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Monitoring Methotrexate Levels in Childhood Acute Lymphoblastic Leukemia Patients During Maintenance Treatment for Methotrexate Dose Adjustment

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Abstract

Objective: Methotrexate (MTX) is one of the major chemotherapeutic drugs in childhood Acute Lymphoblastic Leukemia (ALL) patients. However the bioavailability of MTX is different in each patient. This study aims to show the bioavailability variances in childhood ALL patients taking MTX as maintenance treatment and thus having a sensitive parameter to determine patients at risk for relapse or toxicity and to determine the optimum plasma MTX dose by measuring the plasma MTX level.

Methods: Thirty-four children with ALL were included in the study. Methotrexate levels were measured before (hour 0), 2 and 72 hours after oral MTX administration. The whole blood cell count, electrolytes, and liver enzymes were tested at hours 0 and 72.

Results: The differences between the plasma MTX levels at 0 and 2 hours and 0 and 72 hours were statistically significant (P<0.001). There was no statistical difference between the plasma MTX levels of patients receiving less than 20 mg/m^2 MTX or higher dosages [hour 0 (P= 0.242), hour 2 (P= 0.242), and hour 72 (P= 0.218)]. Plasma MTX levels do not correlate with the dose of MTX administered. At equal MTX dosages, different MTX plasma levels and at different MTX dosages equal MTX plasma levels might be achieved. There was statistically no significant difference in the plasma MTX levels at 0, 2, and 72 hours between patients having WBC count less than 3x 10^9/L or more (P= 0.229) (P= 0.229) and (P= 0.243) respectively.

Conclusion: There are personal differences in the bioavailability of orally administered MTX and the plasma level of MTX. For this reason, plasma MTX levels should be monitored periodically together with the leukocyte count to define the optimum MTX treatment dose for every leukemia patient by measuring the absorption rate.

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Introduction

Oral weekly Methotrexate (MTX) together with oral daily 6-Mercaptopurine (6-MP) is the preferred maintenance treatment of childhood Acute Lymphoblastic Leukemia (ALL). MTX and 6-MP dosages are first calculated according to the body surface area of the patient and tailored during the following weeks according to the WBC counts and hepatotoxicity. MTX is rapidly, but incompletely, absorbed after oral administration with marked variation between individuals. MTX reaches peak serum levels within an hour of oral administration, and about half is protein-bound to serum albumin [1]. Patients receiving the same drug dosage show significant variations in their tolerance to methotrexate. This is due to the bioavailability of the drug, which is different in each patient. Even when given equal doses of HDMTX, patients vary significantly in their pharmacokinetics, patterns of toxicity, and response to therapy [2]. The bioavailability of oral methotrexate is lower than parenteral routes [3].

This study aims to show the bioavailability variances among the patients taking MTX as maintenance treatment and thus having a sensitive parameter to determine patients at risk for relapse or toxicity and to determine the optimum MTX dose of every patient by measuring the plasma MTX level.

Methods

Thirty-one boys and three girls with ALL were included in this cross-sectional study. The median age was 6 years (range 2-15 years). Twenty-four patients were L1, nine L2, and one unidentified according to French American British (FAB) morphologic classification. Immunophenotyping studies showed 25 common ALL, **7 T** cell ALL, and 2 pre-pre B ALL. Patients were treated according to the modified ALL-BFM 90 protocol. There were 8 patients in the Standard Risk (SR), 24 in the Medium Risk (MR), and 2 in the high risk (HR) group. Maintenance treatment was given for a median duration of 282 days (range 18-726 days). The oral MTX dose of the patients was 18.7 mg/m^2 (range 8-50 mg/m^2).

