Bull Yamaguchi Med Sch 35(1-2) : 23-29, 1988

Pharmacokinetic Study on One Case of High Dose Methotrexate Therapy with Leucovorin Rescue

Toshio Fujita, Hiroo Kimoto*, Akira Koshiro*, Norio Yamamoto**, and Jisaburo Sakatoku***

Department of Pharmacy*, Yamaguchi University Hospital and Department of Urology**, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan
(Received January 31, revised March 23, 1988)

Abstract High-dose methotrexate therapy in a testicular cancer was studied pharmacokinetically to obtain safe and effective massive methotrexate theraphy. The drug concentration in serum, cerebrospinal fluid and urinary excretion was analyzed according to two-or three-compartment open models. The ratio of exposure to methotrexate in the central vs. the peripheral compartments was calculated to be 1 : 0.14 by a two-compartment open model. A larger amount of methotrexate permeates into the peripheral compartment by passive transport when a high dose, as compared to the usual dose, is used. However, most of the administered methotrexate is rapidly excreted in urine without any antitumor effects. The urinary excretion showed almost the same pharmacokinetical parameters as obtained from the serum data. The analysis based on a three-compartment open model indicates that the low and sustained serum concentration of methotrexate at the third phase was found to be governed by its release from the third compartment. General consideration about cancer chemotherapy using methotrexate are also discussed.

Key Words : Methotrexate, Pharmacokinetics, Cancer chemotherapy, High dose methotrexate therapy

Introduction

High dose methotrexate therapy combined with leucovorin rescue is applied not only to acute leukemia, choriocarcinoma and malignant lymphoma but also to osteosarcoma which is refractory against methotrexate therapy at ordinary doses. Pharmacokinetical information is essential in order to obtain effective and safe therapy because the antitumor effect of high doses of methotrexate is accompanied by severely adverse or fatal reactions. Much information on the pharmacokinetics of methotrexate is available[1~5]. However, only a few reports give a detailed pharmacokinetic analysis based on physiological pharmacokinetics[6~8]. But it is not practical for clinical application. Therefore, in the current study, one case of high dose methotrexate adminisration was analyzed according to conventional compartment theory.

Methods

A twenty-six-year-old male (50kg) had been experiencing right testicular swelling since January 1984. He visited a physician complaining of a

cough and general fatigue. Later, he received right orchiectomy in July of 1984. Histological findings confirmed mixed type testicular tumors with seminoma, embryonal carcinoma and choriocarcinoma. His chest X-ray showed evidence of multiple tumor metastasis. Human chorionic gonadotropin level rose up to 114,000 units. Gastrectomy was carried out on August 15th, 1985, because of uncontrollable gastric bleeding due to metastasis. On September 4th, he became unconscious due to cerebral metastasis. Consequently, craniotomy was performed to remove intracranial hematoma and metastatic tumor. Because of the elevation of human chorionic gonadotropin and disseminated choriocarcinoma into the lung, intestinal tract and brain, high dose methotrexate therapy combined with leucovorin rescue was selected as the only possible treatment. After being pretreated with hydration and alkalinization of urine using sodiumbicarbonate and sodium acetazolamide injections during 12 h, the patient received 10g of methotrexate in 500 ml of 5% glucose injection containing sodium bicarbonate over a period of 6 h with continuous hydration and alkalinization of urine. From 3 h after the completion of the administration of methotrexate, 15 mg of leucovorin was adminstered parenterally every 3 h according to the recommended protocol.

Blood and cerebrospinal fluid was collected from the drainage tube set in the intracranial cavity. The urine was collected immediately at the initiation of methotrexate administration. The methotrexate level in each body fluid was determined by homogenous enzyme immunoassay (EMIT®). Data analysis was carried out using an ACOS-850 computer (Nippon Electric Co. Ltd., Tokyo).

Results

The methotrexate level of each body fluid is listed in Table I and depicted in Fig. 1.

