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Early Recognition of Patients with Decreased Methotrexate Clearance Following High-Dose Methotrexate Infusion Therapy

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ABSTRACT

The purpose of this study was to identify the patients with decreased methotrexate (MTX) clearance as early as possible after the start of high-dose methotrexate (HD-MTX) infusion. Fifty-six patients (age: 18～83 years) received a HD-MTX infusion (dosage: 1.9～3.8 g/m^2) for 6 h. These patients were retrospectively divided into a low-clearance group and a high-clearance group based on the serum MTX concentration at 48 h (1 μM). Six out of the 56 patients showed decreased MTX clearance. The MTX concentrations in the low-clearance group were significantly higher than those in the high-clearance group even in earlier sampling times than at 48 h. The average MTX concentrations were 330 μM at 6 h, 72 μM at 12 h, and 16 μM at 24 h in the low-clearance group, and those in the high-clearance group were 210 μM, 18 μM, and 1.0 μM, respectively. The estimated elimination half-lives ($t_{1/2}$) at 6～12 h and 12～24 h after the start of the infusion were also significantly longer in the low-clearance group (2.8 vs. 1.7 h and 5.0 vs. 2.8 h, respectively). Therefore, we proposed convenient criteria based on the mean + 1 S.D. of the high-clearance group: the concentration > 270 μM at 6 h and > 32 μM at 12 h; the $t_{1/2}$ value > 2.1 h at 6～12 h. All 6 patients were recognized as belonging to the low-clearance group at an early stage after HD-MTX infusion by using our proposed criteria. These results indicate that patients with decreased MTX clearance could be identified within the first 12 h after the start of HD-MTX infusion. The factors influencing the prolonged elimination of MTX were also investigated. A significant decrease in renal function on day 2 was observed in the low-clearance group. The MTX level at 12 h and the estimated $t_{1/2}$ values were significantly correlated with BUN, Scr and Clcr on the 2nd day after HD-MTX therapy, suggesting that an alteration in renal function occurs within 12 h of the HD-MTX infusion. The prolonged elimination of MTX could be attributable to this decrease in renal function.

Key words: *High-dose methotrexate infusion, Decreased clearance, Criterion, Drug monitoring, Renal function*

Methotrexate (MTX) has been used in the treatment of acute lymphocytic leukemia, malignant lymphoma, osteosarcoma, and various kinds of solid tumors[4,12)]. However, high-dose MTX (HD-MTX) therapy involves the risk of severe toxicity which may be fatal[1,13)]. Hence, HD-MTX treatment has been scheduled with leucovorin rescue and various guidelines based on concentration-time thresholds of MTX have been reported. Nirenberg et al[6)] have classified patients as high risks for severe toxicity with serum MTX concentrations more than 10 μM at 24 h, 1 μM at 48 h, and 0.1 μM at 72 h after the start of infusion. Stoller et al[11)] and Perez et al[7)] have suggested the serum MTX level at 48 h as a reliable indicator. Furthermore, the rate of MTX elimination from plasma may also be useful for identification of high-risk patients, since the cytotoxic effects of MTX might be a function of both concentration and duration of exposure[8)]. Evans et al[3)] reported

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the use of a 24-h serum level and an elimination half-life ($t_{1/2}$) during the first 24 h after the end of infusion as reliable indicators for pediatric patients.

In many cases, the conventional leucovorin rescue is begun within 24 h after the infusion. In order to avoid severe toxicity, an earlier recognition of patients with decreased MTX clearance is required for modification of leucovorin rescue.

The purpose of this study was to identify the patients with decreased MTX clearance at the earliest period after the HD-MTX infusion for adult patients. The factors influencing the prolonged elimination of MTX were also studied.

