No correlation between methotrexate serum level and histologic response in the pre-operative treatment of extremity osteosarcoma

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Our objectives were to evaluate the behavior of different doses of pre-operative methotrexate (MTX) pharmacokinetics, and assess correlations between the osteosarcoma histologic response and MTX serum peak concentrations. In total, 336 patients with osteosarcoma of the extremities were treated with three neoadjuvant protocols of chemotherapy including high-dose MTX (different doses for each protocol), cisplatin and doxorubicin (same doses in all protocols). The doses of MTX were 8 g/m^2 in 124 patients, 10 g/m^2 in 110 patients and 12 g/m^2 in 102 patients. The mean value of peak serum MTX was 801 μmol/l (range 298–1831) with significant intra- and inter-patient variability. For patients treated with 8, 10 and 12 g/m^2 it was 587, 735 and 1114 μmol/l, respectively ($P<0.0001$). The histologic response to pre-operative chemotherapy was 90% or above tumor necrosis in 62.8% of patients and less than 90% in 37.2%. The grade of histologic response significantly correlated with the histologic subtype of the tumor, whereas no significant association was found between the mean peak of serum MTX and the histologic response. Thus, increasing the dose of MTX increases the MTX serum peaks, but does not correlate with the histologic response of the tumor. *Anti-Cancer Drugs* 17:411–415 © 2006 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2006, **17**:411–415

Keywords: chemotherapy, histologic response, methotrexate, osteosarcoma

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Sponsorship: This work was supported by grants from the Instituti Ortopedici Rizzoli (Ricerca Corrente), the Associazione Italiana per la Ricerca Sul Cancro (AIRC), and the Italian Ministry of Health (Ricerca Finalizzata).

Received 14 November 2005 **Accepted** 16 December 2005

Introduction

Neoadjuvant chemotherapy with multiagent regimens has dramatically improved the cure rate for patients with extremity osteosarcoma [1–6]. Tumor necrosis after pre-operative treatment has been clearly demonstrated to be the strongest predictor of outcome [1,2,4–7]. The drugs more commonly used in the pre-operative treatment of osteosarcoma are high-dose methotrexate (HDMTX), cisplatin and doxorubicin (ADM). Since the use of HDMTX requires individual close monitoring due to great inter- and intra-patient pharmacokinetic variability, retrospective data of MTX serum peaks levels are available for each patient, giving the opportunity to investigate the relation between serum peak concentrations of MTX at the end of the infusion and the grade of histologic tumor response. The results of these studies were contrasting; some authors [4,8] found a strict correlation between the serum peak of MTX at the end of the infusion and the grade of histological response, while others [9,10] could not prove any reliable correlation. The aim of this study was to retrospectively evaluate correlations between histologic tumor response and serum peak concentrations of MTX in 336 patients with osteosarcoma of the extremities pre-operatively treated with three different protocols including HDMTX, cisplatin and ADM. We wanted to see whether increasing MTX dose caused an increment in MTX serum peaks and whether 1 cycle of cisplatin between the 2 cycles of MTX could alter MTX pharmacokinetics.