In conformity with the protocol, in the maintenance treatment, a dose of 20 mg/m^2 of MTX was given once a week, and 50 mg/m^2 of 6-MP was given every evening to the patients. The drug dosages were tailored according to the weekly WBC counts. Patients having WBC less than 1x10^9/L were not given MTX or 6-MP, they received half dose if WBC was between 1-2x10^9/L, 100% dosage if WBC was 2-3x10^9/L, and up to 150% of dosage if WBC was over 3x10^9/L. In previous studies, it was shown that approximately 2 hours after the oral MTX dose, plasma MTX levels make a peak, and after that decline rapidly over the first 2 days and tend to stabilize during the rest of the week[1]. The MTX level measured before the weekly MTX shows the stable phase of MTX, the plasma level after 2 hours of administration gives the peak level and the level at 72 hours shows the quick elimination phase of the drug. As these 3 samples indicate the weekly profile of the drug in the body, blood samples were taken before (0 hour), 2, and 72 hours after MTX administration. Plasma MTX levels were measured by a detector attached to the TSP Spectra Phoresis 2000 capillary electrophoresis. Patients who have an increase of plasma MTX concentration of <0.2 µmol/L in 1-2 hours after MTX intake are defined as slow absorbers, those having an increase of >0.2 µmol/L as fast absorbers.

The whole blood cell count, differential of leukocytes, electrolytes, and liver enzymes were tested at hours 0 and 72. Hemogram Coulter Counter MD 18 was used for hematological parameters, and biochemical tests were carried out by an autoanalyzer.

Statistical analyses were done with the Student's t-test.

Results

Plasma MTX levels of the patients at hours 0, 2, and 72 respectively are given in (Figure 1&Table 1). Plasma MTX levels had a mean (SD) of 0.71 µmol/L (0.58) (range 0.2-1.8 µmol/L) before the intake of MTX, 1.17 µmol/L (0.58) (range 0.45-2.12 µmol/L) at hour two, and 0.88 µmol/L (0.45) (range 0.49-1.92 µmol/L) at hour 72. Statistically, the levels of MTX at hours 2 and 72 were significantly different from the levels of hour 0 (P<0.001). There was no difference in the level of MTX at hours 0, 2, and 72 [hour 0 (P= 0.242), hour 2 (P= 0.242), and hour 72 (P=0.218)] between patients receiving more than 20 mg/m^2 and less than 20 mg/m^2 of MTX. There was no correlation between the dose of MTX taken and the plasma level of MTX (Figure 1). Eight of the patients were late absorbers for MTX, while 26 were fast absorbers. In 4 patients, the plasma MTX level was higher at hour 72 in comparison to hour 2. Twelve of the patients were also taking trimethoprim-sulfamethoxazole. There was no significant difference in the MTX levels at hours 0, 2, and 72 between the 2 groups at hour 0 (P= 0.233), hour 2 (P= 0.242), and hour 72 (P= 0.242).

WBC counts of the cases before MTX intake had a mean (SD) of 3.52 (1.38)x10^9/L (range 1.7-6.8x10^9/L). The mean (SD) WBC count 72 hours after MTX was 3.28 (1.12)x10^9/L (range 1.5-6.2x10^9/L). There was no significant difference between the WBC counts before and after MTX intake (P= 0.119).

There was statistically no significant difference in the plasma MTX levels at 0, 2, and 72 hours between patients having WBC count less than 3x10^9/L or more (P= 0.229) (P= 0.229) and (P= 0.243) respectively.

6-MP dosages of the patients had a mean (SD) of 45.5 (16.87) mg/m^2 (range 25-75 mg/m^2). There was no significant difference between hemoglobin levels before and after MTX administration (P= 0.154). Hepatotoxicity and myelotoxicity are seen in (Table 2).

According to the World Health Organization criteria of toxicity, hepatic toxicity of grade 1 was noted in one patient, of grade 2 in four, and grade 3 in one patient before MTX. At 72 hours, liver toxicity progressed from grade 1 to grade 2 in one patient and from grade 2 to grade 3 and to grade 4 in two other patients. The liver toxicity remained to be of grade 3 at 72 hours in the patient with grade 3 toxicity. All these 6 patients had MTX dosages higher than 15 mg/m^2, and three patients with liver toxicity had plasma MTX levels over 0.96 µmol/L. Chemotherapy was delayed in patients with liver toxicity of grade 3 and 4. No clinical toxicity was noted. Biochemical tests showed pathological results of liver function tests in 6 cases (17.6%). Myelotoxicity was observed in 18 patients. 11 cases showed grade 1, 7 cases grade 2 and one case grade 3 WBC toxicity. Platelets were affected in one patient with grade 1 toxicity. Erythroid toxicity was seen in 4 patients 3 of grade 1, one of grade 2. Patients with liver toxicity showed no erythroid toxicity.

