
Serum creatinine (μmol L^{-1})		50–105	77.0	32–433
Creatinine clearance (Cockroft-Gault) (mL min^{-1})		60–140	87.5	40–140

n = number of patients.

(80%) received MTX at doses between 1000 and 5000 mg m^{-2}, and the duration of MTX infusion was between 1–6 h in 61 patients (80%). Twenty-nine patients suffered from malignant pleural mesothelioma, 20 from gastro-oesophageal cancer, 12 from non-Hodgkin lymphoma (NHL), 10 from head and neck cancer, two from choriocarcinoma, and one patient each from acute lymphocytic leukaemia, trophoblastic tumour and osteosarcoma. Foregoing administration of nephrotoxic anticancer treatment included cisplatin in two patients with choriocarcinoma and ifosfamide in one patients with osteosarcoma. All patients with NHL were also being treated with corticosteroids. HDMTX-associated grade 1 creatinine elevations (>ULN to 1.5×ULN) occurred in 22 of 304 treatment cycles (7.2%), grade 2 (>1.5×ULN to 3×ULN) in five courses (1.6%) and grade 3 (>3×ULN to 6×ULN) in nine courses (3%). Benzimidazoles were administered to 13 patients (10 receiving omeprazole 20–40 mg daily and three lansoprazole 30 mg daily). Six patients were being treated with NSAIDs (diclofenac 75–300 mg daily in five patients, ibuprofen 100 mg three times daily in one patient). NSAIDs were discontinued in all patients on the day of HDMTX administration.

Visual inspection of the concentration-time data showed two outliers with markedly increased drug exposure (Figure 1). The patient with the highest MTX concentrations (ID-63) was a 29-year-old male being treated for relapsed malignant pleural mesothelioma with MTX (3 g as a 3 h infusion) combined with intravenous doxorubicin (40 mg). On day two, the patient experienced anuric renal failure CTC grade 3, requiring immediate dialysis. The patient also experienced neutropenia CTC 4, thrombopenia CTC 3 and infectious complications with gram-positive staphylococcal spp. bacteraemia. Basic measures such as urine alkalinization, flushing, drug monitoring and leucovorin rescue were implemented according to guidelines. The patient died 20 days after having received HDMTX, despite immediate continuous veno-venous haemofiltration, antibiotic treatment and intensified intravenous leucovorin and thymidine-rescue as described previously [31]. Haemorrhaghic pericarditis was suspected to be the most probable cause of death. Postmortem examination was not performed.

MTX concentration-time data were best described by a linear three-compartment model with first-order elimination from the central compartment. Those for 7-OH-MTX were best described by a linear two-compartment model (one central and one peripheral compartment) with first-order elimination of the metabolite from the central compartment (Figure 2). Because an independent calculation of the metabolic fraction of MTX to 7-OH-MTX was not possible, we assumed that 10% of MTX was metabolized to 7-OH-MTX, in accordance with literature data [32]. Fixing the metabolic fraction to

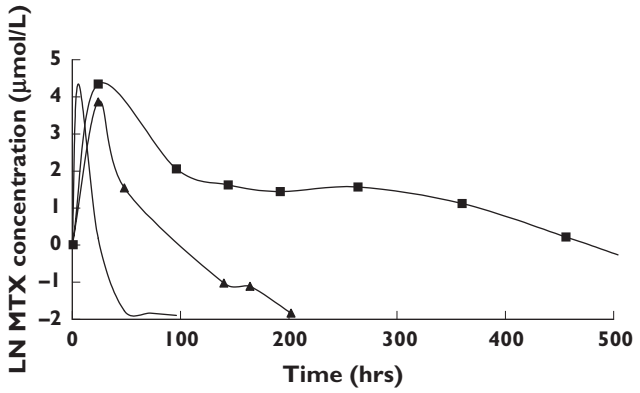
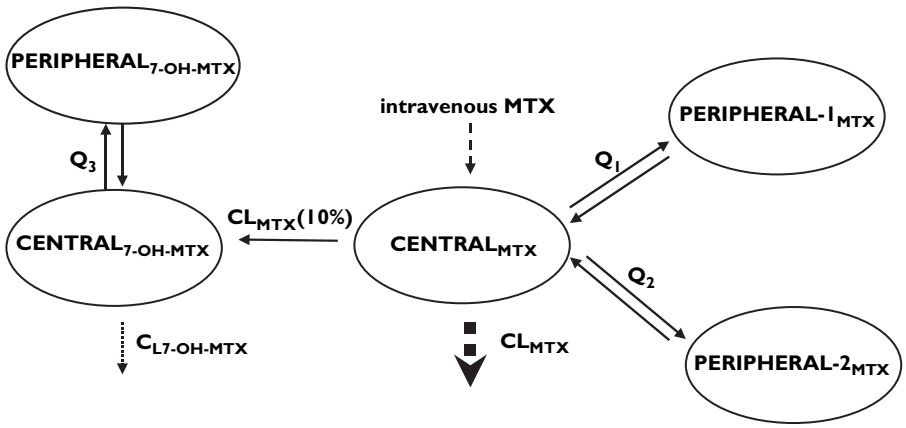


