

소아 골육종 환자에서 고용량 Methotrexate의 Pharmacokinetics

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Pharmacokinetics of High-dose Methotrexate in Pediatric Patients with Osteosarcoma

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Methotrexate(MTX)는 소아 골육종 환자에서 12 g/m²의 고용량을 사용하고 있다. 현재, 소아 골육종 환자에서 신기능에 따른 고용량 MTX의 임상 약동학은 연구되어 있지 않다. 따라서 본 연구에서는 신기능에 따른 MTX 약동학을 이용하여 구내염을 최소화하는 방법을 제시하고자 하였다.

방법: 환자들의 의무기록지를 후향적 방법으로 검토하였다. 한 병원에서 골육종으로 진단받고, 치료 받은 환자들에 대해 정상 신기능군과 비정상 신기능군으로 나누었다. 두 군에 MTX 투여 후 혈중 농도를 각각 비교하였고, 최고 혈중농도도 비교하였다. 혈중 농도와 구내염의 관련성, CL, AUC와 구내염의 상관관계를 분석하였다. 각 군의 terminal half-life, CL, Vss의 평균과 mean residence time(MRT)의 평균을 구하였고, 두 군간 각각을 비교하였다($\alpha=0.05$). 결과: 환자는 6명이었고, 평가 가능한 총 MTX 투여 회수는 34회였다. MTX 투여 후 최고혈중 농도, 24, 48 시간의 혈중농도는 통계적으로 유의성 있는 차이가 있었고, 72, 96 시간에서의 농도는 두 군간 유의성이 없었다. 각 군에서 혈중농도와 구내염의 상관관계, 그리고 CL, AUC와 구내염의 상관관계는 발견되지 않았다. Vss를 제외한 모든 파라미터들(terminal half-life, CL, MRT)은 통계적으로 유의성 있는 차이가 있었다.

결론: 비정상 신기능 군에서 MTX 투여 시작 후 24, 48 시간에서의 혈중농도가 더 높고, 변동이 심했다. 또한 MTX의 CL는 감소했고, 혈중농도는 증가하였다. 이러한 사실로 MTX 투여 전·후 혈중 크레아티닌이나 또는 크레아티닌 청소율 모니터링이 필요하다는 것을 알 수 있으며, MTX 투여가 끝난 직후 그리고 그 이후 24 시간 간격으로 혈중 농도를 측정해야함을 알 수 있다.

□ Key words - 고용량 MTX, 구내염, pharmacokinetics, 신기능

Methotrexate (MTX), the folic acid antagonist, is used for rheumatoid arthritis, severe psoriasis, and various neoplastic diseases, etc.^{1,2)} The dose ranges from 7.5 mg/m² to 33,000 mg/m² with leucovorin rescue considerably depending on the disease state.³⁾ High-dose methotrexate in combination with other cytotoxic agents has been proposed to be used as adjuvant chemotherapy for osteosarcoma.^{2,4)}

Mucositis is one of the most common adverse reactions encountered in radiation therapy for head and

neck cancers, as well as in chemotherapy with antimetabolites (e.g. methotrexate, fluorouracil and cytarabine).^{5,6)} The incidence and the severity of mucositis vary from patient to patient, and from treatment to treatment. It has been estimated that there is 20-40% incidence of mucositis in patients treated with standard chemotherapy,^{7,8)} and this would not only increase with the number of treatment cycles, but also with previous episodes.

After intravenous (IV) administration, eighty to ninety percent of the dose is excreted unchanged in the urine within 24 hours.²⁾ Clearance occurred via glomerular filtration and active tubular secretion.⁹⁾

MTX itself causes nephrotoxicity. Nephrotoxicity occurs with high-doses of MTX when the concentration

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of excreted drug exceeds the drug solubility in the renal tubule.^{10,11)} Because MTX concentrations above 0.01 μM inhibit DNA synthesis in bone marrow and intestinal epithelium, it is reasonable to continue leucovorin rescue in patients who experience toxicity until plasma MTX falls below this level.¹²⁾

Therefore, MTX should be used with great caution in patients with impaired renal function, and at risk for renal dysfunction. In fact, dosage reduction or an alternative anticancer drug should be considered for patients with preexisting renal dysfunction receiving treatment with methotrexate at any dosage range.¹³⁾ Patients with a creatinine clearance of less than 60 mL/min should not receive high-dose methotrexate.¹⁴⁻¹⁶⁾

There was a pharmacokinetic study of high-dose MTX in children with normal renal function and acute lymphoblastic leukemia (ALL, 5 g/m² in 24 h).¹⁷⁾ Also, Bayesian estimation of MTX pharmacokinetic parameters was conducted in children and young adults with localized osteosarcoma.¹⁸⁾ And there was pharmacodynamic study of high-dose MTX in pediatric patients but not pharmacokinetic study.¹⁹⁾

Currently, there are no available clinical pharmacokinetic data of high-dose MTX in pediatric osteosarcoma patients with normal and abnormal kidney function.