The serum concentration of methotrexate

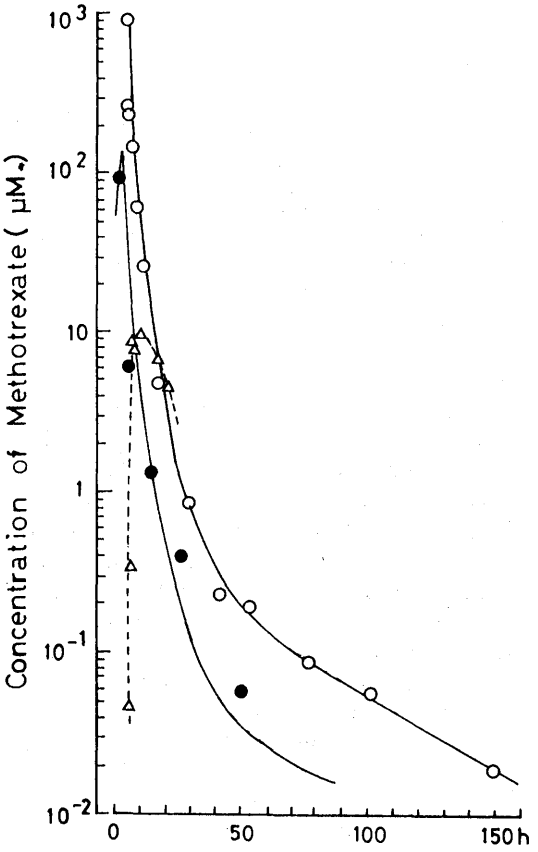

Fig. 1 Time courses of the Concentrations of Methotrexate in Serum and Cerebrospinal Fluid (CSF).
Serum 10g/6h(−○−), CSF 10g/6h(−−△−−), serum 2g/3h(−●−).

Table 1 Concentrations of Methotrexate in Serum, Cerebrospinal Fluid(CSF) and Urinary Excretion after Dosing 0.2g/kg by 6-h Infusion.

Serum															
Time(h)	6	6.65	8	10	12	18	30	42	54	78	102	150			
μM	939.6	227.9	148.0	62.1	26.5	4.70	0.90	0.23	0.019	0.087	0.055	0.0188			
CSF															
Time(h)	3	6	7	8	9	12	15	18	21						
μM	0.0012	0.048	0.345	8.85	7.68	9.69	9.12	6.54	4.38						
Urinary Excretion															
Time(h)	1	2	3	4	5	6	6.5	7	7.5	8	9	10	12	15	21
%	0.02	2.9	7.1	18.3	32.0	52.0	59.7	67.5	72.0	80.9	90.0	92.8	97.6	99.2	99.4
Time(h)	6.5	7	7.5	8	9	10	12	15	18	21	26	27	30	42	
$U_\infty - U_t$ (g)	6.34	5.11	4.41	3.03	1.61	1.14	0.38	0.26	0.13	0.10	0.075	0.043	0.027	0.014	

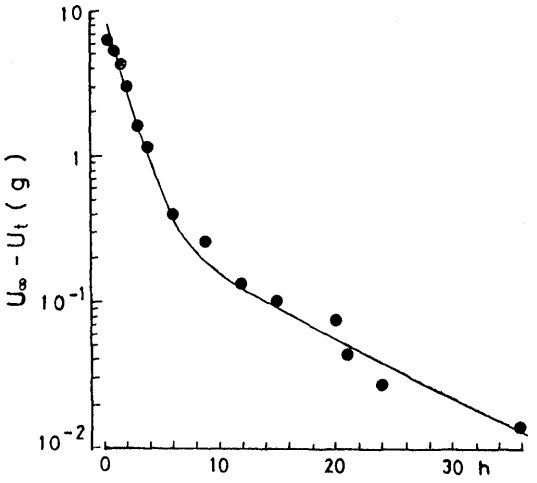

Fig. 2 The Sigma-minus Plots of the Urinary Excretion of Methotrexate after Dosing 0.2g/kg by 6h Infusion.

at 30 h and 50 h were 0.9 μM and 0.19 μM, respectively. These values were similar to those observed by Fujimoto et al [1,2].

The cumulative urinary excretion and sigma-minus values are also listed in Table I. The plots of logarithms of the sigma-minus values versus time shows the typical biexponential pattern (Fig. 2).

About half of the total amount of methotrexate was excreted within 12 h after methotrexate administration while sufficient hydration and alkalinization of the urine was maintained.