Figure 1
Mean MTX plasma concentration time profiles and those of the two most extreme outliers (patients 36 and 63), both of whom received 3 mg MTX as a 3 h infusion. ID-36 (▲); ID-63 (■); mean curve (—)

Figure 2
Five compartment model for MTX and 7-OH-MTX pharmacokinetics; CL = clearance, Q = intercompartmental clearance



higher (up to 50%) or lower (down to 2%) values resulted in a decreased fit with an increased OFV. The combined five-compartment model generated pharmacokinetic parameter estimates as outlined in Table 2. Interindividual variability for the central compartment of MTX ($CENTRAL_{MTX}$), the first peripheral compartment of MTX ($PERIPHERAL\text{-}1_{MTX}$) and the intercompartmental clearance between $CENTRAL_{MTX}$ and $PERIPHERAL\text{-}2_{MTX}$ (Q_2) were not included in the final model as they did not improve the fit. Interoccasion variability for $CL_{7\text{-}OH\text{-}MTX}$ was also not included as it was estimated at <5%.

The two patients with noticeably increased exposure to MTX and 7-OH-MTX (ID-63 and ID-36 in Figure 1) had a markedly decreased CL_{MTX} (Bayesian estimates of 1.70 L^{-1} and 4.11 L^{-1}, respectively). The $CL_{7\text{-}OH\text{-}MTX}$ was also significantly impaired in ID-63 (1.15 L^{-1}), but was almost normal in ID-36 (1.85 L^{-1}). The two patients administered prior cisplatin had moderately decreased CL_{MTX} (7.37 and 5.03 L^{-1}) and an approximately normal $CL_{7\text{-}OH\text{-}MTX}$ (3.36 and 1.91 L h^{-1}). The patient who had been given ifosfamide prior to MTX showed no decrease in CL_{MTX} (9.15 L h^{-1}) or $CL_{7\text{-}OH\text{-}MTX}$ (2.49 L h^{-1}). The addition of cisplatin or ifosfamide as covariates to the population model did not improve the fit. However, there were insufficient data to estimate the influence of these parameters on MTX pharmacokinetics.

Subsequent covariate testing was performed with and without the inclusion of the individuals with markedly decreased CL_{MTX} and $CL_{7\text{-}OH\text{-}MTX}$ to allow the detection of a covariate relationship highly driven by these outliers. The following covariates were identified to be significantly correlated with CL_{MTX} and $CL_{7\text{-}OH\text{-}MTX}$: Creatinine clearance ($CL_{CREA}$), prior administration of NSAIDs, and concurrent administration of benzimidazoles. The inclusion of all three covariates on both CL_{MTX} and $CL_{7\text{-}OH\text{-}MTX}$ led to a decrease in OFV (174.5 points, $P < 10^{-4}$), in the interindividual variability in CL_{MTX} (from 35.2% to 19.6%) and $CL_{7\text{-}OH\text{-}MTX}$ (from 32.5% to 31.0%), and in the interoccasion variability in CL_{MTX} (from 18.6% to 13.3%). Furthermore, an improved fit was observed on inspection of the graphical plots (Figure 3). The concurrent administration of ben-

