High-dose methotrexate in adults with osteosarcoma: a population pharmacokinetics study and validation of a new limited sampling strategy

Charlotte Dupuis[a,c,*], Cedric Mercier[a,c,*], Chenguang Yang[a], Suzanne Monjanel-Mouterde[b,c], Joseph Ciccolini[b,c], Raphaelle Fanciullino[c], Bertrand Pourroy[b], Jean-Laurent Deville[a], Florence Duffaud[a,c], Danielle Bagarry-Liegey[a], Alain Durand[b], Athanassios Iliadis[c] and Roger Favre[a,c]

Preoperative high-dose methotrexate (HD-MTX) with folinic acid (leucovorin) rescue is still a mainstay in the treatment of osteosarcoma. This anticancer agent is characterized by a narrow therapeutic index and wide interpatients variability. To ensure effective and safe administration of HD-MTX, we had earlier developed an adaptive-dosing schedule with a feedback strategy. In our institute, the MTX dosage was tailored according to individual pharmacokinetics parameters, determined in real time both from two blood samples (3.5 and 4.5 h) and from Bayesian population parameters. Up to 20 g of MTX was safely administered as 8-h infusions. Low MTX elimination rate has, however, been reported in 15–20% of the patients, and forecasting the MTX elimination phase and the management of leucovorin rescue is still a challenging issue in clinical oncology. This study aims at identifying the clinical or biological covariates related to impaired MTX clearance, and at validating a new limited sampling strategy (LSS), allowing for the accurate prediction of the MTX terminal elimination phase. This retrospective study was carried out on 49 patients (30 men, 19 women; mean age, 26.7 years) treated for osteosarcoma with HD-MTX. The population and individual pharmacokinetics parameters were computed, before the identification of the relevant covariates. Different LSSs were then tested, to predict accurately when the MTX plasma concentrations would drop below 0.2 μmol/l, the threshold associated with the end of the rescue of leucovorin with alkaline hydration. Two main covariates (creatinemia clearance and alanine aminotransferase) were correlated with MTX clearance. Conversely, the impact of body surface area on MTX pharmacokinetics was weak, suggesting that dosing schedules based on body surface area were inadequate and potentially hazardous. A new LSS predicting accurately when the MTX concentration would reach 0.2 μmol/l has been validated; blood samples are stopped as soon as the MTX concentration drops to 1 μmol/l. With this LSS, our retrospective study suggests that 60% of the patients would have left the hospital earlier than they actually did owing to a better forecasting of the MTX decrease, thus improving their quality of life while improving the cost-effectiveness for the institute. HD-MTX can be administered safely using an adaptive-dosing strategy with drug monitoring. Moreover, pharmacokinetic modeling permits the accurate forecasting of the MTX elimination profile, thus allowing for a better management of the postinfusion care of cancer patients treated with particularly high doses of this drug. *Anti-Cancer Drugs* 19:267–273 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2008, **19**:267–273

Keywords: Bayesian estimation, high-dose methotrexate, leucovorin rescue, osteosarcoma, pharmacokinetics

[a]Oncology Unit and [b]Clinical Pharmacokinetics, La Timone University Hospital, Marseille and [c]EA3286, Pharmacokinetics Laboratory, La Timone Faculty of Pharmacy, Marseilles, France

Correspondence to Dr Cedric Mercier, MD, PhD, Medical Oncology Unit, CHU Timone Adultes, 264, rue Saint Pierre, Marseille Cedex 05, France
Tel: +33 491 384 551; e-mail: cedric.mercier@ap-hm.fr

*Charlotte Dupuis and Cedric Mercier contributed equally to this work.

Received 19 July 2007 **Revised form accepted** 12 September 2007

Introduction

Osteosarcoma is the most common primary malignant bone tumor. Over the past decades, dramatic improvements have been made in the treatment and the final clinical outcomes of this highly aggressive disease. Until the early 1970s, surgery was the only therapy available for osteosarcoma. Even during the apparently localized stages of the disease, radical surgery alone led to a cure, but of very low rate, and relapse was observed in approximately 85–90% of the patients. The concept of administering chemotherapy before definitive surgery on the primary tumor was first introduced by Rosen *et al.* [1,2]. Since then, high-dose methotrexate (HD-MTX) with folinic acid (leucovorin) rescue is still the gold standard therapy in the treatment of osteosarcoma. Today, with the combination of preoperative or 'neoadjuvant' chemotherapy and radical surgery, disease-free survival can reach up to 70% [3]. The challenge that now remains is to improve