The correlation between serum concentrations of MTX and mucositis in normal and abnormal kidney function group and also, other factors that might affect mucositis were investigated in this study. Strategies for minimizing mucositis in pediatric patients are proposed using the pharmacokinetics of high-dose MTX.

Materials and Methods

Patients

Patients' medical charts were retrospectively reviewed. Patients were divided into two groups who had normal and abnormal kidney function diagnosed with osteosarcoma in Seoul National University Children Hospital (Seoul, Korea) and treated before Feb. 2000 because blood samples were not collected before 24 hours from the start beginning of infusion after that time.

The inclusion criteria were the availability of the relevant data in the patients' medical charts. Patients were included whose blood samplings were collected as scheduled appropriately (4, 8, 12, 24, 48, 72, and 96 hours from the starting of infusion). It was not analyzed beyond 96 hours though its blood samples were collected because terminal half life is different as blood sampling time in empirical model.²⁰⁾ Patients were excluded who used concurrently other drugs which potentially interact with MTX (e.g. cotrimoxazole, penicillins, nonsteroidal antiinflammatory drugs-NSAIDs etc).

Data Collection

The following clinical data were obtained by retrospective chart review: age, gender, height, weight, body surface area, presence of distant metastases or relapse of disease, baseline serum creatinine (Scr) and maximum Scr in normal kidney function group, creatinine clearance (CLcr) in abnormal kidney function group, volume of hydration, and lowest recorded urine pH.

MTX administration

Patients were treated on the protocol of Childrens Cancer Group (CCG) - 7921. Some patients received chemotherapy alternating regimen of high-dose MTX and ifosfamide-etoposide (HDMTX-IFO5VP5).

The intravenous administration dose is 12 g/m² (maximum dose is 20 grams) over 4 hours given on days 21 and 28 of each course. All doses should be rounded off to the next highest full gram value (e.g. a calculated dose of 15.2 grams should be rounded up to 16 grams).

Sample analysis

The serum concentrations of MTX were measured by fluorescence polarization immunoassay (FPIA) with a TDx analyzer.

Pharmacokinetic analysis

Pharmacokinetic parameters of MTX were calculated by using the software package WinNonlin Pro program (version 2.1 Pharsight, USA). Pharmacokinetic parameters

ters of MTX were estimated by empirical (exponential decay, noncompartment) model.^{20,21)} Pharmacokinetic parameters estimated were terminal half-life, CL, Vss, MRT, and AUC etc. The pharmacokinetic variables analyzed for each MTX course were dose, each serum concentration, sampling time, and length of infusion time. The serum concentration at each sampling time (24, 48, 72, and 96 hours from the start of infusion of MTX) and the peak serum concentration (after the end of infusion) between two groups were compared. The correlations between serum concentrations and existence of mucositis and, between CL, AUC and mucositis were analyzed. Intraindividual differences were analyzed by comparing clearance of methotrexate between on day 21 and 28. Harmonic mean of terminal half-life, CL, Vss of each group and arithmetical mean of MRT of each group by empirical model were calculated and then compared between two groups.²²⁻²⁵⁾

Toxicity analysis

MTX toxicity was evaluated as gastrointestinal toxicity defined as mucositis scored according to the World Health Organization (WHO) scale (grade 0=no reaction; grade 1=painless ulcers, erythema, or mild soreness; grade 2=painful erythema, edema, or ulcers and can eat solids; grade 3=painful erythema, edema, or ulcers and cannot eat solids; grade 4=requires parenteral or enteral support for alimentation).²⁶⁾ Renal toxicity was assessed by the percentage of increase in Scr (grade 0=<25%, grade 1=25-49%, grade 2=50-74%, grade 3=75-100%, grade 4=>100%).²⁷⁾

Statistical analysis

The serum concentrations at each sampling time and the peak serum concentration were compared between two groups by using students' t-test. It was used multiple linear regression that relationship between serum concentrations and existence of mucositis and, between mucositis and CL, AUC.

Intraindividual differences were analyzed by using paired t-test on day 21 and 28. The means of pharmacokinetic parameters of each group by empirical model

were compared by using students' t-test between two groups. The program used for statistical analyses was SAS for windows release 8.02. In all cases, the significance level chosen was less than 0.05.

