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ORIGINAL ARTICLE

Methotrexate pharmacokinetics in infants with acute lymphoblastic leukemia

Patrick A. Thompson · Daryl J. Murry · Gary L. Rosner · Simon Lunagomez · Susan M. Blaney · Stacey L. Berg · Bruce M. Camitta · ZoAnn E. Dreyer · Lisa R. Bomgaars

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Abstract
Purpose We performed a pharmacokinetic evaluation of methotrexate (MTX) in infants with acute lymphoblastic leukemia enrolled on the Pediatric Oncology Group (POG) 9407 Infant Leukemia Study to evaluate the effects of age on MTX pharmacokinetics and pharmacodynamics.
Methods A pharmacokinetic database of 61 patients was developed by combining MTX data obtained from 16 patients in a pharmacokinetic sub-study with data obtained for clinical care in other patients enrolled on the POG 9407 protocol. The data were analyzed for the first dose of MTX given to patients in induction/intensification therapy. Patients received MTX (4 g/m^2) over 24 h at week 4 of therapy. Toxicity data were also reviewed to evaluate the incidence of common MTX toxicities during the first 6 weeks of therapy (the induction/intensification phase).
Results Steady-state clearance (mean ± standard deviation) for infants aged 0–6 months was 89 ± 32 ml/min/m^2 compared to 111 ± 40 for infants aged 7–12 months ($P = 0.030$). In the subgroup of infants aged 0–3 months the mean steady-state clearance was 84 ± 30 ml/min/m^2 ($P = 0.026$ vs. the 7–12-month group). The incidence of renal toxicity (all grades) during induction/intensification therapy was 23% in the 0–3 months age group compared to 0% (for $n = 27$) in the group 7–12 months of age ($P = 0.029$). There were no significant differences in hepatoxicity or mucous membrane toxicity between age groups.
Conclusions A modest difference in steady-state MTX clearance is observed between younger infants (0–6 months) and older infants (7–12 months). Very young infants (0–3 months) also experienced a slightly higher incidence of renal toxicity during induction/intensification therapy. Steady-state clearance for the older infants is similar to values reported for children in other studies.

Keywords Methotrexate · Pediatrics · Infants · Leukemia · Pharmacokinetics

P. A. Thompson · S. M. Blaney · S. L. Berg · Z. E. Dreyer · L. R. Bomgaars (✉)
Texas Children's Cancer Center,
Baylor College of Medicine, 6621 Fannin St MC,
Houston, TX 77030-2399, USA
e-mail: lbomgaars@txccc.org

D. J. Murry
Department of Pharmacology,
University of Iowa, Iowa City, IA, USA

G. L. Rosner
Department of Biostatistics, University of Texas,
MD Anderson Cancer Center, Houston, TX 77030, USA

S. Lunagomez
Institute of Statistics and Decision Sciences, Duke University,
Durham, NC 27708, USA

B. M. Camitta
Midwest Children's Cancer Center,
Medical College of Wisconsin, Milwaukee, WI 53226, USA

Introduction

Methotrexate (MTX) is one of the most widely used agents in the treatment of childhood cancer. It is an important component of treatment for acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma, osteosarcoma, and other malignancies. MTX is a folate

analogue that inhibits the enzyme dihydrofolate reductase, which is responsible for converting folate to its active, chemically reduced tetrahydrofolate form [6, 13]. In the presence of excess MTX, the intracellular tetrahydrofolate pool is depleted, leading to the depletion of purines and thymidylate and inhibition of DNA synthesis [1, 4, 8].

Methotrexate is eliminated primarily by renal excretion, undergoing glomerular filtration and renal tubular reabsorption and secretion. Approximately 70–90% of a dose is excreted unchanged in the urine [14, 15]. In patients with significant renal dysfunction, MTX clearance is delayed, resulting in prolonged drug exposure and a greater risk of toxicities [2]. The plasma disappearance of MTX is multiphasic, and the terminal half-life is typically in the range of 4–8 h [3]. In patients with increased extravascular fluid, particularly in the presence of pleural fluid or ascites, the drug can be retained, elimination slowed, and the risk of serious toxicity increased [8].