Table 1: MTX doses and plasma levels.

MTX dose (mg/m²)	Hour 0 plasma level (µmol/L)	Hour 2 plasma level (µmol/L)	Hour 72 plasma level (µmol/L)
8	0, 95	2, 06	1, 14
8, 3	0, 8	0, 89	0, 85
8, 3	1, 15	1,38	1, 18
10	0, 97	1, 11	1, 07
12, 5	0, 8	1, 06	0, 85
12, 5	1, 2	1, 5	1, 33
13, 8	0, 33	0, 57	0, 5
15, 3	0, 4	1, 68	0, 41
15, 3	0, 66	1, 08	0, 9
15, 6	0, 56	0, 96	0, 72
15, 6	0, 7	0, 8	0, 75
15, 6	0, 92	1, 34	1, 28
16, 6	0, 44	0, 79	0, 58
16, 6	0, 52	0, 96	0, 72
17, 8	0, 88	0, 93	0, 89
18, 6	0, 25	1, 22	0, 62
18, 7	1, 78	2, 12	1, 82
18, 75	0, 28	2, 1	0, 34
19, 4	0, 45	0, 56	0, 77
20	0, 6	0, 67	0, 63
20	0, 74	1, 15	1, 02
21, 4	0, 3	2, 16	0, 41
22, 2	1, 9	2, 65	2, 16
22, 9	0, 39	0, 43	0, 48
25	0, 28	0, 9	0, 4
25	0, 42	0, 45	0, 75
25	0, 77	1, 13	0, 99
27, 5	0, 51	0, 9	0, 75
29, 7	1, 11	1, 69	1, 65
30	0, 3	0, 5	0, 35
30	0, 5	0, 72	0,6 1
30	1, 8	2, 07	1, 92
37,5	0, 2	0,4 5	0, 49
50	0, 47	0, 84	0, 71

Table 2: Hepatotoxicity and myelotoxicity before and after methotrexate.

Case	Before MTX dose					After MTX dose				
				Toxicity grade					Toxicity grade	
	ALT (U/L)	AST (U/L)	Bilirubin (mg/dl)	Liver	WBC	ALT (U/L)	AST (U/L)	Bilirubin (mg/dl)	Liver	WBC
1	76	43	1	I	I	142	78	1.5	II	I
2	271	202	1.5	III	0	288	156	1.2	III	0
3	54	165	0.5	II	II	111	211	0.5	III	III
4	98	53	0.31	II	II	87	44	0.31	II	II
5	114	58	0.4	II	II	156	84	0.08	II	I
6	142	65	0.6	II	I	413	127	0.5	IV	II

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; WBC: White Blood Cell.

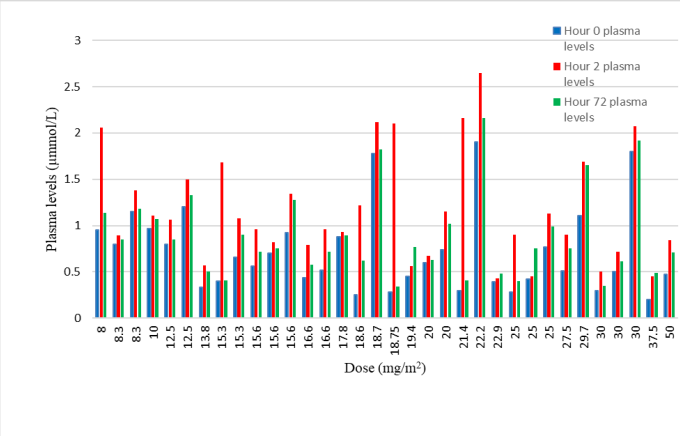

Figure 1: Oral MTX doses and plasma levels.

Discussion

Oral MTX is one of the most preferred chemotherapeutics in the maintenance treatment of childhood ALL. Many chemotherapy protocols monitor MTX dosage according to WBC counts. MTX is a widely used drug, and there are many sensitive and specific methods to determine plasma levels, but they are not used yet for therapeutic dose modification and to monitor therapy for each patient during maintenance [4]. There is a peak of MTX at hour 2 after intake of the drug, and then the level decreases to a median of 0.7 µmol/L before the new weekly MTX intake. Plasma MTX levels differ for each patient during the week, but there is always a detectable level of MTX at hour 0. Shiozawa et al. demonstrated that the mean maximal serum MTX concentration was achieved at 1-2 h after the intake of the first dose of the week [5].