The concentration data for methotrexate in serum up to 42 h and those in cerebrospinal fluid were preliminarily analyzed according to two-compartment open model (Fig. 3).

The time courses of decline of the amount of methotrexate in the central compartment (X_1) and that in the peripheral compartment (X_2) are represented as Eq.1 and Eq.2, respectively.

$$X_1 = C_1 V d_1 = k_0 \left[\frac{(e^{\alpha T}-1)(k_{21}-\alpha)}{\alpha(\beta-\alpha)} e^{-\alpha t} + \frac{(e^{\beta T}-1)(k_{21}-\beta)}{\beta(\alpha-\beta)} e^{-\beta t}\right] \quad (1)$$

$$X_2 = C_2 V d_2 = k_0 k_{12} \left[\frac{(e^{\alpha T}-1)}{\alpha(\beta-\alpha)} e^{-\alpha t} + \frac{(e^{\beta t}-1)}{\beta(\alpha-\beta)} e^{-\beta t}\right] \quad (2)$$

$$\alpha = 0.5(k_{12}+k_{21}+k_e+((k_{12}+k_{21}+k_e)^2-4k_{21}k_e)^{0.5}) \quad (3)$$

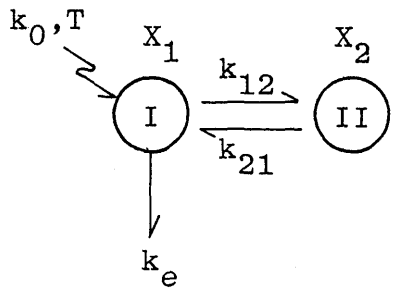

Fig. 3 Two-Compartment Open Model
X_1 and X_2 : amounts of methotrexate in central and peripheral compartment, respectively ; k_{12} and k_{21} : transport rate constants between central and peripheral comartments ; k_e : excretion rate constant ; k_0 : infusion rate ; T : infusion time (h).

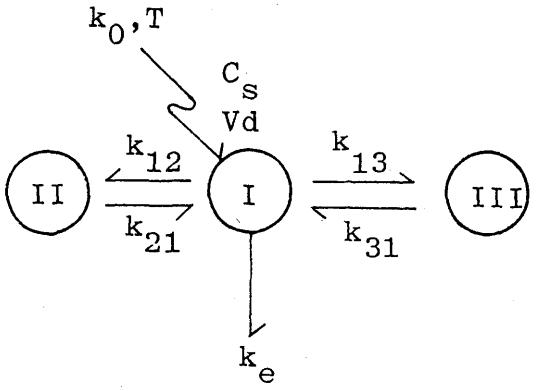

Fig. 4 Three-Compartment Open Model
C_s : Concentration of central compartment ; Vd : distribution volume of central compartment ; k_0 : infusion rate ; T : infusion time ; k_{12} and k_{21} : transport rate constants between central compartment and peripheral comartment II ; k_{13} and k_{31} : transport rate constants between central compartment and peripheral compartment III.

$$\beta = 0.5(k_{12}+k_{21}+k_e-((k_{12}+k_{21}+k_e)^2-4k_{21}k_e)^{0.5}) \quad (4)$$

where k_0 is infusion rate (μM/h), T is infusion time. α and β are represented as Eq. 3 and Eq. 4, respectively.

The obtained data were analyzed according to Eq. 1 and Eq. 2 using nonlinear least-squares method program "MULTI" [9] written in FORTRAN. The calculated values of parameters were Vd 22.7 L/body, $k_{21}=0.102$ h^{-1}, $\alpha=0.455$ h^{-1}, $\beta=0.098$ h^{-1}, $k_{12}=0.247$ h^{-1}, respectively. These values correspond to the

degrees of exposure to methotrexate in each compartment.

As for the cerebrospinal fluid data, physically reliable parameters could not be calculated.

The analysis of sigma-minus data ($U_\infty - U_t$) of the urinary excretion according to Eq. 5 yielded $\alpha = 0.547\ h^{-1}$, and $\beta = 0.092\ h^{-1}$ which are similar to values obtained by serum data according to Eq.1 and Eq.2.

$$U_\infty - U_t = Ae^{-\alpha t} + Be^{-\beta t} \quad (5)$$

This suggests that the urinary data also may give comparatively useful pharmacokientic parameters, although less precise compared to serum data. Furthermore, the cumulative urinary excretion data is important in the evaluation of the urinary excretion and the tissue accumulation.