Table 2
Population pharmacokinetic data

Pharmacokinetic parameter	Full data set Estimate	RSE
CL_{MTX} (L h^{-1})	8.85 (see equation 1)	1.96
$CL_{7\text{-}OH\text{-}MTX}$ (L h^{-1})	2 (see equation 2)	0.417
Volume of $CENTRAL_{MTX}$ (L)	23.0	4.04
Volume of $CENTRAL_{7\text{-}OH\text{-}MTX}$ (L)	21.6	3.16
Volume of $PERIPHERAL\text{-}1_{MTX}$ (L)	185	25.5
Volume of $PERIPHERAL\text{-}2_{MTX}$ (L)	5.34	2.01
Volume of $PERIPHERAL_{7\text{-}OH\text{-}MTX}$ (L)	27.7	2.93
Q1 (L h^{-1})	0.444	8.78×10^{-2}
Q2 (L h^{-1})	0.716	0.371
Q3 (L h^{-1})	0.429	4.79×10^{-2}
Interindividual variability		
CL_{MTX} (%)	19.6	6.66
$CL_{7\text{-}OH\text{-}MTX}$ (%)	31.0	9.77
Volume of $CENTRAL_{7\text{-}OH\text{-}MTX}$ (%)	8.22	3.80
Volume of $PERIPHERAL\text{-}2_{MTX}$ (%)	31.0	16.7
Volume of $PERIPHERAL_{7\text{-}OH\text{-}MTX}$ (%)	41.4	10.2
Q_1 (%)	32.0	21.1
Q_3 (%)	27.1	18.5
Interoccasion variability		
CL_{MTX} (%)	13.3	4.28
Residual variability		
MTX plasma concentration (%)	52.3	27.8
7-OH-MTX plasma concentration (%)	57.1	27.3

RSE, relative standard error; Q_1, intercompartmental clearance between $CENTRAL_{MTX}$ and $PERIPHERAL\text{-}1_{MTX}$; Q_2, intercompartmental clearance between $CENTRAL_{MTX}$ and $PERIPHERAL\text{-}2_{MTX}$; Q_3, intercompartmental clearance between $CENTRAL_{7\text{-}OH\text{-}MTX}$ and $PERIPHERAL_{7\text{-}OH\text{-}MTX}$.

