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# Pharmacokinetics of high-dose methotrexate in adult osteogenic sarcoma

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**Abstract.** The pharmacokinetics of 222 infusions of high-dose methotrexate (MTX) with leucovorin rescue were studied in 22 adults with osteosarcoma. To reduce the variability of plasma concentration, we individualized dose regimens using a Bayesian method to reach a concentration of $10^{-3}$ *M* MTX at the end of an 8-h infusion. The mean concentration observed at the end of the infusion was $1016 \pm 143$ µmol/l. The mean dose delivered was $13.2 \pm 2$ g/m$^2$. The clearance was $49.1 \pm 11.7$ ml min$^{-1}$ m$^{-2}$. The decay of the plasma concentration of MTX after completion of the infusion followed a two-compartment model with a $t_{1/2\alpha}$ of $2.66 \pm 0.82$ h and a $t_{1/2\beta}$ of $15.69 \pm 8.63$ h. The volume of distribution was $0.32 \pm 0.08$ l/kg. As compared with previously published data, the interindividual and intraindividual variations in the concentration at the end of the infusion were reduced, with values of 14% and 5.9%–21%, respectively, being obtained. Severe toxicities were avoided, and there were only 3 hematologic and 8 digestive grade 3 side effects and no grade 4 complication. The $t_{1/2\alpha}$ and the MTX plasma concentrations at 23 and 47 h were correlated with renal toxicity ($P < 0.001$). However, no correlation was found between the pharmacokinetic parameters and other signs of toxicity. There was no significant difference in pharmacokinetics between the toxic and nontoxic groups. In the same manner, the parameters of the group of patients sensitive to MTX were not statistically significantly different from those of the group of nonsensitive patients.

## Introduction

The use of high-dose methotrexate (HDMTX) with citrovorum factor rescue in the treatment of osteogenic sarcoma represents a major advance in the treatment of this tumor [17]. Serum concentration has been demonstrated as the most significant therapeutic factor for a good response in this disease [24]. Severe side effects of MTX vary with both the concentration and the duration of exposure to this drug [21]. In spite of considerable intraindividual and interindividual variabilities of serum concentration that have reached even 900% [20], most administrations of HDMTX with folinic acid rescue have empiric rather than pharmacokinetic bases. To reduce these variabilities, we individualized doses by Bayesian estimation of pharmacokinetic parameters so as to reach a predetermined plasma level at the end of the infusion for each patient. In this study, we examined the pharmacokinetic (pk) parameters of HDMTX in 22 adults with osteosarcoma who received 222 infusions of HDMTX lasting 8 h each to achieve a theoretical plasma concentration ($C_{max}$) of $10^{-3}$ *M* at the end of the infusion.

## Patients and methods

*Patients.* A total of 22 adult patients with primary osteosarcoma were treated using a standardized therapy protocol (SO587) derived from the T10 protocol of Rosen et al. [27]. According to this protocol, HDMTX with citrovorum factor rescue was used preoperatively as a single agent in the treatment of these tumors. Patients showing a good or medium response to preoperative chemotherapy were selected for resumption of the HDMTX-containing regimen postoperatively. The remaining patients were selected as poor responders in whom the HDMTX-containing regimen was discontinued following surgical resection of the primary tumor (Table 1). In this study, we defined a group of patients as being sensitive to HDMTX, which included good and medium responders, as compared with poor responders, who were considered to be nonsensitive. The protocol is shown in Table 2.

The age of the patients varied from 15.7 to 62.5 years (mean ± SD, $24.5 \pm 11.7$ years). A total of 228 infusions of HDMTX were performed during primary treatment and when tumors relapsed. Six courses were rejected from this study due to insufficient information (measured level not available) or to the choice of a desired $C_{max}$ value different from $10^{-3}$ *M*.

*Drug administration.* HDMTX was given for each treatment according to the individualization of the dose protocol described below. Infusions

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**Table 1.** Assigned values for evaluation of the effect of preoperative chemotherapy

| | Response | | | Decrease | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Excellent | Good | Medium | No | ≤25% | >25% ≤50% | >50% ≤90% | >90% <100% | 100% |
| Clinical signs | +1 | +½ | 0 | −1 | – | – | – | – | – |
| Volume of tumor on CT scan or MRI | – | – | – | −1 | 0 | +1 | +2 | +2 | +2 |
| Vascularization of tumor on arteriography | – | – | – | −1 | 0 | +1 | +2 | +3 | +3 |
| Histologic necrosis | – | – | – | 0 | 0 | 0 | +2 | +3 | +4 |