0959-4973

the treatment of the 30% of the patients with poor clinical outcomes. Various factors including the presence of metastases, the histological response to chemotherapy (e.g. Huvos grade) and the dose of chemotherapy have been demonstrated to impact on the final outcomes of osteosarcomas, regardless of their stages. Others studies have focused on the relationship between MTX peak serum concentration and histological tumor response or disease-free survival [4–6]. It has been shown that MTX antitumor efficacy mainly depends on both MTX plasma concentration and exposure duration. Indeed, when MTX was infused for over 6 h, it was demonstrated that the response rate increased when an MTX serum peak level of 700 μmol/l was reached [7]. Other studies have suggested that an MTX concentration of 1000 μmol/l was needed to maximize the tumor response rate [8–10]. Conversely, a decrease of overall survival was observed when MTX serum concentrations exceeded 1500 μmol/l, partly owing to the occurrence of morbidities linked with the severe toxicities encountered [3]. To limit the occurrence of such severe toxicities, rescue with leucovorin combined with alkaline hydration is a standard practice with HD-MTX protocols. Considering the narrow therapeutic index of MTX, we have developed and validated a protocol derived from Rosen's T10 method, using Bayesian adaptive dosing with feedback strategy [11]. With this schedule, MTX concentrations were accurately monitored to reach the optimal target of 1000 μmol/l; the incidence of life-threatening toxicities dramatically decreased in our patients. This model was, nevertheless, not designed to predict the MTX terminal elimination phase, and the management of the leucovorin rescue was based on the daily monitoring of the MTX decay. Owing to the wide interpatient variability in the MTX terminal elimination phase, the adaptation of leucovorin doses indeed requires several, time-consuming blood samples analyses, with the subsequent impact on the patient's quality of life. To improve the cost-effectiveness of our protocol, the first goal of this study was to identify the covariates that were potentially implicated in the interindividual variations of the HD-MTX pharmacokinetics (PK) profile. The next goal was to validate a new limited sampling strategy (LSS) enabling an easier management of the leucovorin rescue.

Patients and methods

Patients

Retrospective data from 49 patients (30 men and 19 women), treated for a newly diagnosed osteosarcoma in the department of Medical Oncology of La Timone University Hospital of Marseille, France, from February 1991 to January 2004, were entered in this study. The main patient characteristics are summarized in Table 1.

Complete kinetics built from at least eight samples were available for each patient. Only the first course was considered for each patient. Fourteen biological and clinical covariates, which had been collected before the first MTX administration, are defined and coded as follows: age (AGE); sex (SEX = 0, male; 1, female); weight (WGT); height (HGT); body surface area (BSA); creatinine (CREA); creatinine clearance (CRCL); aspartate aminotransferase (ASAT); alanine aminotransferase (ALAT); γ-glutamyl transferase (GGT); alkaline phosphatase (PAL); total bilirubin (BT); hemoglobin (HB) and neutrophil count (PNN).

Table 1 Patient characteristics

Covariates	Mean ± SD	(Minimum–Maximum)
AGE (years)	27.58 ± 10.59	14–57
WGT (kg)	65.38 ± 11.32	45–93
HGT (cm)	173.14 ± 8.16	155–192
BSA (m^2)	1.76 ± 0.17	1.46–2.00
CREA (μmol/l)	86.68 ± 28.77	54–229
Creatinine clearance (ml/min)	84.25 ± 22.41	42–160
ASAT (UI/l)	17.66 ± 11.77	5–61
ALAT (UI/l)	28.33 ± 41.42	3–95
GGT (UI/l)	31.03 ± 32.91	4–140
PAL (UI/l)	1.44 ± 2.24	0.02–10
BT (μmol/l)	9 ± 4.44	3–15
HB (g/l)	128.32 ± 17.75	92–156
PNN ($103/mm^3$)	5.07 ± 2.01	2.64–12.58

AGE, age; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BSA, body surface area; BT, total bilirubin; CREA, creatinine; GGT, γ-glutamyl transferase; HB, hemoglobin; HGT, height; PAL, alkaline phosphatase; PNN, neutrophil count; WGT, weight.

CRCL was estimated using Wright's formula [12]:

$$CRCL = \{[6580 - (38.8 \times AGE)]*BSA*[1 - (0.168 \times SEX)]\}/CREA$$ (male: SEX = 0; female: SEX = 1).

Real-time high-dose methotrexate monitoring

High-dose methotrexate administration

All the patients had been treated using a standardized schedule (SO587) derived from Rosen's T10 protocol [13]. According to this protocol, HD-MTX with leucovorin rescue was used preoperatively as a single agent.