MATERIALS AND METHODS

Patients

From April 1989 to September 1993, 56 patients at Hiroshima University Hospital participated in this study after giving their informed consent in accordance with institutional guidelines. The numbers of patients with malignant lymphoma, acute myelocytic leukemia, and acute lymphocytic leukemia were 44, 8, and 4, respectively. All patients showed a creatinine clearance of > 55.0 ml/min and had no clinical features such as pleural effusions, ascites, or other 'third spaces' prior to HD-MTX therapy[2].

HD-MTX therapy

Intravenous hydration and urine alkalization (pH 7.0~8.0) with sodium bicarbonate were started 12 h prior to HD-MTX therapy as previously recommended[9]. The dosage of MTX ranged from 1.9 to 3.8 g/m^2. The infusion schedule was as follows: one-half of the total dose was infused over 1 h and the remaining dose over 5 h. The leucovorin rescue for all patients was begun 24 h after the initiation of MTX infusion. Leucovorin was administered by eight intravenous doses of 12 mg/body every 6 h for patients with high MTX clearance. For the patients with low MTX clearance, leucovorin was administered as an increased dose (15~30 mg/body) until their MTX serum levels dropped below 0.1 μM to 0.05 μM.

Serum MTX concentration

Blood samples were drawn at 1, 6, 12, 24, 48, 72 and 96 h after the initiation of the infusion and were centrifuged to obtain serum samples. The serum MTX concentrations were determined in duplicate by the enzyme inhibition assay (Methotrexate Kit, Iatron Co. Ltd., Tokyo, Japan). This assay allows detection of 0.03 μM MTX. The concentration measure was made in 10 min for one sample and results were reported immediately to the clinicians.

Clinical data

Blood urea nitrogen (BUN), serum creatinine (Scr), creatinine clearance (Clcr), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), red blood cell count (RBC), white blood cell count (WBC), and platelet count (PLT) before and after the MTX infusion were obtained at the Clinical Laboratory Services, Hiroshima University Hospital, with an automatic analyzer (Model 7250, Hitachi Co. Ltd., Tokyo, Japan).

Pharmacokinetic analysis

The elimination rate constants (k) from 6 to 12 h and from 12 to 24 h were calculated by using the negative slope of the natural logarithms of the concentrations. The elimination half-life ($t_{1/2}$) was calculated according to the equation: $t_{1/2} = 0.693/k$.

Classification of the high-clearance and low-clearance groups

The patients were retrospectively divided into the two groups based on the criterion reported by Perez et al[7]; the high-clearance group and low-clearance group with a 48-h MTX concentration of < 1 μM and ≧ 1 μM, respectively.

Statistical analysis

The statistical analysis of the data was determined by the Mann-Whitney test. A p value of < 0.05 was considered to be statistically significant. Correlation between variables was studied by linear regression analysis.

RESULTS

The demographic characteristics of the patients are presented in Table 1. Fifty out of the 56 patients were recognized as belonging to the high-clearance group, and six patients were deliberated as belonging to the low-clearance group. There was no significant difference in age and dosage of MTX between the two groups. The patients in the high-clearance group received conventional leucovorin rescue, and the patients with decreased MTX clearance received an increased dose and prolonged duration of leucovorin rescue.

Figure 1 shows the time courses of serum MTX concentration in the two groups after the start of HD-MTX infusion. The MTX elimination was markedly prolonged in the low-clearance group, and the MTX concentrations at 6, 12, 24, 48, 72 and 96 h in the low-clearance group were significantly higher than those in the high-clearance group.

The MTX concentrations and estimated $t_{1/2}$ values during the first 24 h after the start of infu-

Table 1. Characteristics of the Patients

	Patients[a]		
	High-clearance	Low-clearance	p[b]
Number of patients	50	6	
Age (years)	45.1±17.4 (18~83)	55.8±15.8 (32~74)	NS[c]
Disease			
Malignant lymphoma	40	4	
Acute myelocytic leukemia	6	2	
Acute lymphocytic leukemia	4	0	
Dosage of MTX (g/m^2)	3.0±0.4 (1.9~3.8)	3.1±0.4 (2.6~3.5)	NS

Each value represents the mean ± S.D. Values in parentheses indicate the range.
a) High-clearance and Low-clearance; patients with serum MTX concentrations of < 1 μM and ≧ 1 μM at 48 h, respectively. b) Level of significance. c) Not significant.