The response to preoperative chemotherapy was assessed according to the 4 items reported in the table. They were evaluated before and after the chemotherapy. The score was calculated by adding the results obtained for each item. Score: ≥6, good responders; ≥3 but <6, medium responders; <3, poor responders

**Table 2.** Osteosarcoma treatment regimen

| | |
|---|---|
| All patients: | |
| Weeks 1, 2, 3, 4, 9, 10 | HDMTX $10^{-3}$ *M* |
| Week 6 | en bloc resection of the primary tumor |
| Weeks 6, 16 | Bleomycin 15 mg/m$^2$ |
| | Cyclophosphamide 600 mg/m$^2$ |
| | Dactinomicin 600 μg/m$^2$ |
| Week 11 | Pirarubicin 60 mg/m$^2$ |
| Good Responders: | |
| Weeks 19, 20, 24, 25, 29, 30, 34, 35 | HDMTX $10^{-3}$ *M* |
| Weeks 21, 31 | Pirarubicin 60 mg/m$^2$ |
| Weeks 26, 36 | Bleomycin 15 mg/m$^2$ |
| | Cyclophosphamide 600 mg/m$^2$ |
| | Dactinomicin 600 μg/m$^2$ |
| Medium responders: | |
| Weeks 19, 20, 24, 25, 29, 30, 34, 35 | HDMTX $10^{-3}$*M* |
| Weeks 21, 26, 31, 36 | Ifosfamide 3 g/m$^2$, days 1, 2 |
| | Cisplatin in 120-h infusion with dose adjustment |
| | Pirarubicin 40 mg/m$^2$ |
| Poor Responders: | |
| Weeks 19, 25, 31 | Ifosfamide 3 g/m$^2$, days 1, 2 |
| | Cisplatin in 120-h infusion with dose adjustment |
| | Pirarubicin 40 mg/m$^2$ |
| Weeks 22, 28, 34 | Ifosfamide 3 g/m$^2$, days 1, 2 |
| | Etoposide 100 mg, days 1–6 |
| | Pirarubicin 40 mg/m$^2$, day 3 |

were given using a pump to obtain a constant rate of infusion. Each course consisted of three phases. The first corresponded to a period of 12 h for hydration and urine alkalinization (perfusion of 3 l of 5% glucose solution containing a total of 40 mol sodium bicarbonate and 20 mol potassium chloride). If the urinary output and pH were adequate (output of ≥200 ml/h, pH ≥7) at the end of the first phase, the second phase was begun. This consisted of an 8-h infusion of MTX given at a constant rate so as to reach the desired $C_{max}$ level of $10^{-3}$ *M*. MTX pk parameters were assessed by Bayesian estimation (BE) from two plasma concentrations measured after 4 and 5 h of infusion as described below, and a dose adjustment was performed in real time after the 6th h to reach as nearly as possible the desired $C_{max}$ value [6]. The pk parameters of MTX were estimated using the APIS program [13]. The third phase was begun 36 h later with folinic acid rescue, which was continued every 6 h until the MTX concentration fell below $10^{-7}$ *M*. The calculation of the dose of folinic acid was made from the following formula [28]:

Folinic acid (mg) = 10 × [MTX] (mg/l) × 0.76 × weight (kg).

Hydration and urine alkalinization were continued for the total duration of the three phases. For the first infusion, all patients were considered to have a theoretical clearance (CL) of 6 l/h, which gave a predicted starting dose of 24 g that could be adjusted at the 6th h after BE of pk parameters from the 4- and 5-h samples. The dose of MTX for each course was then assessed from the individual pharmacokinetics of the previous infusion and adjusted if necessary at the 6th h.

*Blood samples.* During the infusions, plasma samples were taken at the following theoretical times: 4, 5, 8 (end of infusion), 14, 23, 29, 47, 53, and 72 h and then every 24 h thereafter until an MTX serum level below $10^{-7}$ *M* was recorded.

*Drug analysis.* The particular nature of HDMTX administration monitoring makes it necessary to use a technique with a quick response for plasma MTX determination. MTX concentrations were determined by an enzymatic method adapted for application with a centrifugal Cobas-Bio (Roche) analyzer [16]. The limiting quantification of this method is $9\times10^{-9}$ mol/l.

*BE and dose adjustment.* For all patients, BE was performed in real time to allow dose adjustment. As previously described, BE was carried out by a minimizing function that takes into account prior information on the distribution of pk parameters in a given population and the partial and individual information obtained on drug concentrations at the 4th and 5th h [6]. The macrocoefficients and exponents of the model were then used for a simulation permitting the determination of the optimal dose needed to reach the desired MTX $C_{max}$ value.