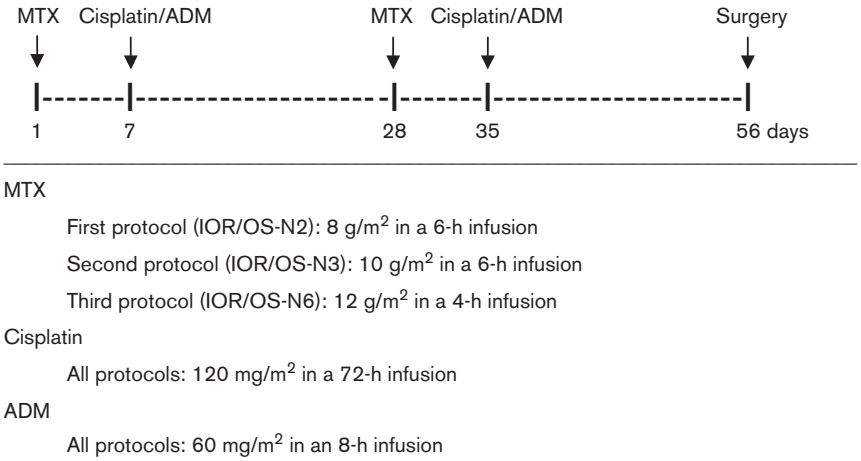
Methods

Patient selection and pathology

Between 1983 and 2004, 898 patients with newly diagnosed high-grade osteosarcoma of the extremities were treated at the authors' institution according to six different chemotherapy protocols previously reported in detail [7,11–13]. Three of these protocols included pre-operative cycles of HDMTX, cisplatin and ADM (Fig. 1). While the doses of ADM and cisplatin were the same, MTX doses varied in the three regimens. Overall, 588 patients received treatments. We excluded 245 patients due to lack of complete data on serum MTX concentrations or to dose reductions during the pre-operative phase. Seven patients who had a severe delayed MTX clearance were also excluded from this analysis. Thus, 336 patients were eligible for this retrospective study; 15 of them had metastatic disease at diagnosis.

0959-4973

Fig. 1



Scheme of pre-operative treatment of the three protocols considered.

Chemotherapy

All cycles of chemotherapy were performed inside the hospital. The hydration and alkalization during and after MTX infusion were well standardized according to the guidelines suggested by Rosen and Niremberg [13]. Regardless of MTX dose, patients received a 1-h i.v. infusion of 250 ml/m^2 glucose 5% with 100 mol/l $NaHCO_3$ and 20 ml KCl solution. MTX was dissolved in 500 ml/m^2 NaCl 0.9%, with 40 µmol/1 $NaHCO_3$ and 20 µmol/l KCl. The doses of MTX were 8, 10 and 12 g/m^2, respectively. The MTX solution was infused for 6 h in the first two protocols and for 4 h in the last protocol. The post-hydration was performed i.v. using 5% glucose with 40 mol/l $NaHCO_3$ plus 20 µmol/l KCl. The total fluid input (including pre-alkalinization, MTX infusion and oral fluids) for the day until the serum MTX concentrations were less than 2 µmol/l was 1500 ml/m^2 in the first day, and 2000 ml/m^2 in the second and third days. Folinic acid rescue consisted of 8 mg/m^2 i.v. or orally every 6 h, beginning 24 h after the start of MTX infusion for 11 administrations. Hydration, alkalinization and leucovorin rescue were modified in case of delayed MTX elimination. Starting from day 6 after MTX infusion, patients received 120 mg/m^2 of cisplatin delivered over a 72-h period followed by ADM 60 mg/m^2 administered in 8 h.

Pathology

The diagnosis of osteosarcoma, established by clinical and radiological findings, was always confirmed on histologic slides of tumor tissue obtained from an open or trocar biopsy, as well as from the resected specimen. Osteosarcomas were classified as classic, telangiectatic, surface osteosarcoma and small cell osteosarcoma. On the basis of predominant cell intercellular material, classic osteosarcomas were classified into osteoblastic, fibroblastic and chondroblastic subtypes. This distinction, always made on surgical specimens, was possible in all but 29 cases that were defined as 'not classifiable'. The histologic response to chemotherapy was evaluated following the criteria previously reported in detail [14] and graded as 'good' (tumor necrosis 90% or above) or 'poor' (tumor necrosis below 90%). Our 'good' and 'poor' responses roughly correspond to grades III/IV and I/II of the descriptive classification proposed by Huvos *et al.* [15]. In order not to create a bias on necrosis evaluation over a 20-year period, the histologic response to pre-operative treatment for all patients considered in this analysis was re-evaluated in January 2005 by the same pathologist, who found different results in 10% of cases as compared with original classification.

Serum MTX concentration analyses

Venous blood samples, not taken from the veins used for the MTX infusion, were obtained at the end of the infusion (peak value), and after 14, 24 and 38 h since the beginning of the infusion. In case of delayed elimination of the drugs, other samples were taken. In this study, we evaluated the MTX concentrations at the peak of the first and second MTX cycles, and the mean of these two values. Serum MTX concentrations were estimated by means of an ELISA (Emit Syva, Palo Alto, California, USA).