Results

Six patients were included and the numbers of evaluable total courses of MTX were 34 (normal: 20, abnormal: 14). Three patients had normal kidney function and the others had abnormal kidney function. Demographic and clinical data on the 6 subjects are listed in Table 1. There were 3 patients with distant metastases, and were 2 patients with relapse. All of patients were treated with cisplatin plus doxorubicin even though number of courses administered were different before chemotherapy containing high-dose MTX treatment; cisplatin 130 mg/m² intra-arterial infusion over 2 hours plus doxorubicin 20 mg/m² continuous infusion for 66 hours after completion of cisplatin or cisplatin 120 mg/m² iv infusion over 6-8 hours plus doxorubicin 30 mg/m² iv. The chemotherapy regimen of CCG-7921A was conducted on patients with number of 1, 3 and that of HDMTX-IFO5VP5 was conducted on patient with number of 6. That of CCG-7921 was substituted to HDMTX-IFO5VP5 in patients with number of 4, 5.

The chemotherapy was delayed in two courses in normal kidney function group, 5 days and 7 days respectively, because of myelosuppression. There was not delay in chemotherapy in abnormal kidney function group, but in one patient (number 6) treated with HDMTX-IFO5VP5, no high-dose of MTX was administered on days 28 because of myelosuppression (thrombocytopenia) throughout the chemotherapy regimen of HDMTX-IFO5VP5.

The creatinine clearance of abnormal kidney group recorded to the nearest administration of MTX were below 60 mL/min/1.73 m². There was no medicine administered concurrently with MTX which can cause nephrotoxicity except chemotherapeutic agents (e.g. aminoglycosides, vancomycin, and diuretics etc).

The dose of MTX in osteosarcoma is 12 g/m², but

substantially was administered slightly above 12 g/m² because of rounding off to the next highest dose.

Pharmacokinetic analysis

As a result of fitting by WinNonlin Pro software, the terminal phase was unified as 48 hours from the start of infusion of MTX as possible.

The serum concentrations of MTX at 24, 48 hours were 13.91(± 19.34) µM, 0.6990(± 1.298) µM in normal kidney group, respectively and 68.44(± 57.34) µM, 2.325(± 2.232) µM in abnormal kidney group, respectively. In abnormal kidney group, the elimination of the MTX was delayed late.²⁾ It was statistically different between two groups. The serum concentrations at 72, 96 hours were not significantly different between two groups. This result coincided with the fact that serum methotrexate concentrations during the terminal phase were not dose dependent.²⁸⁾

The peak serum concentration was 1.302×10³(± 278.5) µM in normal kidney group and 1.760×10³(± 548.0) µM in abnormal kidney group and was below 1,000 µM in two courses of abnormal kidney group. The peak serum concentration was significantly different between two groups. If the peak serum concentration does not exceed 1,000 µM, the dose may be escalated to 15 g/m² in subsequent courses.²⁾

It was not significant that relationship between serum concentrations and mucositis and also, between CL, AUC and mucositis in each group; Scale of mucositis

(from grade 0 to 4) was dependent variable, and mucositis was independent variable and also CL, AUC was dependent, mucositis independent variable. If mucositis was not recorded in the medical charts, it was estimated from the diary of nursing care of the charts. Because, it was not found that the relationship between serum concentrations and mucositis and also, between CL, AUC and mucositis, so it was divided into two groups according to the existence of mucositis in each group at 24, 48 hours from the start of infusion because its concentration of MTX was statistically different. And then the concentration of MTX was compared in each group, but it was not statistically different.

The harmonic means of each terminal half-life, CL, Vss were 22.43 hours, 2.766 L/h/m², 13.16 L/m² in normal kidney group, respectively and was 14.08 hours, 1.866 L/h/m², 11.65 L/m² in abnormal kidney group, respectively. The terminal half-life reported, and volume of distribution at steady state is eight to fifteen hours, and approximately 0.4 to 0.8 L/kg (40% to 80% of body weight) respectively.²⁾ In another study, the terminal half life was 10 hours to 27 hours^{6,33)} and volume of distribution approximate those of total body water.⁶⁾

Pharmacokinetic parameters except Vss were significantly different. The clearance of MTX was larger in normal kidney group than abnormal kidney group.