Each course consisted of the following two phases:

- The first phase (hyperhydration and urine alkalinization) involved a 12-h hydration period (3 l/24 h of 5% dextrose in water with 15 ml of 10% KCl) and urine alkalinization (360 ml of 1.4% sodium bicarbonate). Urine pH was checked throughout this period.
- The second phase (MTX infusion) started immediately after the first phase if and only the pH of urine was above 7 and urine flow was at least 200 ml/h. MTX infusion was given through a central venous catheter at a constant rate of 3000 mg/h for the first 6 h. The dose was then adapted, after the Bayesian estimation of the individual pharmacokinetics parameters from the

sixth hour to the eighth, to reach a target MTX concentration at the end of the infusion (C_{max}) of 1000 μmol/l [10].
- The last phase (leucovorin rescue) started 36 h after the start of the MTX infusion and consisted of a 15-mg leucovorin infusion every 6 h until the MTX concentration dropped below 0.2 μmol/l. In case of low elimination of MTX, leucovorin doses were increased according to standard recommendations.

Hydration and urine alkalinization were monitored for the total duration of the three phases.

Sampling times

Venous blood samples were all collected into heparinized tubes at the following times: before the beginning of the infusion (T0), then 3.5 and 4.5 h into the infusion, at the end of the MTX infusion (C_{max}), 30 and 47 h after the beginning of the infusion, and thereafter, every 24 h until the residual MTX concentrations dropped below 0.2 μmol/l.

Methotrexate assay

Blood samples were immediately centrifuged at 3000*g* for 5 min at room temperature. MTX plasma concentrations were measured by fluorescence polarization immunoassay with a TDX FLX analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). The limit of quantification of this assay was 0.01 μmol/l with an interday precision of 6.7% [14].

Bayesian estimation and dose adjustment

For all patients, Bayesian estimation using Apis software [15] had been performed in real time with the two MTX concentration values collected at 3.5 and 4.5 h and using a reference population as described by Bruno *et al.* [16]. Individual PK parameters were computed, and the dose administered from the sixth to the eighth hour was tailored to reach the desired target C_{max}.

Retrospective NONMEM analysis

Pharmacokinetic parameters

Retrospective determination of population and individual pharmacokinetics parameters was performed with NONMEM 5.0 software. As recommended by Sheiner *et al.* [17–19], two-compartment and three-compartment models, additive, multiplicative or mixed error models, first order (FO) and first order conditional estimation (FOCE) methods were tested and compared. The choice of the best model was determined by comparisons of the objective function criterion (decrease > 4), analyses of the goodness of fit including observed MTX values (dependent variable) versus population predicted values (PRED) and individual predicted values (IPRED) and by residuals analysis. The goodness of fit was analyzed using Xpose (version 3.1) under S + (version 6.1) [20]. Using the best model obtained for each patient, Bayesian estimates were computed for clearance (CL), area under the time-concentration curve (AUC), volume of distribution (V_d) and intercompartment constants (K_{ij}).

Covariates analysis

Correlation between pharmacokinetics parameters and the 14 covariates defined above were tested with Xpose 3.01 software [20], using three different statistical tools:

- General additive models (GAM), for which all covariates that lead to a decrease of Akaike criterion greater than 4 are considered to be significant.
- Bootstrap of the GAM (BOOT), which tests the inclusion frequency of the covariates in linear and nonlinear models (covariates improved the model significantly if their inclusion frequency was greater than 50%).
- Tree-based modeling (TREE), an algorithm that splits the whole population into two subsets of patients in which the mean of the PK parameter is statistically different after the cutoff value of a covariate is reached.

All covariates statistically correlated with MTX clearance were then integrated into the NONMEM base structural model.

The monitoring/achievement of the target level of 0.2 μmol/l needed for leucovorin rescue

To validate the best estimation of the end time of leucovorin rescue, we randomly split our whole data set into a validation subset (15 patients) and a population subset (34 patients).

We used the population model built from the pharmacokinetics data of the 34 patients to perform a Bayesian estimation of the pharmacokinetic profile of the validation subset, using two different LSS procedures:

- The first LSS retained only the concentration times performed before T47 (h).
- The second LSS used all the samples withdrawn until the MTX concentration was found to be equal to or below 1 μmol/l.

The time at which the simulated MTX concentrations reached the 0.2-μmol/l threshold was compared with the actual time at which the concentration reached this level.

Results

Performance of tailored dosage: C_{max} targeting

The mean MTX dose effectively delivered in the 49 patients was as follows: 24.4 g (range: 18–30). Clearances ranged from 3.05 to 8.15 l/h (mean value of 6.57 ± 0.97 l/h)

and the central volume of distribution ranged from 39.68 to 48.51 (mean value of 42.75 ± 1.471).

The performance of the adaptive-dosing protocol of MTX was determined by comparing the theoretical target $C_{max} = 1000$ μmol/l with the experimental one actually assayed at the end of the infusion. The mean experimental C_{max} was 986.6 ± 132.3 μmol/l and the mean bias was −3 ± 16%, with an overall precision of 8%. No significant difference was found between experimental and target exposure ($P = 0.24$, paired t-test).