*Pharmacokinetic analysis.* Since pk parameters obtained by BE could not be considered as reference parameters, we also estimated pk parameters from MTX concentrations measured during MTX-phase decay. All the individual MTX blood-concentration data sets were fitted to a two-compartment model using the APIS program. Estimation of individual parameters was based on the maximum-likelihood criterion [1]. Drug concentrations were expressed by a general complex formula developed by Iliadis et al. [14, 15]. The macrocoefficients (A, B) and macroexponents (α, β) of the model were estimated and then converted to pk parameters (CL, $t_{1/2\alpha}$, $t_{1/2\beta}$, Vd, and AUC).

*Toxicity.* Toxicity was evaluated according to World Health Organization guidelines [19].

*Statistical analysis.* For all parameters, statistical analysis was performed using the *t*-test to assess the significance of differences between mean values of grouped data. Coefficients of correlation were obtained by linear regression analysis. Statistical calculation was performed with the MEDLOG program (Logisoft France, Avon-Fontainebleau).

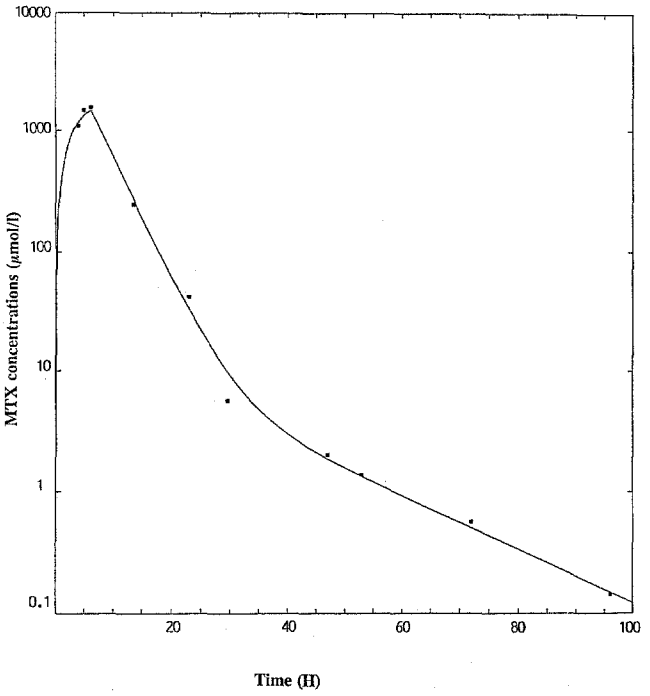


**Fig. 1.** Typical plasma MTX disappearance curve in a patient given a continuous 8-h infusion of HDMTX so as to reach a concentration of $10^{-3}$ *M* at the end of the infusion. The *solid symbols* represent observed values, whereas the *line* was obtained by computerized curve fitting

**Table 3.** Pharmacokinetic parameters of MTX as evaluated from 222 infusions

| | |
|---|---|
| Age (years) | 24.5 ±11.7 |
| Number of infusions | 222 |
| Dose (g/m²) | 13.2 ±2 |
| Concentration at the end of the infusion (µ*M*) | 1016 ±143 |
| CL (ml min⁻¹ m⁻²) | 49.1 ±11.7 |
| $t_{1/2\alpha}$ (h) | 2.66±0.82 |
| $t_{1/2\beta}$ (h) | 15.69±8.63 |
| Vd (l) | 22.4 ±5.6 |
| AUC (µmol l⁻¹ h) | 4780 ±1630 |

**Table 4.** Toxicities observed as graded according to WHO guidelines

| Toxicities | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| Renal | 4 | 0 | 0 | 0 |
| Mucositis | 0 | 13 | 0 | 0 |
| Digestive | 116 | 52 | 8 | 0 |
| Hematologic | 37 | 21 | 3 | 0 |

## Results

The pk parameters of MTX were evaluated in 22 patients for a total of 222 infusions and are summarized in Table 3. During this study, severe toxicities were avoided (Table 4). The mean duration of the infusions was 8.07±0.72 h. The mean dose delivered was 22.6±3.2 g (13.2±2 g/m²). The dose was increased (1.6±2.25 g; range, 2–10.5 g) for 103 infusions and was decreased (0.8±0.85 g; range, 0.05–7 g)