The serum data were further analyzed according to a three-compartment open model. The model depicted in Fig. 4 and described by Eq. 6 gave the most physically reliable parameters.

$$C_s = \frac{k_0}{Vd}\left[\frac{(e^{\alpha T}-1)(k_{21}-\alpha)(k_{31}-\alpha)}{\alpha(\beta-\alpha)(\gamma-\alpha)}e^{-\alpha t} + \frac{(e^{\beta T}-1)(k_{21}-\beta)(k_{31}-\beta)}{\beta(\alpha-\beta)(\gamma-\beta)}e^{-\beta t} + \frac{(e^{\gamma T}-1)(k_{21}-\gamma)(k_{31}-\gamma)}{\gamma(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t}\right] \quad (6)$$

The results were $Vd = 20.1L$, $\alpha = 0.503h^{-1}$, $\beta = 0.167h^{-1}$, $\gamma = 0.022h^{-1}$, $k_{21} = 0.181h^{-1}$ and $k_{31} = 0.022h^{-1}$.

Substituting these values for parameters in Eq. 6, simulation curve of the time course of methotrexate level was generated when 2g of the methotrexate was administered within 3 h and was compared with measured values. Relatively good fits were observed as shown in Fig. 1, indicating the usefuless of the above model and parameters.

Discussion

The uptake of methotrexate into the cell is said to be intermediated by some active transport mechanism through the cell membrane[10]. Because of the dysfunction of the membrane of the tumor cell, the active transport of methotrexate into a normal cell is higher than in a tumor cell[11]. This phenomenon is one of the favorable properties for leucovorin rescue, but unfavorable in terms of the selective antitumor activity of methotrexate. Moreover, the transport of methotrexate into the deep tissue is insufficient at ordinary concentrations of methotrexate as substantiated by the unsatisfactory response for osteosarcoma by standard methotrexate therapy. High dose administration of methotrexate has overcome these problems by compensating for the deficiency of active transport through the enhancement of passive diffusion by a high concentration of methotrexate, which allowed effective application of this therapy to many other kinds of tumors. Ultra high dose methotrexate therapy has been successfully used even for osteosarcoma. In this therapy, the rescue by leucovorin should be done with careful monitoring of the level of methotrexate in the blood, urinary excretion, and laboratory findings in order to avoid fatal adverse effects.

Homogenous multiplied enzyme immunoassay (EMIT®), fluorescence polarization immunoassay (TDX®) and high performance chromatography are available to determine methotrexate level[12~15]. The homogenous multiplied enzyme immunoassay has been confirmed as being able to determine methotrexate selectively. Cross reactivity in this immunoassay with its main metabolite, 7-hydroxy-methotrexate, is only 4%, so overestimation is hardly likely in the determination of methotrexate level[15].

The cumulative urinary excretion observed in this study was 50% within 6 h after the initiation of methotrexate infusion and almost 100% within 12 h. The reported urinary excretion rates were $45.1 \pm 19.0\%$ within 6 h and $64.2 \pm 28.0\%$ within 24 h and $66.1 \pm 27.8\%$ within 72 h[1] or 55% within 24 h and 65% within 48 h[5]. The comparatively rapid urinary excretion rate observed in this case seemed to be more responsive to the diuresis by sufficient hydration and alkalinization of urine rather than to nonspecific determination of methotrexate and its main metabolite, 7-hydroxy-methotrexate.

The cytotoxicity of methotrexate shows s-phase specificity in the cell cycle. The degree of the exposure to methotrexate is an

important factor in evaluating the antitumor effect of methotrexate. The integration values of Eq.1 and Eq.2 during 0 to infinite times were 5.16 x 10^4 μmole·h for the central compartment and 7.26 x 10^3 μmole·h for the peripheral compartment. This suggests that only a small amount of the administered methotrexate permeates into peripheral compartment by enhanced passive diffusion due to its high concentration, and the greater part is rapidly excreted without any contribution to the antitumor effect on the tissues. From the above stand point, techniques for the enhancement of methotrexate uptake into the cell need to be developed. Some such approaches have already been carried out, although most of them are in vitro experiments. Probenecid[16], indomethacin[17] and non-ionic surfactant vesicle (niosome)[18] are reported to enhance the methotrexate uptake and the antitumor effects.