Dose adaptation scheduled for 6 h after the start of the MTX infusion had been required in almost all patients ($n = 48$). As predicted by the model, four patients had already reached an MTX concentration above the C_{max} target at 6 h, and consequently MTX infusion was stopped. No other patient with low MTX clearance has been overexposed ($C_{max} > 1500$ μmol/l), and only two with high MTX clearances had recorded a C_{max} below 700 μmol/l (630 and 660 μmol/l).

Retrospective NonMEM and Xpose analysis

NONMEM basic model

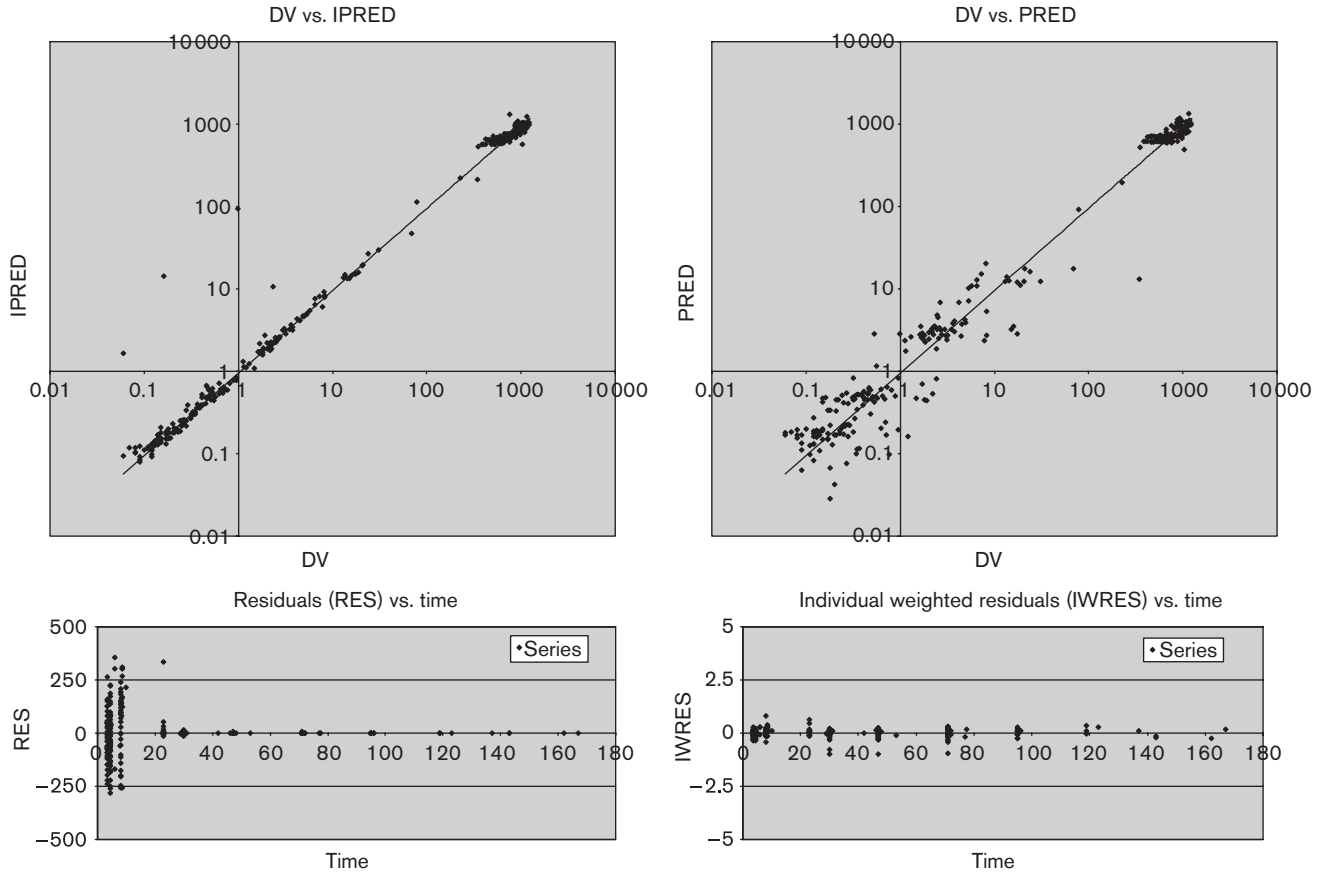
The pharmacokinetics of HD-MTX was best described by a three-compartment model with a mixed error model, using the FOCE method (ADVAN 11, TRANS 4; Fig. 1). Excellent subsequent fitting of the measured concentrations was achieved (Fig. 2). The basic model (i.e. without covariates) gave a typical value of MTX clearance of 6.41 l/h with interindividual variability (CV%) of 20%.