The arithmetical mean of MRT was 4.703 hours and 6.356 hours in normal kidney and abnormal kidney group, respectively. Unexpectedly, terminal half-life of

Table 1. Characteristics of the patients

Number of patients	year	Sex	Ht (cm)	Wt (kg)	BSA (m ²)	metastasis /relapse*	Scr (mg/dL)		CLcr [§] (mL/min /1.73m ²)	lowest recorded urine pH	Number of evaluable MTX courses	Dose of MTX administered substantially (g/m ²)
							Baseline	Maximum				
1	4	F	101.1	14.22	0.6315	-/-	0.43	0.50	-	7.375	4	12.70
2	11	M	152.8	38.13	1.270	+/-	0.62	0.80	-	6.500	5	12.29
3 [#]	14	F	153.0	33.02	1.184	-/+	0.58	0.54	-	6.590	11	12.36
4	16	M	169.2	40.34	1.376	+/+	0.90	1.00	35.00	7.333	4	12.35
5	10	M	155.0	44.76	1.432	-/-	0.70	0.85	56.50	6.375	4	12.22
6	14	M	165.6	46.99	1.468	+/-	0.78	0.96	19.40	6.333	6	12.25

Ht: Height, Wt: Weight, BSA: Body surface area

The value of following parameters is means: Ht, Wt, BSA, Scr, lowest recorded urine pH, dose of MTX administered substantially

*: presence of metastasis yes +, no -; relapse yes +, no -

§: The creatinine clearance by 24-hour urine collection recorded was the nearest measured value of the high dose MTX administration.

#: Numbers of evaluable MTX courses were 11, but because in one course blood sample at 72 hours was not collected, it excluded analyzing terminal half-life and included analyzing other parameters.

MTX was longer in abnormal kidney group. This might be why the model chosen was empirical and terminal phase was inappropriately chosen. But, MRT of MTX was longer in abnormal kidney group. MRT means average time of the intact drug molecules (only parent drug) residing in the body. Most of the MTX (about 80-90%) is excreted unchanged through the kidney.²⁾ It's reasonable that MRT of MTX in abnormal kidney group be inverse to the CL of MTX. So, it's reasonable using MRT instead of terminal half-life.

Toxicity analysis

Severe mucositis (grade 3-4) happened in only one course in normal kidney group. Most of patients experienced no or mild mucositis.

There were mild nephrotoxicity in some courses of MTX. There has no changed in serum creatinine concentrations in most courses.

Discussion

In this study, the difference of pharmacokinetics of MTX was observed as kidney function. The serum concentrations of MTX from 4 hours to 48 hours were higher and also, the clearance of MTX was decreased in abnormal kidney function group. Log transformation of serum mean concentrations of MTX versus time plotting is shown Fig. 1. The terminal half-life was rather longer in normal kidney group, but this might be due to the empirical (exponential decay) model. As shown in Fig. 1, the serum MTX level in normal kidney group rapidly decreased to the 48 hours from the infusion of MTX, but in abnormal kidney group it decreased to the 72 hours rapidly but more slowly than in normal kidney group. It's reasonable that the terminal phase start at 48 hours in normal kidney group and 72 hours in abnormal kidney group. So to speak, the terminal phase in abnormal kidney group was late than in normal kidney group.

Another parameter, MRT not influenced by model was calculated. Because most of MTX is cleared by the kidney as parent drug²⁾, so MRT reflects more correctly the substantial residing time of the MTX in the body.

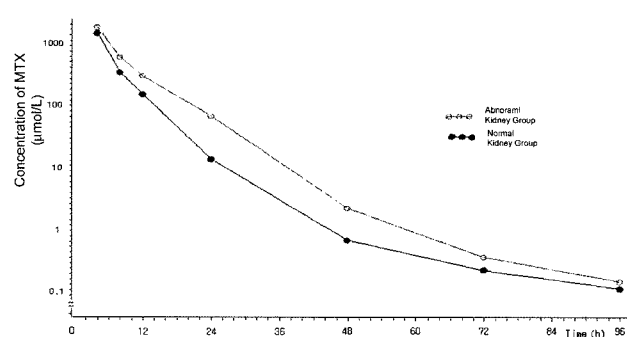


Fig. 1. Mean concentrations (log-transformed) of serum MTX and time plotting (dose: 12 g/m²) in normal and abnormal kidney function group. As shown in this figure, the terminal phase starts at 48 hours in normal kidney group, and at 72 hours in abnormal kidney group (Black circle indicates serum concentrations of MTX in normal kidney group, open circle does serum concentrations of MTX in abnormal kidney group). The numbers of MTX courses at 24, 48, 72 hours were 23, and at 96 hours were 22, respectively in normal kidney group. That of MTX courses at 24, 48 hours were 18, and at 72, 96 hours were 17, respectively in abnormal kidney group.