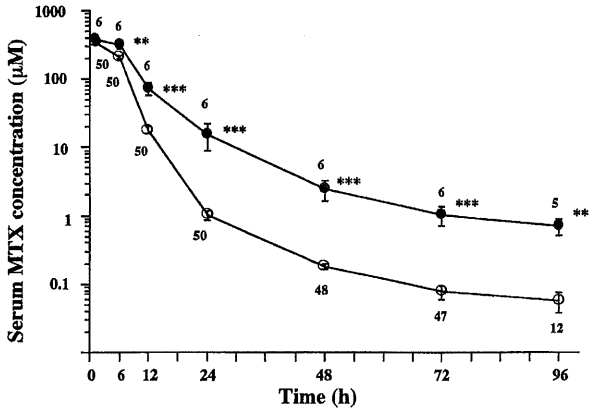

Fig. 1. Time Courses of Serum MTX Concentration in the High-(○) and Low-clearance (●) groups after 6-Hour High-dose Infusion of MTX.
Each point represents the mean±S.E.M. Numbers next to symbols refer to numbers of observations. **p<0.01; ***p<0.001.

Table 2. Serum Concentration and Elimination Half-life of MTX in the High- and Low-clearance Groups after 6-Hour High-dose Infusion of MTX

	Patients[a]		
	High-clearance (n=50)	Low-clearance (n=6)	p[b]
	Serum MTX concentration (μM)		
6 h	207 ± 62	325 ± 108	< 0.01
12 h	18 ± 14	72 ± 34	< 0.001
24 h	1.0 ± 1.1	16 ± 16	< 0.001
	Elimination half-life (h)[c]		
6–12 h	1.7 ± 0.4	2.8 ± 0.7	< 0.001
12–24 h	2.8 ± 0.8	5.0 ± 1.7	< 0.001

Each value represents the mean ± S.D. a) High-clearance and Low-clearance; patients with serum MTX concentrations of < 1 μM and ≧ 1 μM at 48 h, respectively. b) Level of significance. c) Calculated from the slope of serum MTX levels at 6 and 12 h, and 12 and 24 h, respectively.

sion are summarized in Table 2. The $t_{1/2}$ values at 6~12 h and 12~24 h after the infusion in the low-clearance group were 2.8±0.7 and 5.0±1.7 h, respectively. These values were significantly greater than those in the high-clearance group (1.7±0.4 h at 6~12 h and 2.8±0.8 h at 12~24 h, respectively).

The clinical characteristics of the two groups before and after the infusion are shown in Table 3. No significant difference was observed between the two groups in all the laboratory values before the infusion. On the other hand, the BUN and Scr values on days 2 and 7 in the low-clearance group were significantly higher than those in the high-clearance group. Furthermore, the Clcr and RBC values on days 2 and 7 and the PLT value on day 7 were significantly decreased in the low-clearance group. No significant difference was observed in the GOT, GPT, and WBC values after the HD-MTX infusion between the two groups.

The correlations of blood chemical and hematological profiles on the serum MTX concentrations and the $t_{1/2}$ values during the first 24 h after the HD-MTX infusion were investigated by using a linear regression analysis. None of the blood chemical and hematological data before the infusion was significantly correlated with the MTX concentrations and the $t_{1/2}$ values. On the contrary, renal functions monitored on the 2nd day after the infusion were significantly correlated with the MTX concentrations: the MTX concentrations at 12 and 24 h vs. the BUN values; those at 6, 12 and 24 h vs. the Scr values; those at 12 h vs. the Clcr values (Table 4). Furthermore, the $t_{1/2}$ values correlated significantly with the BUN, Scr, and Clcr values on day 2 (Table 4).