Covariates analysis and NONMEM final model

Methotrexate clearance

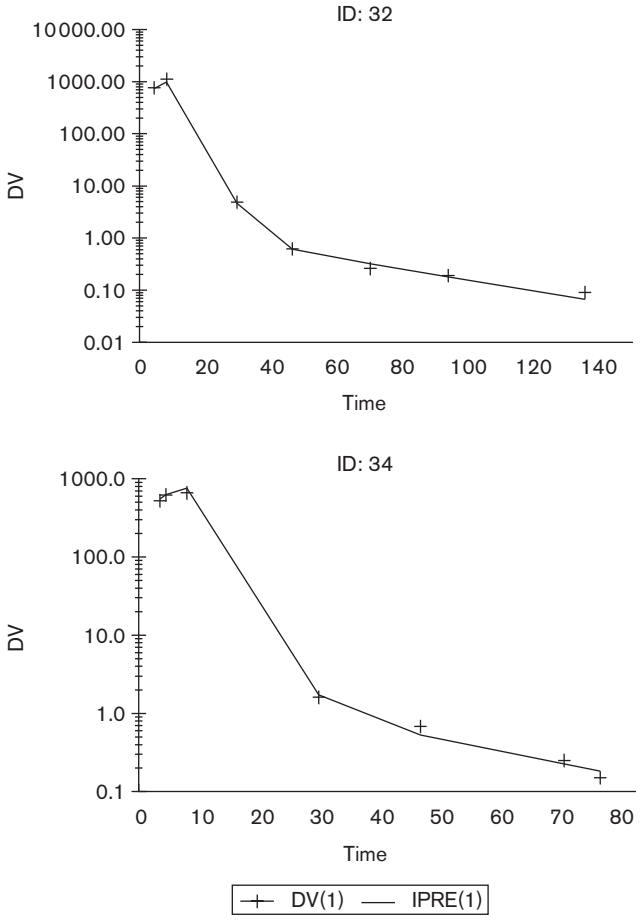
Among the 14 covariates tested using GAM and bootstrap of the GAM in Xpose v3 software, only two (ALAT and CRCL) have been found to be correlated with MTX clearance. It is worth noting that no relationship between

Fig. 1



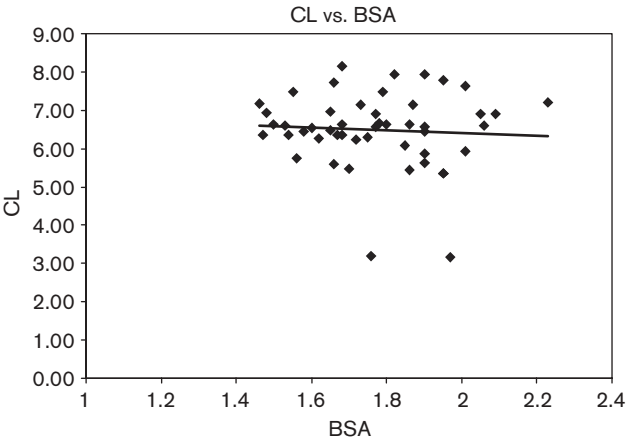
Correlation between experimental and predicted MTX concentrations in plasma (DV vs. IPRED). Graphic display of the relationship between experimental (dependent variable = DV) and predicted MTX concentrations in plasma, along with the residuals [weighted (WRES) and nonweighted (RES)] throughout the time period. DV, experimental concentration; MTX, methotrexate; PRED, population model prediction; IPRED, individual model prediction. Values were equally distributed around the identity line; no bias was observed between experimental and predicted values throughout the time period.

Fig. 2



Goodness of fit of individual predicted MTX concentrations vs. experimental values (two typical patients, one therapeutic course). Data generated by the model showed excellent fit with measured values. Data are from two representative patients. MTX, methotrexate.

Fig. 3



Impact of BSA on HD-MTX total clearance. No correlation was observed between the BSA of patients and MTX clearance. BSA, body surface area; HD-MTX, high-dose methotrexate.

Table 2 Impact of covariates on MTX clearance

	Cutoff value	<Cutoff value	>Cutoff value	Difference (%)
Mean MTX clearance (l/h) when covariates are				
CRCL (ml/min)	89	5.945	6.607	10
ALAT (UI/l)	20	6.671	5.692	15
Mean volume of distribution (l) when covariates are				
HGT (cm)	181	21.76	22.63	4
BSA (m^2)	1.64	21.35	22.08	3
HB (g/dl)	13.45	21.75	22.45	3

ALAT, alanine aminotransferase; BSA, body surface area; CRCL, creatinine clearance; HB, hemoglobin; HGT, height; MTX, methotrexate.

the BSA and MTX clearance has been demonstrated, as shown in Fig. 3.