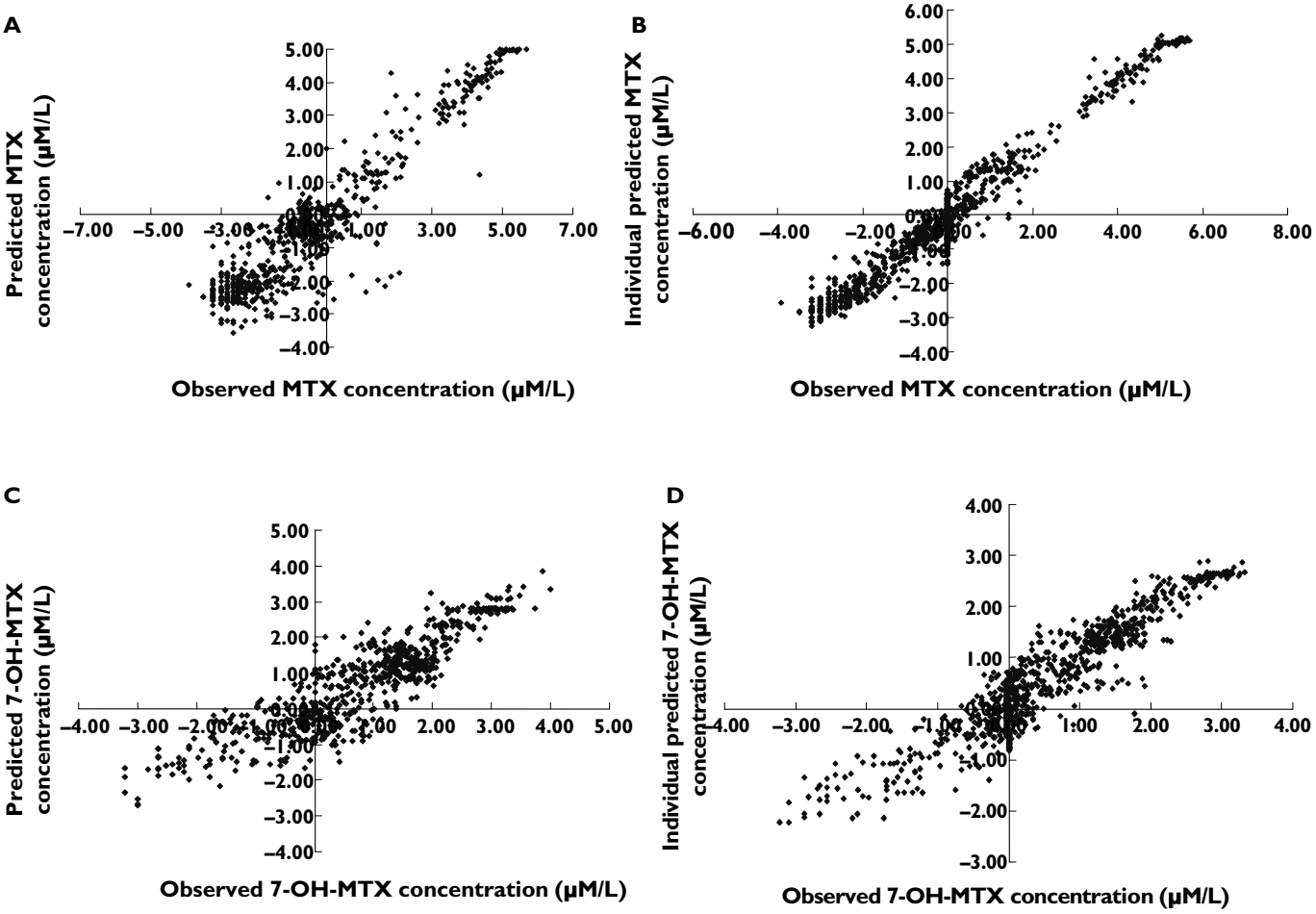


Figure 3

Goodness-of-fit plots from the final population pharmacokinetic model (all data are log-transformed). (A) Observed *vs.* predicted MTX concentrations. (B) Observed *vs.* individual Bayesian predicted MTX concentrations. (C) Observed *vs.* the model predicted 7-OH-MTX concentrations. (D) Observed *vs.* individual Bayesian predicted of 7-OH-MTX concentrations.

zimidazoles led to a 27% and 39% decrease in CL_{MTX} and $CL_{7\text{-OH-MTX}}$, respectively, and prior administration of NSAIDs led to a 16% and 38% decrease in CL_{MTX} and $CL_{7\text{-OH-MTX}}$, respectively. These relationships were consistent in the datasets containing or not containing the two individuals with the highest exposure to MTX. No difference in the effect on CL_{MTX} or $CL_{7\text{-OH-MTX}}$ could be demonstrated for the two benzimidazoles (omeprazole and lansoprazole) or the two NSAIDs (diclofenac and ibuprofen). Furthermore, inclusion of the dosage of the benzimidazoles and NSAIDs as covariates on CL_{MTX} and $CL_{7\text{-OH-MTX}}$ did not improve the fit. Third-space fluid collections, comedication with corticosteroids or anticancer drugs, anthropometric parameters and surrogate markers of liver function (AST, ALT, AP, total bilirubin) had no influence on the pharmacokinetics of MTX and 7-OH-MTX.

Equations 1 and 2 describe CL_{MTX} and $CL_{7\text{-OH-MTX}}$ as a function of CL_{CREA} (median value = 87 mL min^{-1}) and comedication (benzimidazoles (PPI) and NSAID is 1 with concurrent comedication and 0 without comedication):

$$CL_{MTX} = 8.85 + 0.0423 \times (87\ \text{mL min}^{-1} - CL_{CREA}) - 2.45 \times PPI - 1.46 \times NSAID \quad (1)$$

$$CL_{7\text{-OH-MTX}} = 2 + 0.0123 \times (87\ \text{mL min}^{-1} - CL_{CREA}) - 0.369 \times PPI - 0.357 \times NSAID \quad (2)$$