MRT is longer about twenty six percent in abnormal kidney group.

Three patients had renal function decreased by cisplatin.^{29,30)} GFR continued to decline throughout the cycles of chemotherapy containing cisplatin.³¹⁾ And they also had ototoxicity induced by cisplatin.³²⁾ So, they could not receive CCG-7921 any more, then they changed to the HDMTX-IFO5VP5 in two patients and the other was not treated with CCG-7921 because he already had his kidney failure on the account of regimen of cisplatin plus doxorubicin.

MTX clearance was decreased after cisplatin therapy, after cisplatin in combination with ifosfamide therapy, and after MTX therapy. And mean peak serum concentrations above 1,000 µM were achieved in 96% of patients. But, unexpectedly patients who had a mean peak MTX plasma concentration above 1500 µM were found to have a worse outcome (lower event free survival) compared with patients who had a mean peak concentration less than 1,500 µM.³³⁾

In another study, patients exposed to the mean AUC above 4,000 µmol·h/L presented a high disease-free survival. A close and linear correlation was observed

between the MTX peak concentration and the AUC, with a highly significant relationship between a mean AUC of 4,000 $\mu\text{mol}\cdot\text{h}/\text{L}$ and a mean peak concentration of 700 $\mu\text{mol}/\text{L}$.³⁴ Also, patients with a slow creatinine clearance (less than 85 mL/min) or with an AUC greater than 1,100 $\mu\text{mol}\cdot\text{h}/\text{L}$ survived significantly longer than patients with a fast creatinine clearance or with lower levels in the other study.³⁵⁾

Delayed early MTX elimination refers serum MTX level 50 μM or more at 24 hours, or 5 μM or more at 48 hours after administration.²⁾ This corresponded to the profile of serum concentrations of MTX in abnormal kidney group. High-risk MTX concentrations were significantly associated with low MTX clearance, low urine pH etc.³⁶⁾

The clearance of MTX in abnormal kidney group was about 68% of that in normal kidney group. Therefore, serum concentrations of MTX in abnormal kidney group were higher than in normal kidney group. This fact was similar to the study that elevation of serum creatinine (measured 12-24h after the start of MTX infusion) was a better predictor of delayed elimination.³⁷⁾ Therefore, serum creatinine must be measured routinely at least 24 hours after the start of MTX infusion. But, in this study, lowest recorded urine pH was not different.

Mucositis, characterized by mouth soreness, stomatitis has been reported with methotrexate therapy. Mucositis usually occurs 7 to 14 days (most commonly associated with a low white blood cell count) after therapy and lasts 4 to 7 days.

In this study, severe mucositis (grade 3-4) happened to only one course in normal kidney group. Mucositis almost always occurred in patients with nephrotoxicity.³⁸⁾ But, in this study the results did not agree with the fact. This might be why leucovorin rescue, hydration, urine alkalinization, small size of patients, difference of pharmacogenetics for MTX³⁹⁾ and younger patients.⁴⁰⁾

About 10% to 12% of the patients with the lower 5,10-methylenetetrahydrofolate reductase (MTHFR: converts 5,10-methylenetetrahydrofolate to 5,10-methyltetrahydrofolate)

activity had higher oral mucositis index. And platelet counts recovered more slowly among patients with decreased MTHFR activity.³⁹⁾

Younger patients had lower plasma MTX levels than older patients. In addition, the urinary excretion of MTX was faster in younger children. Younger patients for the most part had mild, tolerable toxicities when treated with high-dose MTX⁴¹⁾, whereas older patients exhibited significant toxicities.⁴²⁾ But, Crews et al. found that age did not appear to affect peak serum concentration and clearance of MTX, but older age (more than or equal to 12 years) was associated with higher 24-hour MTX concentrations.⁴³⁾

In one study, high-dose leucovorin was enough sole therapy for MTX toxicity.⁴⁴⁾ But, leucovorin overdosing could compromise the antitumor effect.⁴⁵⁾ Therefore, increasing the dose of leucovorin unqualifiedly in abnormal kidney patients might be inappropriate.

Conclusions

The serum concentrations of MTX were higher and more fluctuant at 24, 48 hours from the starting of infusion in abnormal kidney group. The clearance of MTX was decreased and MRT of MTX was increased in abnormal kidney group. These results suggest that monitoring of Scr or CLcr be needed before and after giving MTX. Therapeutic drug monitoring of MTX should be needed at the intervals of 24 hours including the end of the infusion and more conducted with caution in patients with abnormal kidney function.

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