Tree-based modeling demonstrated that patients with CRCL below 89 µmol/l and those with ALAT above 20 UI/l had statistically significant decreases of MTX clearance of 10 and 15%, respectively (Table 2).

The inclusion of ALAT [$CL = \theta1*(1 + \theta7*ALAT)$] or CRCL (if CRCL ≤ 89 µmol/l, $CL = \theta1$; if CRCL > 89 µmol/l, $CL = \theta3$) in the NONMEM model yielded statistically significant improvements, with respective decreases in the objective function of -22 and -19 ($P < 0.05$).

The final model has been defined as follows: If CRCL ≤ 89 µmol/l, [$CL = \theta1*(1 + \theta8*ALAT)$] and if CRCL > 89 µmol/l, [$CL = \theta3*(1 + \theta8*ALAT)$]. The inclusion of these covariates improved the NONMEM model significantly, with a decrease in the objective function of -42 ($P < 0.005$) and of interindividual variability (CV% = 11.8).

Volume of distribution

Only three covariates (HGT, BSA and HB) were correlated with the volume of distribution (Table 2).

Limited sampling strategies for leucovorin rescue monitoring

Among the different LSSs tested, the one that used all the samples withdrawn, until one MTX concentration was equal to or below 1 µmol/l, gave the best results for the estimation of the terminal elimination phase; this LSS allowed us to determine accurately the next time when the MTX concentrations reached 0.2 µmol/l. Comparison between the estimated time to reach 0.2 µmol/l and the actual time to do so showed a mean error of 0.3 h, without any statistical bias. Among the 15 patients in our validation set, 10 would have their hospitalization times reduced by at least 1 day (Table 3)

Table 3 Comparison between simulated 0.2 μmol/l reaching time and actual values

Patients	Theoretical time MTX < 0.2 (h)	Real time MTX < 0.2 (h)	Difference (h)	Bias (%)	Real out time (h)	Difference (h)
1	85	95	10	10.5	124	39
2	60	60	0	0.0	76	16
3	48	48	0	0.0	52	4
4	50	50	0	0.0	76	26
5	48	48	0	0.0	76	28
6	50	48	−2	−4.2	76	26
7	62	59	−3	−5.1	76	14
8	76	72	−4	−5.6	76	0
9	62	56	−6	−10.7	76	14
10	76	74	−2	−2.7	82	6
11	60	60	0	0.0	76	16
12	72	70	−2	−2.9	76	4
13	65	63	−2	−3.2	76	11
14	135	145	10	6.9	172	37
15	68	65	−3	−4.6	76	8
Mean			−0.3	−1.43		16.60
SD			4.4	4.92		11.69

MTX, methotrexate.

and the average reduction in the number of blood samples would have been two.

Discussion

The introduction of HD-MTX administration with leucovorin rescue by Jaffe in 1972 dramatically improved the management of osteogenic sarcoma. Several studies have demonstrated that the response to HD-MTX is correlated with the delivered dose and the serum level of MTX, thus stressing the need for developing adaptive-dosing methods [3,4,6,21]. Our institute has developed over the past decade Bayesian, dynamic adaptive dosing with feedback methods, which can be applied to various anticancer drugs such as CDDP, CBDCA, etoposide and MTX [11,22–24]. Dose adjustment is normally performed, to reach a predetermined plasma concentration that is associated with optimal response and fully manageable toxicities.

The toxicity of HD-MTX is, in main part, related to its concentration in the terminal elimination phase [25]. Correlations between MTX PK parameters and tumor responses have been examined in several studies [3,26]. We previously demonstrated that therapeutic drug monitoring of HD-MTX was feasible in a routine clinical setting in patients with inflammatory breast cancer, and that it did indeed reduce interpatient variability [11,14]. No multivariate analysis has, however, been performed to identify the covariates associated with MTX clearance; unpredictably, some patients with impaired elimination were forced to stay longer in our institute for extra leucovorin rescue with daily sampling until the MTX concentrations fell below 0.2 μmol/l, which is the threshold usually associated with toxicity and requiring leucovorin rescue. In this study, we retrospectively examined the possible correlation between MTX clearance, distribution volume and a number of covariates, as an attempt to detect patients with reduced elimination patterns.

To examine the critical terminal elimination phase, we used a three-compartment model that takes better account of patients with a low MTX elimination rate [27]. Among the 14 examined covariates, only ALAT and CRCL were correlated with MTX elimination clearance. Importantly, we found that BSA was not correlated with drug clearance, thus confirming that adjusting doses of this anticancer drug with BSA is certainly not adequate: considering the narrowness of the MTX therapeutic window, this type of dose adjustment is potentially hazardous for cancer patients.