Statistics

The endpoint of the study was to investigate the correlation between serum peak of MTX and several variables: MTX dose, gender and age, tumor site, size and histologic subtype, the mean peak values of MTX, and the peak values in the first and second pre-operative cycle. The relationship between the grade of necrosis and serum MTX peak levels treated as a continuous variable or dichotomized at 1000 µmol/l was analyzed by ANOVA or Fisher's exact test. The frequency of distribution of different parameters among groups of patients was

compared by means of the χ^2-test. Significance was set at $P < 0.05$. A multivariate logistic regression analysis was performed to evaluate the influence on tumor response of variables that were statistically significant at the univariate analysis.

Results

Delayed MTX clearance

In the 681 pre-operative MTX cycles considered, there was delayed MTX clearance in 21 patients (3.1%) with a mean serum value of MTX after 38 h of 19.7 μmol/l (range 2–150). The rate of delayed MTX clearance was 2.4% for the 125 patients treated with 8 g/m^2, 7.1% for the 112 patients treated with 10 g/m^2 and 9.4% for the 106 patients treated with 12 g/m^2, i.e. 1.2, 3.5 and 4.7%, respectively. Delayed elimination was observed in 12 cases after the first cycle of MTX and in nine after the second. Among these 21 patients, seven did not complete the pre-operative treatment due to renal toxicity. These patients, as reported before, have been excluded from this study; the characteristics of the 336 patients evaluated are outlined in Table 1.

Table 1 Characteristics of the 336 patients evaluated and rates of good histologic response to pre-operative treatment

	No. of cases	Good histologic response (%)	*P*
Age (years)			
<15	133	61.8	0.85
≥ 15	203	63.4	
Site			
Femur	161	63.9	0.94
Tibia	105	62.8	
Humerus	48	62.5	
Fibula	18	55.6	
Other	4	50	
Histologic subtype			
Osteoblastic	209	63	0.0001
Chondroblastic	42	28	
Fibroblastic	27	74	
Telangiectatic	25	100	
Non-classifiable	29	58	
Other	4	100	
Volume (ml)[a]			
<150	55	65.9	0.03
≥ 150	121	53.9	
Peak serum MTX level (mean) (μmol//l)[b]			
<1000	53	65.9	0.06
≥ 1000	283	53.9	
Staging			
Localized	321	64.5	0.007
Metastatic	15	26.7	

[a]Data missing for 179 patients.
[b]Mean of the 2 pre-operative cycles considered.

Effect of doses and other factors on MTX concentrations at the end of the infusion

The overall median of the 674 peaks in MTX serum levels was 801 μmol/l (range 298–1831). Significant inter-patient variability was observed, as indicated by the wide range and even considering single cycles. In fact the mean serum values of MTX after the first cycle (median 756 μmol/l) ranged from 233–1980 μmol/l and in the second cycle (median 835 μmol/l) from 235–1980μmol/l. The maximum difference for a single patient between the first and second cycle was 986 μmol/l (1938 and 952 μmol/l). As shown in Table 2, these peak levels were not related to gender and age, but significantly correlated with the dose of MTX used. The mean peaks were 578 μmol/l for patients treated with 8 g/m^2 and 735 μmol/l for patients treated with 10 g/m^2 ($P < 0.0001$). MTX was delivered in a 6-h infusion in both groups. The peak observed in patients treated with 12 g/m^2 of MTX was also significantly higher than in patients treated with 10 g/m^2 (1114 versus 735 μmol/l, $P < 0.0001$). It must be stressed that in this group of patients MTX was delivered in a 4-h infusion. As reported in Table 2, these differences were highly significant also considering the first and second cycles of MTX infusion separately. The rates of mean serum peak 1000 μmol/l or above in the three groups were 1.6, 11.8 and 72.5%, respectively. These differences are highly significant ($P < 0.0008$ and $P < 0.0001$).

Effect of previous cisplatin treatment on MTX concentrations

As shown in Fig. 1, the second MTX cycle was infused 3 weeks after the cycle of cisplatin (120 mg/m^2 in 72 h) and ADM (60 mg/m^2 in 8 h). The mean values at the end of infusion of MTX serum levels were 756 μmol/l after the first cycle and 835 μmol/l after the second cycle. This difference is statistically significant ($P < 0.003$). The

Table 2 Peak serum MTX levels after first and second cycle, and total mean, according to several variables