Skoglund et al. in their report, 17 children showed that plasma MTX levels decreased rapidly during 2 days and were stable after that during the week [6]. In our study, stable MTX levels appeared much later than 72 hours. The last decrease of MTX is due to the long half-life of MTX, and this phase is probably due to the low distribution of the MTX stored in polyglucomized form intracellularly. Balis et al. gave a peak level for MTX between 0-5 hours, with a median of 2 hours [4]. In 3 of our patients, plasma MTX levels measured at 72 hours were higher than the level at hour 2. In these 3 patients, the peak time of MTX is probably between 2 and 5 hours. In patients with MTX dose higher than 12 mg/m^2, the peak time was found to be between 2 to 5 hours. But to show this in all patients with MTX over 12 mg/m^2 plasma MTX levels should be measured during the first 5 hours.

Reports on oral MTX intake showed no correlation between plasma MTX level and the dosage given. Different MTX levels were measured in patients who received the same MTX dosage. MTX absorption was different from patient to patient. The oral bioavailability of methotrexate is highly variable and averages 73% of that of subcutaneous administration [7]. Methotrexate bioavailability is affected adversely if high doses are absorbed all at once, thus affecting the rate of response by the patient receiving the drug [8].

Lower MTX plasma levels were recorded in patients taking higher MTX dosages. Patients with similar MTX levels also had quite different MTX dosages. This is probably due to the absorption and bioavailability of the drug [9,10]. This difference in the absorption noted between patients is also seen in the same patient when different dosages of MTX are administered. Balis et al. gave 2 different dosages of MTX to 2 different patients and recorded their plasma MTX levels; giving different dosages did not affect their plasma MTX level. The absorption was delayed in these 2 patients, and in the second patient, less absorption was noted when a higher dose of MTX was administered [4]. This observation shows us that absorption and bioavailability do not increase in parallel to increased dosage. In work done by Balis et al., plasma MTX levels measured in 15 cases and the rate of absorption had a range of 23 to 95%. Maximum levels did not have any correlation with the dose given [4]. Skoglund [6] and Teresi [10] obtained similar results. These results suggest us to think that some patients cannot reach adequate systemic MTX levels after oral drug intake, and this may be a cause of systemic relapse. Besides this, it was shown that subtherapeutic drug dosages might lead to drug resistance in the leukemic cells [11]. High drug concentration and toxicity are also related [6].

Doses between 25 and 40 mg MTX per week, administered orally, result in limited bioavailability [9]. Oral absorption of MTX was found incomplete, dose-dependent, and highly variable (absolute bioavailability range: 13-76 %) [10]. MTX absorption was shown to be higher in patients taking less than 40 mg/m^2 of oral MTX [10]. In twelve patients receiving less than 12 mg/m^2 of oral MTX, the mean (SD) bioavailability was higher than those taking MTX between 12 and 28 mg/m^2. Balis et al. reported that the mean and median maximum MTX concentrations after oral methotrexate was found to be 1.1 µmol/L (0.1-4.3) at a dose range of 21-36 mg/m^2 and 1.2 µmol/L (0.21-3.1) at a dosage range of 17.5 to 22.3 mg/m^2 [4,12]. In the study of Teresi et al. at 13 to 40 mg/m^2 of MTX, the median bioavailability was 41.7%, and in those at 43-76 mg/m^2 of MTX, it was 17.5% [10]. We had only one patient taking MTX over 40 mg/m^2. The MTX dose was 50 mg/m^2, and his plasma MTX level was less than the level of patients who had low MTX intake. His plasma MTX level was 42% of the expected MTX level. In another patient with MTX doses of 37.5 mg/m^2, the plasma MTX level was lower than that of the other patients and his MTX level was 33% of the expected level. These findings are in correlation with Teresi's results and indicate that bioavailability is lower in doses over 30 mg/m^2 of MTX, and if dosages over 40 mg/m^2 are required, intramuscularly administration should be an option. Disease-free survival is lower in slow absorbers [10,11]. Eight of our patients were slow absorbers, 26 found to be fast absorbers. By now, 6 patients have relapsed; three of them were slow absorbers, and the other three were fast absorbers. There is some evidence that at higher doses oral bioavailability declines, a phenomenon most likely because uptake of methotrexate from the gastrointestinal tract is mediated by a saturable transporter, Reduced Folate Carrier 1 (RFC11) [13]. The clinical importance of the concept of bioavailability rests on two main principles. First, that measurement of the active component at the site of action is generally not possible and, secondly, that some relationship exists between the efficacy or safety and concentration of the active compound or its active metabolite(s) in the systemic circulation [14]. Currently, there is no international consensus on drug dosing. Because of significant interindividual and intraindividual variations in drug disposition and pharmacodynamics, vigorous dose adjustments are needed to obtain a target degree of myelosuppression. As the normal white blood cell counts vary by patients' ages and ethnicity, and also within age groups, the same white blood cell levels for 2 patients may not reflect the same treatment intensity [15]. There was no statistical difference between the leukocyte counts of our patients before and 72 hours after MTX intake, but the MTX