Table 3. Laboratory Values in the High- and Low-clearance Groups before and after High-dose MTX infusion

Item[a]	Before [b]		2nd day[c]		7th day[d]	
	High-clea.[e]	Low-clea.[e]	High-clea.	Low-clea.	High-clea.	Low-clea.
BUN (mg/dl)	12 ± 4	13 ± 7	11 ± 4	19 ± 8**	13 ± 10	20 ± 10**
Scr (mg/dl)	0.77 ± 0.26	0.80 ± 0.09	0.78 ± 0.20	1.21 ± 0.48**	0.76 ± 0.18	1.15 ± 0.46**
Clcr (ml/min)	99.5 ± 31.9	81.0 ± 34.2	88.4 ± 31.9	55.1 ± 5.4**	98.1 ± 44.8	61.8 ± 11.1**
GOT (IU/l)	27 ± 22	30 ± 17	53 ± 88	71 ± 54	45 ± 68	41 ± 43
GPT (IU/l)	43 ± 44	41 ± 29	74 ± 106	86 ± 81	79 ± 102	85 ± 89
RBC ($\times 10^4/\mu l$)	330 ± 64	284 ± 68	320 ± 65	276 ± 54*	317 ± 64	258 ± 32*
WBC ($\times 10^3/\mu l$)	4.1 ± 2.0	4.2 ± 2.2	4.1 ± 1.6	4.6 ± 3.7	4.0 ± 1.9	4.4 ± 2.4
PLT ($\times 10^4/\mu l$)	24.6 ± 13.8	25.0 ± 20.8	28.1 ± 15.5	26.0 ± 26.7	33.8 ± 29.6	19.7 ± 24.4*

Each value represents the mean ± S.D. *p<0.05 and **p<0.01; vs. high-clearance group.
a) BUN, blood urea nitrogen; Scr, serum creatinine; Clcr, creatinine clearance; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; RBC, red blood cell; WBC, white blood cell; PLT, platelet. b) Before MTX Infusion. c) 2 days after MTX Infusion. d) 7 days after MTX Infusion. e) High-clearance and Low-clearance; patients with serum MTX concentrations of < 1 μM (n=50) and ≧ 1 μM (n=6) at 48 h, respectively.

Table 4. Correlation between Pharmacokinetic Parameters of MTX and BUN, Scr or Clcr on 2nd Day after 6-Hour High-dose Infusion of MTX

Parameters	BUN[a]		Scr[b]		Clcr[c]	
	r[d]	p[e]	r	p	r	p
Serum Concentration (n=56)						
6 h	0.260	NS[f]	0.365	0.01	–0.115	NS
12 h	0.358	0.01	0.546	0.001	–0.297	0.05
24 h	0.399	0.01	0.522	0.001	–0.247	NS
Elimination half-life[g] (n=56)						
6–12 h	0.357	0.01	0.524	0.001	–0.343	0.01
12–24 h	0.370	0.01	0.539	0.001	–0.304	0.05

a) Blood urea nitrogen. b) Serum creatinine. c) Creatinine clearance. d) Correlation coefficient. e) Level of significance. f) Not significant. g) Calculated from the slope of serum MTX levels at 6 and 12 h, and 12 and 24 h, respectively.

DISCUSSION

Various guidelines have been reported for preventing severe toxicity following HD-MTX therapy. In this study, 6 out of the 56 patients were considered to be at high risk for toxicity by using the criterion reported by Perez et al[7]. Their serum levels at 48 and 72 h also exceeded the toxic levels demonstrated by Nirenberg et al[6] and Crom et al[2].

Since there were no significant differences in age, dosage of MTX and laboratory values observed before HD-MTX infusion between the two groups, the prediction of patients with a decreased MTX clearance was difficult before HD-MTX treatment.