As a consequence, plasma MTX concentrations at 24 h were significantly higher in patients taking benzimidazoles (geometric mean 2.01 μmol L^{-1}, 95% CI 1.44–2.81 μmol L^{-1}) compared with patients not taking benzimidazoles (0.66 μmol L^{-1}, 0.52–0.84 μmol L^{-1}, $P < 10^{-4}$), as were plasma MTX concentrations at 48 h in patients taking benzimidazoles (0.25 μmol L^{-1}, 0.17–

Table 3
MTX- and 7-OH-MTX plasma concentrations at 24 and 48 h in patients also taking benzimidazoles or who had been treated with NSAIDS prior to MTX

		Geom. mean (95% CI) (μmol L^{-1})			
		24-h plasma conc.		48-h plasma conc	
Patient subgroup	*n*	MTX	7-OH-MTX	MTX	7-OH-MTX
+Benzimidazoles	13	2.01 (1.44–2.81)	4.47 (3.17–6.31)	0.25 (0.17–0.35)	1.11 (0.73–1.68)
No benzimidazoles	63	0.66 (0.52–0.84)	2.52 (1.99–3.20)	0.12 (0.09–0.15)	0.72 (0.53–0.98)
Ratio		3.03 (2.19–4.19)	1.77 (1.27–2.48)	2.10 (1.50–2.95)	1.53 (1.04–2.26)
P-value		$<10^{-4}$	0.0009	$<10^{-4}$	0.031
Prior NSAIDs	6	0.98 (0.63–1.51)	1.74 (1.10–2.76)	0.17 (0.11–0.28)	1.61 (0.93–2.80)
No NSAIDs	70	0.77 (0.62–0.97)	2.83 (2.25–3.55)	0.13 (0.10–0.16)	0.73 (0.55–0.99)
Ratio		1.26 (0.84–1.91)	0.61 (0.40–0.95)	1.34 (0.84–2.13)	2.20 (1.33–3.65)
P-value		0.26	0.27	0.21	0.0023

0.35 μmol L^{-1}) compared with patients not taking benzimidazoles (0.12 μmol L^{-1}, 0.09–0.15, $P < 10^{-4}$). Plasma 7-OH-MTX concentrations at 24 h were also significantly higher in patients taking benzimidazoles (4.47 μmol L^{-1}, 3.17–6.31 μmol L^{-1}) compared with patients not taking concurrent benzimidazoles (2.52 μmol L^{-1}, 1.99–3.20 μmol L^{-1}, $P = 0.0009$), as were plasma 7-OH-MTX concentrations at 48 h in patients taking benzimidazoles (1.11 μmol L^{-1}, 0.73–1.68 μmol L^{-1}) compared with patients not taking concurrent benzimidazoles (0.72 μmol L^{-1}, 0.53–0.98 μmol L^{-1}, $P = 0.031$). For patients who had been administered NSAIDs, the differences were not statistically significant except for plasma 7-OH-MTX concentrations at 48 h which were higher in patients given NSAIDs compared with patients not given NSAIDs (1.61 μmol L^{-1} *vs.* 0.73 μmol L^{-1}, $P = 0.0023$) (Table 3). Plasma MTX concentrations ≥ 0.1 μmol L^{-1} at 48 h were found in 78.1% of patients taking benzimidazoles, in 71.4% of patients who had been given NSAIDs, but in only 33.5% of patients not taking comedication. Compared with patients without the respective comedication, median 24- h plasma MTX concentrations were not different in patients receiving 5-fluorouracil (0.59 μmol L^{-1} *vs.* 1.09 μmol L^{-1}, $P = 0.063$), vinca alkaloids (1.79 μmol L^{-1} *vs.* 0.66 μmol L^{-1}, $P = 0.38$), L-asparaginase (4.27 μmol L^{-1} *vs.* 0.68 μmol L^{-1}, $P = 0.12$), corticosteroids (1.30 μmol L^{-1} *vs.* 0.66 μmol L^{-1}, $P = 0.96$) or in patients treated for third-space fluids (0.51 μmol L^{-1} *vs.* 0.70, $P = 0.51$).