Moreover, CRCL was correlated with MTX clearance. Not surprisingly, ALAT was strongly correlated with MTX clearance. One of the principal metabolic pathways of MTX is hepatic, as MTX is metabolized into 7-OH-MTX, which is a less active metabolite [28]. Regarding the impact of ALAT on the MTX PK profile, liver dysfunction could lead to impaired metabolism and explain the increased toxicities (e.g. renal and hematologic) observed in patients with liver disease. These data are fully consistent with previous observations by Evans *et al.* [29], which demonstrated that change in hepatic and renal function could explain delayed MTX clearance, even when creatinine and bilirubin counts were normal [30].

We found that three covariates did correlate with distribution volume. Interestingly, the mean distribution volume increased with hemoglobin level, thus highlighting the fact that erythrocytes could be considered as storage compartments for this drug, with several pharmacological implications. Polyglutamation of MTX occurs via intracellular conversion. The resulting metabolites are at least as potent as MTX itself in inhibiting DHFR, but with a slower dissociation rate from this enzyme [28,30]. Like MTX, 7-OH-MTX is also polyglutamylated in the cells, and the retention of these polyglutamated forms in the erythrocytes could therefore contribute to MTX toxicity. One of our principal aims was to estimate when the MTX concentration dropped below 0.2 μmol/l [14,30], as soon as possible after the event. Accurate early estimation of this reaching of the 0.2-μmol/l threshold would indeed avoid daily sampling of the patients. On the basis of our Bayesian prediction, we found that 10 out of the 15 patients in our study (60%) would have been authorized to leave the institute safely at least 1 day before they actually did, thus improving their quality of life and being cost-effective. Importantly, we found that early samples collected during the infusion were sufficient to make an accurate estimation of the late MTX elimination phase; therefore, it would not be necessary to sample patients on a daily basis anymore to manage the use of leucovorin.

Conclusion

Taken altogether, this study proves that it is possible to administer HD-MTX with a good prediction of the MTX elimination pattern, thus allowing for an early forecast of each patient's hospital stay, on the basis of a minimal number of early samples. This strategy should reduce not only the total number of samples to be withdrawn to monitor MTX exposure, but also the overall hospital stay in patients with osteosarcoma.

References

1 Rosen G, Marcove RC, Caparros B, Nirenberg A, Kosloff C, Huvos AG. Primary osteogenic sarcoma: the rationale for preoperative chemotherapy and delayed surgery. *Cancer* 1979; **43**:2163–2177.

2 Winkler K, Beron G, Kotz R, Salzer-Kuntschik M, Beck J, Beck W, *et al*. Neoadjuvant chemotherapy for osteogenic sarcoma: results of a cooperative German/Austrian study. *J Clin Oncol* 1984; **2**:617–624.

3 Crews KR, Liu T, Rodriguez-Galindo C, Tan M, Meyer WH, Panetta JC, *et al*. High-dose methotrexate pharmacokinetics and outcome of children and young adults with osteosarcoma. *Cancer* 2004; **100**:1724–1733.

4 Aquerreta I, Aldaz A, Giraldez J, Sierrasesumaga L. Methotrexate pharmacokinetics and survival in osteosarcoma. *Pediatr Blood Cancer* 2004; **42**:52–58.

5 Bacci G, Ferrari S, Delepine N, Bertoni F, Picci P, Mercuri M, *et al*. Predictive factors of histologic response to primary chemotherapy in osteosarcoma of the extremity: study of 272 patients preoperatively treated with high-dose methotrexate, doxorubicin, and cisplatin. *J Clin Oncol* 1998; **16**:658–663.

6 Graf N, Winkler K, Betlemovic M, Fuchs N, Bode U. Methotrexate pharmacokinetics and prognosis in osteosarcoma. *J Clin Oncol* 1994; **12**:1443–1451.

7 Borsi JD, Schuler D, Moe PJ. Methotrexate administered by 6 and 24-h infusion: a pharmacokinetic comparison. *Cancer Chemother Pharmacol* 1988; **22**:33–35.

8 Delepine N, Desbois JC, Delepine G, Cornille H, Astier A, Alkallaf S. Individualization of the dosage of high-dose methotrexate by assaying plasma concentration. Therapeutic value in osteogenic sarcoma. *Bull Cancer* 1989; **76**:913–918.

9 Delepine N, Delepine G, Desbois JC. A monocentric therapy study: an approach to optimize the results of the treatment of osteosarcoma by protocols based upon HDMTX, associated with systematic conservative surgery. *Cancer Treat Res* 1993; **62**:327–332.

10 Zelcer S, Kellick M, Wexler LH, Shi W, Sankaran M, Lo S, *et al*. Methotrexate levels and outcome in osteosarcoma. *Pediatr Blood Cancer* 2005; **44**: 638–642.