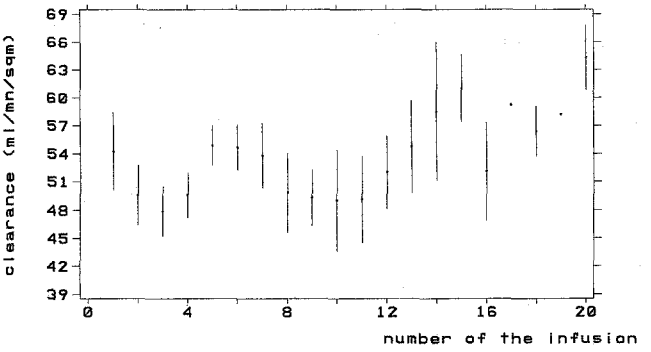


**Fig. 2.** Mean values ± SD for the clearance obtained in 222 infusions. The points represent the mean values obtained for the 22 patients at each course and the *bars* indicate the SD

for 104 infusions. For 15 infusions, dose adjustment was unnecessary. The mean concentrations observed at the 4th and 5th h were, respectively, 725±172 and 800±153 µmol/l. At the end of the infusion, the mean concentration observed was 1016±143 µmol/l, and this value did not significantly differ from the desired $C_{max}$ value of 1000 µmol/l. The interindividual coefficient of variation of concentrations observed at the end of the infusion was 14%, and the intraindividual variability ranged from 5.9% to 21%.

The plasma concentration decay of MTX at the end of the infusion followed a two-compartment model with a $t_{1/2\alpha}$ value of 2.66±0.82 h and a mean terminal half-life $t_{1/2\beta}$ of 15.69±8.63 h. The mean concentrations determined at 23 and 47 h were 30.3±29.9 and 2.12±3.14 µmol/l, respectively. A typical elimination curve is shown in Fig. 1. The mean CL value was 49.1±11.7 ml $min^{-1}$ $m^{-2}$ or 1.33±0.31 ml $min^{-1}$ $kg^{-1}$ with an interindividual variability coefficient of 24.6% and an intraindividual variability coefficient ranging from 5.2% to 49.4%. Figure 2 shows the evolution of CL values with respect to the number of infusions given to the patients. It appears that the interindividual variability of clearance was low. The mean total volume of distribution (Vd) was 0.32±0.08 l/kg (22.4±5.6 l) and the mean area under the curve (AUC) was 4780±1630 µmol $l^{-1}$ h.

There was no correlation between toxicity and the concentration measured at the end of the infusion, the CL value, or the AUC value, respectively. In contrast to CL, $t_{1/2\alpha}$ was significantly ($P < 0.001$) correlated with the concentration of MTX at 23 ($r = 0.54$) and 47 h ($r = 0.48$), respectively.

The MTX concentration determined at 23 h was significantly ($P < 0.001$) correlated with $t_{1/2\beta}$ ($r = 0.35$) and AUC ($r = 0.26$). Pk parameters and MTX plasma concentrations were not correlated with hematologic or digestive toxicities. On the other hand, renal toxicities were correlated with the $t_{1/2\alpha}$ value ($r = 0.25$; $P < 0.001$) and the MTX concentration measured at 23 ($r = 0.23$; $P < 0.001$) and 47 h ($r = 0.38$; $P < 0.001$), respectively. The parameters of the group of toxic infusions and those of the group of infusions without toxicity did not significantly differ and were similar to the parameters of the entire group. The same observation was made for the sensitive versus non-

**Table 5.** Pharmacokinetic parameters of MTX obtained in the groups of nontoxic and toxic infusions and those obtained in sensitive and nonsensitive groups of patients

| | Nontoxic infusion | Toxic infusion | Sensitive | Nonsensitive |
|---|---|---|---|---|
| Age (years) | 23.9 ±2 | 25 ±12.4 | 24.9 ±11.9 | 23.55±11.4 |
| Number of infusions | 117 | 96 | 145 | 77 |
| Dose (g/m$^2$) | 13 ±1.7 | 13.3 ±2.4 | 13.7 ±2 | 12.7 ±2.4 |
| Concentration at the end of the infusion (μ*M*) | 991 ±150 | 997 ±131 | 980 ±148 | 1018 ±126 |
| Concentration at 23 h (μ*M*) | 29.5 ±30.9 | 31.5 ±28.4 | 31.75±31 | 27.8 ±25.8 |
| Concentration at 47 h (μ*M*) | 1.79±2.4 | 2.5 ±3.9 | 2.16±3.33 | 2.01±2.76 |
| CL (ml min$^{-1}$ m$^{-2}$) | 48.8 ±12 | 49.4 ±12 | 50.5 ±11.7 | 46.47±12.47 |
| $t_{1/2\alpha}$ (h) | 2.6 ±0.75 | 2.73±0.88 | 2.69±0.77 | 2.6 ±0.9 |
| $t_{1/2\beta}$ (h) | 14.8 ±7.7 | 16.8 ±9.7 | 16.5 ±9.5 | 14.2 ±6.3 |
| Vd (l) | 21.7 ±7 | 21.7 ±7 | 22 ±5.3 | 18.8 ±5.9 |
| AUC (μmol l$^{-1}$ h) | 4812 ±1800 | 4738 ±1380 | 4688.8 ±975 | 4953 ±2430 |

Differences among all values are not significant

sensitive groups. The pk parameters obtained in these groups are listed in Table 5.