Methotrexate is an important part of therapy in the treatment of infants with ALL. Although chemotherapy is generally perceived to be more toxic in infants, there have been no large studies evaluating MTX toxicity in infants. Small retrospective studies have not noted a significant difference in toxicity between infants and older children [9, 16]. However, based on the physiologic differences between infants and older children, differences in the pharmacokinetics and toxicity of MTX between infants and children might be expected.

Renal immaturity is present at birth with renal tubular function, glomerular filtration rate, and renal blood flow all changing significantly during the first year of life [17, 19]. Renal tubular function in infants is comparable to adults by 3–7 months of age, glomerular filtration rate (normalized for body surface area) by 5–12 months, and renal blood flow by about 5 months [17, 19]. A number of non-specific mechanisms that also affect maturation of renal function develop during the first 6–12 months [20]. This suggests that antineoplastic agents like MTX that are dependent upon renal function for elimination will be cleared more slowly in the young infant leading to the potential for an increased risk of pharmacologic effects and toxicity.

In addition to maturing renal function, the body composition of infants can also affect MTX pharmacokinetics. In the neonatal period total body water content is approximately 75%, decreasing to about 60% at 1 year of age, and 55% by adulthood [12]. Furthermore, in neonates 45% of total body water is extracellular, while for adults only 20% is extracellular [12]. Therefore, differences in both total water content and the fraction of extracellular water could affect the clearance of MTX in younger infants much the same way that extravascular fluid accumulations prolong MTX half-life [8].

Limited information is available on the pharmacokinetic behavior of antineoplastic agents in infants. Because of the therapeutic importance of MTX in treating infant ALL, its potential toxicity, and the possible impact of infant physiology on its disposition, characterizing pharmacokinetics in infants is a clinically important step toward improving the safety and efficacy of ALL therapy for this vulnerable patient population. Previous studies of MTX pharmacokinetics in young children have included only small numbers of patients, and very few infants <6 months of age [9, 16]. We present results of a study of the pharmacokinetics of MTX in infants.

Methods

Patients

The pharmacokinetic analysis was performed with data collected from patients enrolled in the Pediatric Oncology Group infant ALL protocol (POG 9407) from May 1996 to September 2003. Study eligibility criteria included: (1) newly diagnosed ALL or acute undifferentiated leukemia; (2) age <366 days; (3) gestational age ≥36 weeks for infants with congenital ALL; and (4) enrollment on POG 9900 biology classification study. Patients were not eligible for the study if they had mature B-cell ALL or were on chronic steroid therapy for another disease.

Participation in the pharmacologic study was not required for participation in the treatment portion of the 9407 study. Informed consent was obtained in accordance with federal and individual institutional guidelines.

Drug administration

Patients received their first cycle of MTX at week 4 of induction/intensification following initial induction therapy with prednisone, vincristine, daunorubicin, cyclophosphamide, and L-asparaginase. The infusion was administered as a 200 mg/m^2 loading dose given over 20 min, followed by a 3.8 g/m^2 given over the remainder of the 24 h. The dose was then repeated at week 5 of therapy (the end of the induction/intensification phase) and again at weeks 11 and 12 of therapy (the consolidation phase). Pre- and post-dose hydration with alkalinized intravenous fluids was given at 2,400 cc/m^2/day. Leucovorin rescue was initiated 42 h

after the start of the infusion and given IV at 10 mg/m^2 every 6 h for two doses then PO or IV every 6 h for an additional three doses. Patients with MTX levels ≥0.18 μM but <5 μM 48 h after the start of the infusion received increased hydration (200 cc/m^2/h) and extended leucovorin rescue (10 mg/m^2 PO every 6 h) until the MTX level was <0.18 μM. Patients with MTX levels >5 μM at 48 h received increased hydration (200 cc/m^2/h) and had leucovorin increased to 10 mg/m^2 every 3 h. For these patients MTX levels were also repeated every 12–24 h until the MTX level was <0.18 μM. Liver function tests and creatinine were obtained weekly throughout induction/intensification and consolidation. Toxicity was graded using the toxicity grading scales outlined in Table 1.