	Mean MTX level (μmol/l)	*P*	Levels after first MTX cycle (μmol/l)	*P*	Levels after second MTX cycle (μmol/l)	*P*
Gender						
Female	905	0.37	825	0.07	947	0.58
Male	940		898		971	
Age (years)						
<15	946	0.40	888	0.44	981	0.45
≥ 15	913		858		949	
Pre-operative dose MTX (g/m^2)						
8	578	0.0001	559	0.0001	617	0.0001
10	735	0.0001	705	0.0001	780	0.0001
12	1114		1048		1158	
Total (range)	801 (298–1831)		756 (233–1980)		835 (235–1980)	

mean difference in the peak of MTX at the end of infusion between the first and second cycles was 204 μmol/l (1–986). The highest values of MTX were observed in 125 patients (37.2%) in the first cycle and in 211 patients (62.7%) in the second cycle ($P < 0.0001$). In the first group the mean difference was 169.9 μmol/l (6–986) and in the second group the mean difference was 225.5 μmol/l (1–708) ($P < 0.003$). The rate of patients with a difference in serum value of peak MTX between the 2 cycles of more than 200 μmol/l was 31.2% after the first cycle and 47.4% after the second cycle ($P < 0.005$). According to the doses of MTX used, the mean values of the serum peak of MTX in the first and second cycles were 559 versus 617 μmol/l for patients treated with 8 g/m^2 of MTX, 705 versus 780 μmol/l for patients treated with 10 g/m^2 ($P < 0.21$) and 1084 versus 1158 μmol/l for patients treated with 12 g/m^2. These differences are not statistically significant.

Factors influencing histologic response

Histologic response was good in 211 patients (62.8%) and poor in 125 patients (37.8%). As shown in Table 1, at univariate analysis, the rate of good histologic response was proven to correlate with histologic subtype, tumor size and stage of disease. At multivariate analyses only, the histologic subtype kept its statistical significance ($P = 0.028$, 95% confidence interval 1.109–6.165). The mean peak serum MTX levels in good and poor responder patients were 790 and 820 μmol/l. This difference is not statistically significant ($P = 0.35$). The rate of good histologic response in the two groups was 53.9% for patients with mean peaks above 1000 μmol/l and 65.9% for patients with serum peaks below 1000 μmol/l. This difference is not statistically significant ($P = 0.06$).

Discussion

The role of HDMTX in the successful treatment of osteosarcoma is well established and the serum peak to achieve the optimal efficacy is generally set at 1000 μmol/l.

All the same, a strong correlation between histologic response of primary tumors and prognosis is well recognized, and the grade of histologic response to pre-operative treatment can be considered a reliable predictor of final outcome. Several studies investigated the relationship between peak serum MTX levels and histologic tumor response with contradictory results. Delepine *et al.* [8] reported that individualized pre-operative doses designed to achieve a serum concentration above 1000 μmol/l in a 6-h infusion led to improved histologic response. Saeter *et al.* [4] found a correlation between histologic tumor response and MTX concentrations after 24 and 48 h at univariate and multivariate analyses. On the contrary, Zelcer *et al.* [10] did not find any demonstrable correlation between peak serum concentrations of MTX and the histologic response or event-free survival. In between these positions, Graf *et al.* [9], in a retrospective analysis of 108 patients treated with three different chemotherapy regimens, showed that a threshold concentration of 1000 μmol/l was associated with a greater probability of a good histologic tumor response only in one protocol, when MTX was used as single pre-operative drug. A correlation with peak serum MTX was not demonstrable, however, when pre-operative chemotherapy was a multiagent regimen.

The results of our study seem to confirm this hypothesis. Despite significant inter- and intra-patient variability, peak serum MTX levels did not correlate with the histologic response. These data also contradict the results of a previous paper by us [16]. Our previous study however, analyzed a smaller number of patients, including those who had received incomplete pre-operative treatment in terms of number of cycles and/or doses of drugs, and the serum peak level was set at 700 μmol/l.

On the other hand, in the present study, regardless of the doses used, the mean peak serum MTX levels of the second cycle, given after cisplatin/ADM, were significantly higher than those of the first MTX administration. This is in accordance with other authors [9,17] and confirmed that the use of cisplatin may alter MTX pharmacokinetics.

In conclusion, divergence remains about the influence of MTX serum levels on histologic response to primary treatments. Moreover, the great inter- and intra-patient variability represents a major potential confusing variable for interpretation of data, together with MTX pharmacokinetics alterations induced by other drugs of pre-operative regimens.

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