November 1998

Accumulation of methotrexate in human tissues following high-dose methotrexate therapy

M P. Iqbal
Aga Khan University, perwaiz.iqbal@aku.edu

Accumulation of Methotrexate in Human Tissues Following High-Dose Methotrexate Therapy

Pages with reference to book, From 341 To 343

M. Perwaiz Iqbal (Department of Biochemistry, The Aga Khan University, Karachi.)

Abstract

Methotrexate concentration was analyzed in a number of tissues of a patient of osteogenic sarcoma who had been on high-dose methotrexate therapy for nearly 6 months. Gall bladder and kidney contained the highest concentration of the drug, followed by testis, small intestine, skeletal muscle, bone marrow, lung, spleen, heart and liver, Although, compared to kidney the liver contained relatively small amount of the drug, yet nearly 115th of the total drug in liver was in bound form, This bound form of methotrexate is most likely associated with multiple forms of dihydrofolate reductase. The total concentration of methotrexate in kidney is 80 fold higher than the concentration of the drug in liver and 28 fold higher than the concentration in bone marrow. This suggests that in high-dose methotrexate therapy,nephrotoxicityis the more immediate threat to the patient than hepatotoxicity and bone marrow suppression (JPMA 4&341 ,1998).

Introduction

Antifolate drug methotrexate (MTX) has wide application in the treatment of a variety of neoplasms[1]. It is widely used in high dosages along with “leucovorin rescue” in the treatment of osteogemc sarcoma This therapy has been quite effective[2-8], however, occasional reports of clinical toxicity do appear in the literature[4,9]. Despite extensive use of the drug in the treatment of cancer, data pertaining to its accumulation in various human tissues is scanty especially after high dosage protocols[10,11]. In this paper, we report the accumulation of MTX in human tissues alter multiple cycles of high-dose MTX in a patient suffering from osteogenic sarcoma.

Patients and Methods

Tissue samples were provided by the University of California at Los Angles Center for Health Sciences, Los Angeles, of a patient who died of metastatic osteogenic sarcoma. This patient was a 16 year old boy and was being treated with 4-hour infusions of MTX (200 mg/kg). He had received thirteen cycles of MTX over a period of six months. Each infusion of the drug was preceded by intravenous hydration and urinary alkalinization and Ca leucovorin rescue was initiated 4 hours following the completion of MTX infusion. A week following the last infusion, the patient died of sepsis. An autopsy was performed and tissue specimens of liver, kidney, spleen, bone marrow, heart, skeletal muscle, jejunum, thyroid, lung, testis and gall bladder were obtained for analysis of free and protein-bound MTX.

Tissues were rinsed with cold 0.15 M NaCI and dried on a filter paper. A piece of each tissue was weighed and homogenized in a homogenizer using 3 volumes of 0.06 M citrate buffer, pH 7.4 per gram of tissue using a procedure described by Rothenberg et al[12]. The homogenate was centrifuged at 30,000xg for 15 ruin and the supernatant solution was divided into two parts. One part was subjected to exhaustive dialysis overnight against 0.06 M citate buffer, pH 7.4 containing an anion exchange resin Ag 2x8 (Bio-Rad Laboratories) and the non-dialyzable MTX in the dialysand was assayed by a sensitive ligand binding radioassay[13]. Total MTX in the other half (not subjected to dialysis) was also determined by the same procedure. Protein concentration in the cytoplasmic contents of the tissues was determined by the method of Lowry et al[14]. Hemoglobin contents in some of these tissues (bone marrow, heart, spleen and skeletal muscle) were determined by the benzidine method[15] and subtracted from the total protein concentration of that particular tissue.

Results

Table. Methotrexate concentration in various human tissues following repeated cycles of high-dose methotrexate therapy.