It has been reported that the cytotoxic effects of MTX depend on both MTX level and duration of exposure[8]. According to this point of view, it is important to consider not only the concentration but also the $t_{1/2}$ value for the classification of patients with decreased MTX clearance. Evans et al[3] have used the initial $t_{1/2}$ > 3.5 h and the MTX concentration > 5 μM at 24 h as indexes for high risk of toxicity. Table 5 lists the serum concentrations and $t_{1/2}$ values of the 6 patients with decreased MTX clearance identified by the MTX concentrations at 48 h as reported by Perez et al[7]. Not only the serum MTX concentration at 48 h but also those at early stages, and the $t_{1/2}$ values at 6~12 h and 12~24 h were significantly higher in all 6 patients. Based on these kinetic data, we examined the applicability of the mean + 1 S.D. of the high-clearance group as convenient criteria: MTX concentration > 270 μM at 6 h, > 32 μM at 12 h, > 2.1 μM at 24 h; and $t_{1/2}$ value > 2.1 h during 6~12 h and > 3.6 h during 12~24 h. Using the serum concentration at 12 h, 5 of the patients were identified as belonging to the low-clearance group. In addition, the $t_{1/2}$ values at 6~12 h of these 5 patients exceeded our

Table 5. Serum Concentration and Elimination Half-life of MTX in the Patients with Decreased MTX Clearance[a)]

Patient	Serum MTX Concentration (μM)			Elimination Half-life (h)[b)]	
	6 h	12 h	24 h	6–12 h	12–24 h
Criterion[c)]	270	32	2.1	2.1	3.6
M.M.	460	130	48	3.3	8.4
E.M.	450	90	16	2.6	4.8
M.T.	300	59	6.3	2.6	3.7
O.O.	290	30[d)]	4.4	1.8[d)]	4.3
N.S.	260[d)]	62	11	2.9	4.8
Y.W.	190[d)]	62	8.1	3.7	4.1

a) The patients with serum MTX level ≧ 1 μM at 48 h were identified as the low-clearance group. b) Calculated from the slope of serum MTX levels at 6 and 12 h, and 12 and 24 h, respectively. c) Each criterion was established as mean + 1 S.D. of the high-clearance group in the Table 2. d) Lower than our criteria.

setting. Moreover, all 6 patients were recognized as belonging to the low-clearance group at an early stage after HD-MTX infusion by using more than two of our proposed criteria. These findings indicate, as a result, that patients with decreased MTX clearance can be identified during the first 12 h through careful therapeutic drug monitoring after the start of HD-MTX infusion.

It is also important to elucidate the factors influencing the kinetics, including the prolonged elimination of the MTX. MTX is filtered, secreted, and reabsorbed by the nephron, secretion being via the organic anion transport system[5)]. In addition, it has been reported that more than 70% of the MTX dosage was excreted during the first 48 h period from the start of MTX infusion[14)]. In the present study, a significant decrease in renal function was observed in the low-clearance group on the 2nd day after HD-MTX infusion, whereas all patients showed normal renal functions before the treatment (Table 3). Moreover, renal functions on day 2 showed significant correlations with the MTX concentrations and the $t_{1/2}$ values compared to hepatic function and hematological values. These results indicate that the alteration in renal function begins during the early period after the start of infusion, and that the higher MTX level and the prolonged MTX elimination might be attributable to the decrease in renal function. It has been reported that an early increase in the Scr value may be useful as a sign of impending toxicity[10)]. On the other hand, there is a report suggesting that Scr measurements may not be sensitive enough to detect this functional impairment[11)]. In the present study, the Scr values showed a higher correlation with the MTX concentrations and the $t_{1/2}$ values than the BUN and Clcr values (Table 4).

It was concluded that the patients with decreased MTX clearance could be identified by use of our criteria during the first 12 h after the start of infusion: MTX concentration > 270 μM at 6 h, > 32 μM at 12 h; $t_{1/2}$ value > 2.1 h during 6～12 h. The prolonged elimination of MTX might be attributable to a decrease in renal function. Careful monitoring at the initial phase of MTX elimination would contribute to the effectiveness of HD-MTX therapy with leucovorin rescue.

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