level before intake was statistically different from levels at hour 2 and 72. These results show that either MTX level affects WBC counts with a delay or WBC counts are not a perfect reflection of the plasma MTX level. There was no statistical difference in the plasma MTX levels at hours 0, 2, and 72 between the two groups of patients having WBC counts less than and over $3x10^9$ g/L. This result also shows that WBC count is not a reflection of plasma MTX level. WBC counts cannot tell us that plasma MTX levels are at therapeutic or toxic levels. But currently, that is the method used to monitor the MTX level. We must measure plasma MTX levels of the patients, and MTX intake should be monitored according to its rate of absorption together with the leukocyte count. MTX intake did not affect hemoglobin levels. This is because the MTX plasma levels did not reach the toxicity level on hemoglobin.

There were pathological results in the liver function tests of 6 of our cases (24%) (Table 2). This is a lower rate in comparison to Skoglund et al. who report 30% of liver function abnormality [6]. All of our patients with liver toxicity received MTX doses over 15 mg/m^2; they had no sepsis, viral hepatitis, or other illnesses. The patients were also taking another hepatotoxic agent, 6-MP together with MTX. As seen in (Table 2), myelotoxicity does not reflect hepatotoxicity. In 2 patients, hepatic and leucocytic toxicity were similar. There was hepatotoxicity of grade 1 before MTX, of grade 2 after MTX and WBC toxicity was of grade 1. Hepatotoxicity of grade 3 before and after MTX was noted in 1 patient and there was no leukocytic series toxicity in this patient. In another patient who had grade 2 hepatotoxicities before and after MTX, myelotoxicity was of grade 2 before and 1 after MTX. Hepatotoxicity of grade 2 before MTX became of grade 4 in one patient, and granulocytic toxicity was of grade 1 before and 2 after MTX. These examples show that it is not possible to foresee hepatotoxicity according to the WBC counts. Three patients showing hepatotoxicity had no low WBC counts. This is due to different toxicity thresholds of the liver and the bone marrow. Normal leukocyte counts do not mean normal liver function tests. Plasma MTX levels and liver function tests should be followed at regular intervals.

In patients taking weekly oral MTX, plasma MTX levels measurements are adequately reflected by levels at hours 0, 2 and 72. There is no correlation between oral MTX intake and plasma MTX levels at hours 0, 2 and 72. There are personal differences in the bioavailability of orally administered MTX. Bioavailability is lower in patients who receive MTX at doses higher than 30 mg/m^2 MTX in comparison to patients receiving smaller doses.

Limitations

The limitation of the study was the relatively small number of patients and the fact that it was single-centered.

Conclusion

There are personal differences in the bioavailability of orally administered MTX and the plasma level of MTX. WBC counts cannot reflect whether the plasma MTX level is at a therapeutic or toxic dose. For this reason, plasma MTX levels should be monitored periodically together with the leukocyte count to define the optimum MTX treatment dose for every leukemia patient by measuring the absorption rate. Liver toxicity should be followed up regularly. It would be an excellent option to define the optimum MTX dose for every leukemia patient by measuring the absorption rate.

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