Other successful approaches such as combined use of vinca alkaloids with verapamil[19,20], a calcium channel blocker, and esterification of chlorambucil with estradiol (estrabucil)[21] have been reported.

Because of the nonspecific activity of antitumor agents dissimilar to antibiotics such as β-lactam antibiotics against infection, sophisticated pharmacokientic study is also essential for the determination of dosage schedule to obtain more effective and less toxic antitumor therapy. For a representative example, rescue with leucovorin is necessary to prevent adverse effects such as bone marrow depression. Rescue with leucovorin is said to be based on the differences of active transport of cell membrane. It is difficult for leucovorin to permeate the membrane of the tumor cells due to the lack of active transport, whereas it can selectively permeate into the normal cells. Therefore, leucovorin rescue is possible. It has been reported, however, that the excess administration of leucovorin attenuates the antitumor activity of methotrexate[22]. The protocol for leucovorin rescue, therefore, has many difficult problems. Shirotnak et al[22] has reported the relationship between the survival rate and interval of leucovorin rescue after treatment with various dose of methotrexate in mice. The results show that a too-rapid administration of leucovorin reduced the survival rate compared to a gradual administration. This suggests that leucovorin dose not enter the cell when sufficient methotrexate remains in the medium because of competition with methotrexate for the active transport. Favre at al [23] reported the protocol of high doses of methotrexate therapy with 24h continuous infusion and initiation of leucovorin rescue at 36 h.

Another important problem in cancer chemotherapy is the tissue distribution of antitumor agents. When methotrexate is administered in high doses, the prolongation of plasma concentration level is observed independently of administered dose. This phenomenon was explained by the strong binding of methotrexate to dihydrofolate reductase in the cells. To clarify the precise kinetics of methotrexate in the body including the binding to dihydrofolate reductase, pharmacokinetic technique based on physiological knowledge instead of conventional compartment theory is most useful. Dedrick and Bischoff[7] have developed the physiological pharmacokinetics and clarified the disposition of methotrexate in bone marrow, spleen and small intestine, where the time courses of the concentration in tissue are not parallel to those in plasma. It is desired, therefore, that we develop a practical application of physiological pharmacokientics for cancer chemotherapy in the near future.

We thank Dr. J. Kariya, Mrs. E. Niki and Mr. T. Munechika of the Data Processing Center, Yamaguchi University, School of Technology, for their kind advice in computations and Prof. T. Ban of the Department of Phamacology, Yamaguchi University, School of Medicine, for his kind advice in manuscription.

References

1) Sasaki, K. and Fujimoto, T. : Massive dose with citrovorum factor rescue : MTX-pharmacokinetics, excretion pattern and techniques for safety administration. *J. Jpn. Soc. of Cancer Therapy,* **14** : 988-966, 1979 (in Japanese).
2) Fujimoto, T. and Sasaki, K. : Pharmacokinetics of high dose methotrexate in solu-