11 Pignon T, Lacarelle B, Duffaud F, Guillet P, Catalin J, Durand A, *et al*. Pharmacokinetics of high-dose methotrexate in adult osteogenic sarcoma. *Cancer Chemother Pharmacol* 1994; **33**:420–424.

12 Wright JG, Boddy AV, Highley M, Fenwick J, McGill A, Calvert AH. Estimation of glomerular filtration rate in cancer patients. *Br J Cancer* 2001; **84**:452–459.

13 Rosen G, Caparros B, Huvos AG, Kosloff C, Nirenberg A, Cacavio A, *et al*. Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. *Cancer* 1982; **49**:1221–1230.

14 Monjanel-Mouterde S, Lejeune C, Ciccolini J, Merite N, Hadjaj D, Bonnier P, *et al*. Bayesian population model of methotrexate to guide dosage adjustments for folate rescue in patients with breast cancer. *J Clin Pharm Ther* 2002; **27**:189–195.

15 Iliadis A, Brown AC, Huggins ML. APIS: a software for model identification, simulation and dosage regimen calculations in clinical and experimental pharmacokinetics. *Comput Methods Programs Biomed* 1992; **38**: 227–239.

16 Bruno R, Iliadis A, Favre R, Lena N, Imbert AM, Cano JP. Dosage predictions in high-dose methotrexate infusions. Part 2: Bayesian estimation of methotrexate clearance. *Cancer Drug Deliv* 1985; **2**:277–283.

17 Karlsson MO, Beal SL, Sheiner LB. Three new residual error models for population PK/PD analyses. *J Pharmacokinet Biopharm* 1995; **23**: 651–672.

18 Mandema JW, Verotta D, Sheiner LB. Building population pharmacokinetic–pharmacodynamic models. I. Models for covariate effects. *J Pharmacokinet Biopharm* 1992; **20**:511–528.

19 Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. III. Monoexponential model: routine clinical pharmacokinetic data. *J Pharmacokinet Biopharm* 1983; **11**:303–319.

20 Jonsson EN, Karlsson MO. Xpose: an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Comput Methods Programs Biomed* 1999; **58**:51–64.

21 Bacci G, Loro L, Longhi A, Bertoni F, Bacchini P, Versari M, *et al*. No correlation between methotrexate serum level and histologic response in the pre-operative treatment of extremity osteosarcoma. *Anticancer Drugs* 2006; **17**:411–415.

22 Ciccolini J, Monjanel-Mouterde S, Bun SS, Blanc C, Duffaud F, Favre R, *et al*. Population pharmacokinetics of etoposide: application to therapeutic drug monitoring. *Ther Drug Monit* 2002; **24**:709–714.

23 Mercier C, Ciccolini J, Pourroy B, Fanciullino R, Duffaud F, Digue L, *et al*. Dose individualization of carboplatin after a 120-h infusion schedule: higher dose intensity but fewer toxicities. *Ther Drug Monit* 2006; **28**:212–218.

24 Salas S, Mercier C, Ciccolini J, Pourroy B, Fanciullino R, Tranchand B, *et al*. Therapeutic drug monitoring for dose individualization of cisplatin in testicular cancer patients based upon total platinum measurement in plasma. *Ther Drug Monit* 2006; **28**:532–539.

25 Blum R, Seymour JF, Toner G. Significant impairment of high-dose methotrexate clearance following vancomycin administration in the absence of overt renal impairment. *Ann Oncol* 2002; **13**:327–330.

26 Ferreri AJ, Guerra E, Regazzi M, Pasini F, Ambrosetti A, Pivnik A, *et al*. Areas under the curve of methotrexate and creatinine clearance are outcome-determining factors in primary CNS lymphomas. *Br J Cancer* 2004; **90**:353–358.

27 Sabot C, Debord J, Roullet B, Marquet P, Merle L, Lachatre G. Comparison of 2- and 3-compartment models for the Bayesian estimation of methotrexate pharmacokinetics. *Int J Clin Pharmacol Ther* 1995; **33**: 164–169.

28 Fabre G, Fabre I, Matherly LH, Cano JP, Goldman ID. Synthesis and properties of 7-hydroxymethotrexate polyglutamyl derivatives in Ehrlich ascites tumor cells *in vitro*. *J Biol Chem* 1984; **259**:5066–5072.

29 Evans WE, Relling MV, Boyett JM, Pui CH. Does pharmacokinetic variability influence the efficacy of high-dose methotrexate for the treatment of children with acute lymphoblastic leukemia: what can we learn from small studies? *Leuk Res* 1997; **21**:435–437.

30 Relling MV, Fairclough D, Ayers D, Crom WR, Rodman JH, Pui CH, *et al*. Patient characteristics associated with high-risk methotrexate concentrations and toxicity. *J Clin Oncol* 1994; **12**:1667–1672.