Data collection and sample analysis

Pharmacokinetic data was collected for the first cycle of MTX given at week 4 of therapy. All patients had MTX levels at the end of drug infusion and 24 h later. Subsequent levels were checked every 12–24 h until the MTX level was <0.18 μM. The 17 patients enrolled in the pharmacokinetic sub-study had additional serial MTX levels obtained at 1, 6, 12, and 23 h after the start of the infusion. The additional pharmacokinetic samples were used to develop a population pharmacokinetic model for MTX, which will be described in a separate publication.

For the pharmacokinetic sub-study, samples were centrifuged at 2,500 rpm or higher for 5–10 min immediately following collection. Plasma from each tube was transferred to a polypropylene vial, shipped and frozen at −70°C until analysis. Samples obtained from patients enrolled in the pharmacokinetic sub-study were analyzed at the central study laboratory at Texas Children's Hospital using a previously described high-performance liquid chromatography (HPLC) method [5].

The routine monitoring samples collected for clinical care were analyzed in the clinical laboratories of the treating hospitals. Fluorescence polarization immunoassay and the enzyme-multiplied immunoassay are the two most commonly utilized and universally employed assays for measurement of plasma levels of MTX in children [11]. Both methods have been shown to correlate well with HPLC, although the less commonly used enzyme-multiplied immunoassay does have some cross-reactivity with 7-hydroxymethotrexate (7-OH MTX), a plasma metabolite of MTX [11].

Pharmacokinetic data analysis

To minimize any effect of prior treatment and to focus on age and developmental effects on MTX pharmacokinetics, the pharmacokinetic data analysis was done for the first cycle of MTX given at week 4 of therapy. MTX clearance was calculated from the ratio of intravenous infusion rate to the 23-h MTX level (for pharmacokinetic sub-study subjects) or to the end of infusion concentration (for other subjects). The appropriateness of this approach was assessed using the pharmacokinetic sub-study data. Clearance estimates obtained by fitting the complete concentration time profile were compared to those calculated from the infusion rate and the 23-h concentration.

Additional analyses were done to examine the percentage of patients in each age group having elevated MTX concentrations at either the end of infusion (>90 μM—the level at which the protocol required the study coordinator to be contacted) or at 24 h after the end of the infusion (>0.18 μM—the level at which the protocol required increased hydration and extended leucovorin rescue).

Toxicity data analysis

The toxicity data was abstracted from the main POG database for the 9407 study. The database is structured so that toxicity is reported as the highest grade experienced for each separate toxicity during different phases of treatment. Consequently, we looked at common adverse events associated with MTX administration (including renal, hepatic, and mucous membrane toxicity) recorded for the first 6 weeks of therapy (induction/intensification) because this period included the first two cycles of MTX given at weeks 4 and 5.

Table 1 Toxicity grading criteria

Toxicity type	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Renal	Serum Cr WNL	Serum Cr <1.5 × nl	Serum Cr 1.5–3.0 × nl	Serum Cr 3.1–6.0 × nl	Serum Cr >6.0 × nl
Hepatic	AST/ALT WNL	AST/ALT <2.5 × nl	AST/ALT 2.6–5.0 × nl	AST/ALT 5.1–20.0 × nl	AST/ALT >20.0 × nl
Stomatitis (mucous membrane toxicity)	None	Erythema, or mild soreness	Painful/edema can eat	Cannot eat or drink	Requires parenteral or enteral support

Statistical considerations

For the pharmacokinetic data we used the two-sample *t*-test to compare groups with respect to MTX steady-state clearance. Fisher's Exact tests were done to determine if there were significant differences between groups in the fraction of patients with elevated MTX levels at the end of infusion or 24 h post-infusion. For the pharmacokinetic sub-study patient group, the Wilcoxon Matched-Pairs Signed-Ranks Test was used to compare clearance estimates based on the infusion rate and the 23-h concentration to clearance estimates obtained from fitting the full concentration time profile. For the toxicity analysis, Fisher's Exact tests were used to determine if there were significant differences between groups for any of the common MTX toxicities. All quoted *P*-values are two-sided, and we required a *P*-value <0.05 for statistical significance. We provide means with standard deviations.