Organ	ng total MTX/mg protein	ng Bound MTX/mg protein	ng free MTX/mg protein	% bound
Gall bladder (bile)	10,250	-	-	-
Kidney	2,408	337	2,071	14
Testis	167	5	167	3
Jejunum	110	7	103	6
Skeletal muscle	105	8	97	8
Bone marrow	86	8	78	9
Lung	64	5	59	8
Spleen	47	4	43	9
Thyroid	43	6	37	14
Heart	32	3	29	9
Liver	30	6	24	20

Table shows the free and the protein-bound MTX concentration values in the cytoplasmic samples of various human tissues: Highest levels of the drug were found in gall bladder, kidney and testis, while jejunum, skeletal muscle and bone marrowhadlevelsbetween86 ng -110 ngpermgprotein. Liver, spleen, lung and heart did not show a substantial uptake of the drug.

Discussion

High levels of the drug in gallbladder, kidney and small intestine are consistent with the findings of Anderson et al[10] who have shown kidney and gall bladder having the drug concentration more than any other tissue in the body. This is further supported by various models of MTX pharmacokinetics showing elimination of the drug mainly through kidney and biliary secretion[16], therefore, most of the drug was expected to be present in the kidney and the gall bladder. Because of high levels of plasma MTX due to repeated infusions, othertissues such as heart, skeletal muscle, lungs and thyroid did show accumulation of the drug to some degree. This may suggest that at highplasma levels of the drug, the tissues generally spared at low plasma levels of the drug still permit drug intake probably by passive diffusion. Since there is usually very little dihydrofolate reductase (the principal binding detenninant of MTX) inside these tissues[17], only a small component of the total drug is in the bound form, whereas most of the drug is in the free state. On the other hand, liver compared to other tissues has been shown to have the maximum amount of dihydmfolate reductase[17,18] and therefore, nearly 1/5thofthetotal drug inliverwas foundtobe in the bound form. Since the amount of enzyme in most human es ineluding liver has been repoited to be less than 0.08 nmol/gm of it is unlikely that all of the protein-bound MDC is in association with high affinity form of dilry drofolate reductase.

This alludes to the presence of a form of dihydrofolate reductase having low affinity for MTX. We have reported earlierthepresenceof such aformofenzymeinnormal human colon[20] and goat liver[21] High dose methotrexate therapy is now being increasingly used in Pakistan[18,19,22]. Although short-term renal toxicity and hematological and mucosal toxicities are prevented by hydration and alkalinization of urine with $NaHCO_3$ and leucovonn rescue, respectively, yet repeated exposure to high doses of MTX may lead to serious toxic effects due to the accumulation of the drug in various tissues of the body. These side-effects originate primarily because of the inhibition of de novo DNA synthesis.

Because of an 80-fold higher accumulation of total MTX mthe kidney compared to the liver(Table), itis plausible that the chance of nephrotoxicity would be much higher than hepatotoxicity, once multiple cycles of the drug have been administered. This observation is consistent with the results by Pitman et al. who have reported that 60% of their patients on high-dose MTX experience a serum creatinine elevation of more than 50%23. Similarly, the concentration of total MTX in kidney was found to be 28 times more than the concentration of the drug in bone marrow. This again suggests that nephmtoxicity is more likely to occur first in such patients than bone marrow suppression. Another important observation of this study was a very high concentration of MTX in the bile. Biliary excretion of MTX in humans after an intravenous injection of the drug has been reported[19,24]. However, there is not much information available on accumulation of the drug inbile after repeated cycles of therapy. Bile, therefore, could serve as a reservoir of the drug leading to a continuous supply of the drug for several hours after an infusion of MTX and thereby, contributing towards the adverse effect of the therapy. A more detailed investigation on a large number of patients receiving high-dose MDC would be required to have a betterunderstanding of the associated toxic effects.

Acknowledgements

This work was supported by a research grant (No.S-AKU-Bio-239) by the Pakistan Sciences Foundation. Valuable suggestions by Professor M. Anwar Waqar and Dr.
Ikmm Burney in improving this manuscript are gratefully acknowledged.

References

1. Jolivet J, Cowan KH, Curt GA, et al. The pharmacology and clinical use of methotrexate. N. EngI. J. Med., 1983;309: 1094- 1104.
2. JaffeN, FreiE. ill, Traggis D, et al. Adjuvantmethotrexate andcitrovoTumfactor treatment ofosteogenic sarcoma. N. Engi. J. Med., 1974;291:994-97.
3. Jaffe N. Pediatric solid tumors and the introduction of rescue therapy. Cancer, Bull., 1981 ;33:59-62.