tion and cerebrospinal fluid. *Cancer and Chemotherapy,* **105** : 279-286, 1979 (in Japanese).
3) Fujimoto, T., Sutow, W. W., Wang, Y. M. and Kin, H. : High dose methotrexate with citrovorum factor rescue in children. *Cancer and Chemotherapy,* **91** : 525-531, 1977 (in Japanese).
4) Taguchi, H. : Method for administration of lecovorin at high dose methotrexate therapy. *Rinsho Ketsueki,* **22** : 1687-1699, 1981 (in Japanese).
5) Hayata, S., Nomura, S., Hitoshi, T., Tanaka, T. and Hiyoshi, Y. : Pharmacokinetics and toxicity of hig dose methotrexate therapy in children, *Byoin Yakugaku,* **10** : 155-159, 1984 (in Japanese).
6) Bischoff, K. B., Dedrick, D. L., Zaharko, D. S. and Longstrength, J. A. : Methotrexate pharmacokinetics. *J. Pharm. Sci.,* **60** : 1128-1133, 1971.
7) Dedrick, R. L., Zaharko, D. S. and Lutz, R. J. : Transport and binding of methotrexate in vivo. *J. Pharm. Sci.,* **62** : 882-890, 1973.
8) Reich, S. D., Bachur, R. H., Goebel, R. H. and Berman, M. : A pharmacokinetic model for high-dose methotrexate infusions in man. *J. Pharmacokinet.Biopharm.,* **5** : 421-433, 1977.
9) Yamaoka, K. : Analysis of pharmacokinetics of drug by a microcomputer, Nankodo, Tokyo, 1984.
10) Kessel, D., Hall, T. C. and Roberts, D : Uptake as a determinant of methotrexate response in mouse leukemias. *Science,* **150** : 752-754, 1965.
11) Goldman, I. D., The characteristics of the membrane transport of amethopterin and the naturally occuring folates. *Ann. N. Y. Acad. Sci.,* **186** : 400-422, 1971.
12) Tong, W. P., Wisnicki, J. L., Horton, J. and Ludlum, D. B. : A direct analysis of methotrexate and their 7-hydroxymetabolites in plasma by high performance liquid chromatography. *Clinica Chimica Acta.,* **107** : 67-72, 1980.
13) Cairres, D. A. and Evans, W. E., High performance liquid chroamtography assay of methotrexate, 7-hydroxymethotrexate, 4-deoxy-4-amino-N^{10}-methylpteroic acid and sulfamethoxazole in serum, urine and cerberospinal fluid. *J. Chromatogr.,* **231** : 103-105, 1982.
14) Samuels, L. L., Feinberg, A., Moccio, D. M., Sirontag, F. M. and Resen, G. : Detection by high performance liquid chromatography of methotrexate and its metabolites in tumor tissue from osteosarcoma patient treated with high dose methotrexate/leucovorin rescue. *Biochem. Pharmacol.,* **33** : 2711-2714, 1984.
15) So, N., Chandra, D. P. and Alexander, I. S. : Determination of serum methotrexate and 7-hydroxymethotrexate concentrations, method and evaluation showing advantages of high performance liquid chromatography. *J. Chromatogr.,* **337** : 81-90, 1985.
16) Gewirtz, D. A. and Hait, S. A. : Protein binding as a component of drug interaction in cellular pharmacokinetic studies : Effects of probenecid on transport and accumulation of methotrexate in Ehrlich ascite tumor cell in vitro. *Biochem. Pharmacol.,* **34** : 747-754, 1985.
17) Graffen, J. D., Bennet, A. and Barrer, M. R. : A new and its use to show that indomethacin enhances cell killing by methotrexate. *J. Pharm. Pharmcol.,* **37** : 261-263, 1985.
18) Azmin, M. N., Florence, A. T., Handjanivilla R. M., Stuart, J. F. B., Vanterberghe, G. and Whittaker, J. S. : The effects of nonionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. *J. Pharm. Pharmcol.,* **37** : 237-242, 1985.
19) Tsuruo, T., Iida. H., Tsukagoshi, S. and Sakurai, Y. : Overcoming of vincristine resistance in P 388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res.,* **4** : 1967-1972, 1981.
20) Tsuruo, T. : Chemotherapeutic modalities with high selectivity against durg resistant tumor cells. *14th International Congress of Chemotherapy,* Kyoto, Japan, 1985, Abstract, p.11.
21) Ohsawa, N. : Bestrabucil (Estradiol-chlorambucil conjugate) and its carcinotropic feature. *14th International Congress of Chemotherapy,* Kyoto, Japan, 1985, Abstract, p.10.
22) Shirotnak, F. M., Moccio, D. M. and Dedrick, D. M. : Optimization of high dose methotrexate with leucovorin rescue therapy in L 1210 leukemia and sarcoma 180 murine tumor models. *Cancer Chemother. Rep. (Part 3),* **6** : 25-29, 1975.
23) Favere, R., Bruno, R., Lena, N., Imbert, A. M. Iliadis, A., Dracourt, M., Catalin, J., Cano, J. P. and Carcassonne, Y. : MTX-HD Phar-

macokinetics in clinical use : Test dose protocol and Bayesian improvement. *14th International Congress of Chemotherapy,* Kyoto, Japan, 1985, Abstract, p.339.