Results

Patients

Pharmacokinetic data were available for 61 infants receiving their first course of MTX. Sixteen of these patients were enrolled in the pharmacokinetic sub-study, and an additional 45 patients had clinical MTX data available. The mean ± standard deviation age for all included patients was 6.6 ± 3.2 months with a range of 2–12 months. Patient characteristics are outlined in Table 2.

Table 2 Patient characteristics

Characteristic	Pharmacokinetic sub-study	Clinical group
Male/female	8/8	19/26
Age (months)[a]		
Mean ± standard deviation	7.6 ± 3.3	6.2 ± 3.0
Range	2–12	2–12
Weight (kg)[b]		
Mean ± standard deviation	7.9 ± 1.8	7.7 ± 2.1
Body surface area (m^2)[b]		
Mean ± standard deviation	0.38 ± 0.06	0.36 ± 0.06
Serum creatinine (mg/dl)[b]	0.27 ± 0.07	0.26 ± 0.09

[a] At time of study entry

[b] At time of week 4 methotrexate dose

Pharmacokinetics

The comparison of MTX concentrations and steady-state MTX clearance for infants 0–6 months at the time of diagnosis to infants 7–12 months are outlined in Table 3. Results are expressed as mean ± standard deviation. There was a statistically significant difference in the mean steady-state MTX clearance for the two groups, 89 ± 32 ml/min/m^2 vs. 111 ± 40 ml/min/m^2 ($P = 0.030$). Differences between age groups in the 48-h MTX concentration and the fraction of patients having elevated MTX levels at the end of infusion (>90 μM) or at 48 h (>0.18 μM) were not statistically significant. Both groups had a large percentage of patients with an elevated MTX concentration at 48 h (55 and 43%).

The subgroup analysis comparing very young infants (0–3 months) to infants 7–12 months is outlined in Table 4. A slightly more pronounced difference in mean steady-state clearance is observed between these two groups, 84 ± 30 ml/min/m^2 vs. 111 ± 40 ml/min/m^2 ($P = 0.026$). The group of youngest infants also had a higher percentage of patients with elevated MTX levels at the end of infusion (23% vs. 7%) and at 48 h (62% vs. 45%). However, neither difference reached statistical significance.

In the pharmacokinetic sub-study subjects there was no significant difference in the estimate of clearance based on infusion rate and steady-state (23-h) MTX concentration compared to the clearance estimated from fitting the complete concentration time profile. When the two methods were compared using the Wilcoxon Matched-Pairs Signed-Ranks Test no difference was observed (*P*-value approximately equal to 1).

Table 5 shows the results of this study compared to previously reported results of MTX clearance in infants and selected studies of MTX clearance in older children. Clearance for the younger infants (age 0–3 months) is similar to the results reported by McLeod et al. [16] for infants 3–12 months, but significantly lower than the results of Donelli et al. [9]. The results for the older infants (age 7–12 months) are similar to values reported for older children by McLeod et al. [16] and Murry et al. [18], but are also significantly lower from the results reported by Donelli et al. [9].

Toxicity

Fifty-five of the 61 patients were evaluable for toxicity. The incidence of renal, hepatic, and mucous membrane toxicities during induction/intensification therapy is shown in Table 6. Twenty-two of 55 patients (40.0%) had mucous membrane toxicity, and 17 (30.9%) experienced Grade 3 or 4 toxicity.

Table 3 Methotrexate (MTX) pharmacokinetics in infants age 0–6 months compared to infants age 7–12 months

Parameter (units)	Age 0–6 months (n = 33)	Age 7–12 months (n = 28)	P-value
MTX steady-state clearance (ml/min/m^2)	89 ± 32	111 ± 40	0.030
End of infusion MTX level (μM)	74 ± 27; range (27–150); median 73	60 ± 23; range (26–140); median 54	0.034
Twenty-four-hour post-infusion MTX level (μM)	0.69 ± 1.39; range (0.06–6.66); median 0.20	0.35 ± 0.41; range (0.09–1.70); median 0.15	0.143
Percentage with MTX level >90 μM at end of infusion	18% (6)	7.1% (2)	0.269
Percentage with MTX level >0.18 μM at 24-h post-infusion	55% (18)	43% (12)	0.444