4. Isacoff WH. Townsend CT, Eliber FR, eta!. High dose methotrexate therapy of solid tumors: Observations relating to clinical toxicity. Med. Pediatr. Oncol., 1976;2:319-25.
5. Kimura K. High dose methotrexate for adult malignancies. Cancer Bull., 1981;33:67-71.
6. Edmonson JH, Green SJ, Ivins JC, eta!. A controlled pilot study of high-dose methotrexate as postsurgical adjuvant treatment for primary osteosarcoma. 3. Clin. Oncol., 1984;2:152-56.
7. Link MP Goorin AM, Miser AW, eta!. The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N. Engl. J. Med., 1986;314:1600-6.
8. Iqbal MP, Malik IA. Mega doses of methotrexate in the treatment of osteosarcoma. J. Pak. Med. Assoc., 1990;40:248-51.
9. lqbal MP, Khursheed M, Mahboobali N. Methotrexate clearance and clinical toxicity in osteosarcornafollowing high-dosemethotrexate therapy. J. Pak. Med. Assoc., 1989;39:38-42.
10. Anderson LA, Collins GJ, Ojima Y, et al. A study of the distribution of methotrexate in human tissues and tumours. Cancer Res., 1970;30: 1344-48.
11. Oliverio VT, Zaharko DS. Tissue distribution of folate antagonists. Ann. NY. Acad. Sci., 1971;186:387-99.
12. Rothenberg SP, Iqbal MP, daCosta M. Effect of folate compounds on the accumulation of methotrexate and the activity of dihydrofolate reductase in liver, kidney and small intestine of the mouse. J. Pharm. Exp. Therap., 1982;223:631-34.
13. Rothenbcrg SP, daCosta M, Iqbal MP. Ligand-binding radioassay for the antifolate compounds; application in patients receiving methotrexate. Cancer Treat. Rep. 1977;61 :575-84.
14. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folio phenol reagent. 3. Biol. Chem., 1951;193:265-75.
15. Oser EL, Hawk's physiological chemistry, 14th edition, New York, McGraw-Hill Publishing Company Ltd., 1971, p.356.
16. BischoffKB, Dedrick RL, Zaharko DS, etal. Methotrexate pharmacok inetics. J. Pharm. Sci., 1971 ; 60:1128-33.
17. Hall TC, Roberts 0, Kessel DH. Methotrexale and folic reductase in human cancer. Eur. J. Cancer, 1966;2:135-41.
18. Blakley RL. The biochemistry of folic acid and related pteridines, Amsterdam, North-Holland Publishing Company, 1969, pp. 139-41.
19. Creaven P1, Hansen HH, Afford DA, et al. Methotrexate in liver and bile after intravenous dosage in man. Br. J. Cancer, 1973;28 :589-91.
20. Iqbal MP, Mahboobali N, Waqar MA, et al. Heterogeneity of methotrexate binding in human colon tumor cells. J. Pak. Med. Assoc., 1991 ;41 :136-39.
21. Iqbal MP, Mahboobali N, Waqar MA. Multiple forms ofdihydrofolatereductase in goat liver. Biochern. Soc. Trans., 1989;17:561-63.
22. Usman N. Osteogenic sarcoma. 10th Annual Meeting of the Medical Research Society of Pakistan, Lahore, Shaukat Khanam Memorial Hospital and Research Centre, 1996.
23. Pitman SW, Parker LM, Tattersall MHN, et al. Clinical trial of high dose mehotrexate (NSC-740) with citrovorum factor (NSC-3590) - Toxicologic and therapeutic observations. Cancer, Chemother. Rep., 1975;6:43-49.
24. Nuernberg B, Koehnke R, Solosky M, et al. Biliary elimination of low-dose methotrexate in humans. Arthritis and Rheumatism, 1990;33:898-902.