Discussion

HDMTX-schedules are associated with an incidence of nephrotoxicity of 1.8% and a fatality rate of almost 0.1%, despite routine drug monitoring and supportive therapy [24]. Additionally, HDMTX-induced renal impairment exacerbates overall drug toxicity by delaying MTX elimination. Because concurrent administration of NSAIDs has been shown to increase MTX toxicity [10, 33], it is recommended that the two drugs should not be administered together. However, the interaction between benzimidazoles and MTX is less well known, and has been described in only a few case reports [13, 34, 35]. Previously, there have been no published data on the effects of NSAIDs and benzimidazoles on the elimination of MTX and 7-OH-MTX using a Bayesian approach.

Estimates for CL_{MTX} and $CL_{7\text{-OH-MTX}}$ were in accordance with literature data [7, 36]. CL_{CREA} correlated with model-predicted CL_{MTX} and $CL_{7\text{-OH-MTX}}$, internally validating the presented population model. Concurrent administration of benzimidazoles and prior administration of NSAIDs, even if discontinued on the day of HDMTX administration, significantly impaired the elimination of both MTX and 7-OH-MTX. Decreased clearance of MTX has been described in cancer patients on concurrent HDMTX and ketoprofen, with fatalities in three out of 36 patients [10]. Indomethacin was similarly reported to affect the pharmacokinetics of MTX with resulting severe renal failure [33]. No clinically significant interactions were found between ketoprofen, piroxicam and flurbiprofen and MTX if the latter was administered to patients with rheumatoid arthritis at low oral doses [37]. Interactions with NSAIDs result from inhibition of renal prostaglandin synthesis [10], by displacement of 7-OH-MTX from protein binding [38], and by competition between NSAIDs and MTX/7-OH-MTX for renal excretion [10], possibly mediated by

human organic anion transporters [14]. We found six patients in whom NSAIDs were only stopped shortly before HDMTX administration, for unknown reasons.

Two case reports indicated that MTX elimination is impaired with concurrent administration of omeprazole, resulting in sustained, highly toxic plasma MTX concentrations [13, 34]. Additionally, impaired MTX elimination has been reported during treatment with pantoprazole in a patient with cutaneous T-cell lymphoma receiving low-dose intramuscular MTX [35]. Benzimidazoles are potent inhibitors of MTX transport by the drug-transporter ABCG2 *in vitro* [39], which could explain the present clinical findings. It remains unclear if interactions between benzimidazoles and MTX are a class-effect or restricted to individual drugs.

Anthropometrics, biochemical markers, comedication with anticancer drugs or corticosteroids and third-space effusions were not found to affect the elimination of MTX or 7-OH-MTX. The third-space effusions in 15 out of the 76 patients studied were treated before the administration of HDMTX. Whereas corticosteroids, vinca alkaloids and L-asparaginase have been shown to interact with MTX at the cellular level *in vitro* [16–18], these covariates had no influence on the elimination of MTX or 7-OH-MTX in the present work. However, patient subgroups were small except for those taking benzimidazoles and corticosteroids, and there was a considerable lack of homogeneity with respect to MTX dosage and duration of infusion.

The patient who died had significantly decreased CL_{MTX} (0.69 L^{-1}) and $CL_{7\text{-OH-MTX}}$ (0.77 L^{-1}). The reasons for the marked impairment of drug elimination remain unclear, as supportive therapy and intensive leucovorin rescue were performed, comedication was absent and baseline CL_{CREA} was 92 mL min^{-1}. According to a recent meta-analysis, HDMTX-induced nephrotoxicity CTC grade ≥2 occured in 1.8% of patients receiving HDMTX for osteosarcoma, with an overall fatality rate of 0.08%, despite supportive therapy [24]. The latter study did not include data on comedication. A specific mutation within the drug transporter ABCC2 (Arg412Gly) has recently been found in a patient with impaired MTX elimination and nephrotoxicity, who was treated with HDMTX for large B-cell lymphoma [40]. MTX was shown to be a substrate for ABCG2, another drug transporter, *in vitro* [41], and variant alleles of the gene ABCG2 may also account for altered MTX elimination.

In conclusion, concurrent administration of benzimidazoles and HDMTX causes a significant decrease in CL_{MTX} and $CL_{7\text{-OH-MTX}}$, resulting in significantly higher plasma concentrations of MTX and 7-OH-MTX. The data suggest that benzimidazoles should not be administered with HDMTX. Assessment of patient self-medication of over-the-counter drugs before HDMTX is administered, would seem prudent to prevent toxicity.

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