## Discussion

The introduction of HDMTX with citrovorum factor rescue by Jaffe in 1972 [17] dramatically changed the management of osteogenic sarcoma. Rosen et al. [26] have demonstrated that the response of this tumor to HDMTX is correlated with the delivered dose and the serum level of MTX. Although no threshold concentration of MTX for osteosarcoma cells has been reported [4], contrary to a variety of in vitro models [9], a value of $10^{-3}$ *M* seems to be necessary to obtain a good effect on the tumor [4, 11]. On the other hand, few toxicities have been reported with this concentration [4, 27]. However, the wide intra- and interindividual variations observed at a given fixed dose led us to individualize the dose at each course so as to achieve as closely as possible the desired $C_{max}$ value of $10^{-3}$ *M*. This dose adjustment was performed at the 6th h for a total infusion duration of 8 h, a period longer than that chosen by numerous authors [2, 4, 27]. The mean concentration obtained at the end of the infusion for the entire group was 1016±143 μmol/l, and the mean dose delivered (13.2 g/m$^2$) was significantly superior ($P < 0.001$) to that recommended by Rosen for adult patients (8 g/m$^2$). The inter- and intraindividual variability were reduced and were distinctly lower than the results published by Wolfrom et al. [30], who reported values of 98.8% and 8.3%–121%, respectively.

Some differences have been described in the pk parameters of good and poor responders in leukemia [3, 23] and even in osteosarcoma [11]. However, in this series, no significant difference was observed in the mean values for CL, $t_{1/2\alpha}$, $t_{1/2\beta}$, AUC, or Vd between sensitive and nonsensitive patients.

The mean values of 1.33±0.31 ml min$^{-1}$ kg$^{-1}$ for CL and 0.32±0.08 l/kg for Vd obtained in our study were distinctly lower than those reported using low-dose MTX in the literature [7, 8, 20] but were consistent with those reported by other investigators [4, 5] using HDMTX. This suggests a possible dose dependence for the pharmacokinetics of MTX. The mean values of 2.66±0.82 h for $t_{1/2\alpha}$ and 15.69±8.63 h for $t_{1/2\beta}$ obtained in this study was consistent with those published by many authors [10, 18, 22, 25, 30]. The correlation observed between $t_{1/2\alpha}$ and the levels of MTX measured at 23 and 47 h was also reported by Raude et al. [25], who suggested that $t_{1/2\alpha}$ may be an aid in the identification of patients with a risk of prolonged terminal MTX elimination for the purpose of adapting the leucovorin rescue. Effectively, we found in the present study group a significant correlation ($r = 0.471$, $P < 0.0001$) between $t_{1/2\alpha}$ and $t_{1/2\beta}$. However, in practice we do not think that it is possible to predict $t_{1/2\beta}$ with sufficient precision on the basis of knowledge of the $t_{1/2\alpha}$.

The toxicity of MTX after HD infusions has usually been related to the concentration of this drug in the terminal elimination phase, and many thresholds have been defined to identify patients at an increased risk of developing toxicity [25, 29]. In this series, only renal toxicity was correlated with the MTX concentration measured at 23 and 47 h, although the mean values observed were over the limits of the above-mentioned concentrations. On the other hand, it has been reported [12] that patients with a $t_{1/2\alpha}$ superior to 3.5 h are at increased risk for toxicity. The mean $t_{1/2\alpha}$ value obtained in our group of patients was 2.66±0.82 h, and we avoided lethal toxicity and life-threatening side effects, observing only eight digestive and three hematologic grade 3 side effects and no grade 4 complication.

Most published reports indicate that variations in pharmacokinetics are probably responsible for the toxic effects of HDMTX. The present study indicates that dose adjustment performed to achieve a predetermined plasma concentration of MTX makes it possible to reduce the variability of plasma concentrations and toxicities. In addition, this study shows that when a standardized protocol is used in a homogeneous set of patients, the interindividual variability of clearance is low as compared with that obtained in previous studies. We also demonstrated that no significant difference occurred in the pk parameters between the groups of toxic and nontoxic infusions or, for that matter, between the groups of sensitive and nonsensitive patients.

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