Results are shown as mean ± standard deviation unless otherwise specified

Table 4 Methotrexate (MTX) pharmacokinetics in subgroup of infants age 0–3 months compared to infants age 7–12 months

Parameter (units)	Age 0–3 months (n = 13)	Age 7–12 months (n = 28)	P-value
MTX steady-state clearance (ml/min/m^2)	84 ± 30	111 ± 40	0.026
End of infusion MTX level (μM)	77 ± 29; range (40–150); median 72	60 ± 23; range (26–140); median 54	0.083
Twenty-four-hour post-infusion MTX level (μM)	0.84 ± 1.30; range (0.07–5.04); median 0.30	0.32 ± 0.37; range (0.09–1.70); median 0.15	0.172
Percentage w/MTX level >90 μM at end of infusion	23% (3)	7.1% (2)	0.304
Percentage w/MTX level >0.18 μM at 24-h post-infusion	62% (8)	43% (12)	0.326

Results are shown as mean ± standard deviation unless otherwise specified

Table 5 Comparison to previously reported data for methotrexate (MTX) clearance in infants and children

Author	Number of patients	MTX dose (g/m^2)	Patient age range	MTX clearance mean ± standard deviation (ml/min/m^2)
Current study	13	4	0–3 months	84 ± 30
Current study	28	4	7–12 months	111 ± 40
McLeod et al. [16]	4	1.5	3–12 months	80 ± NR
Donelli et al. [9]	7	5	3–12 months	178 ± 83
Murry et al. [18]	18	1	0.2–18 years	101 ± NR
McLeod et al. [16]	108	2	1–19 years	103 ± NR
Donelli et al. [9]	26	5	1–3 years	160 ± 71

NR denotes not reported by the author

Table 6 Incidence of renal, hepatic, and mucous membrane (stomatitis) toxicity during induction/intensification therapy

Parameter	Incidence (%)
Renal toxicity	
Grades 1—2	5.5% (3/55)
Grades 3—4	0% (0/59)
Grades 1—4 inclusive	5.5% (3/55)
Hepatic toxicity	
Grades 1—2	9.1% (5/55)
Grades 3—4	5.5% (3/55)
Grades 1—4 inclusive	14.6% (8/55)
Mucous membrane toxicity (stomatitis)	
Grades 1—2	9.1% (5/55)
Grades 3—4	30.9% (17/55)
Grades 1—4 inclusive	40.0% (22/55)

The incidence of toxicity in the youngest infants (0–3 months) is compared to the older infants (7–12 months) in Table 7. While there were no statistically significant differences in the incidence of hepatic or gastrointestinal toxicities, the younger group had a significantly higher incidence of renal toxicity (P = 0.029). However, renal toxicity in the younger patients was lower grade: two Grade 2 and one Grade 1. In the younger infants experiencing hepatic toxicity, one patient had Grade 4 toxicity and another Grade 3 toxicity. None of the patients in the older group experienced greater than Grade 2 hepatic toxicity during induction/intensification. The incidence of stomatitis was not significantly different between the two age groups. In the younger infants, 15.4% (2/13) had Grade 3 or 4 toxicity, compared to 33.3% (9/27) of the older age group.

Table 7 Comparison of renal, hepatic, and mucous membrane (stomatitis) toxicity during induction/intensification therapy in younger and older infants

Parameter	Age 0–3 months ($n = 13$)	Age 7–12 months ($n = 27$)	P-value
Percentage of patients with renal toxicity			
Grades 1—2	23.1% (3)	0%	
Grades 3—4	0%	0%	
Grades 1–4 inclusive	23.1% (3)	0%	0.029
Percentage of patients with hepatic toxicity			
Grades 1—2	7.7% (1)	7.4% (2)	
Grades 3—4	15.4% (2)	0%	
Grades 1–4 inclusive	23.1% (3)	7.4% (2)	0.307
Percentage of patients with stomatitis			
Grades 1—2	7.7% (1)	11.1% (3)	
Grades 3—4	15.4% (2)	33.3% (9)	
Grades 1–4 inclusive	23.1% (3)	44.4% (12)	0.298

Discussion

Our data show a modest difference in mean steady-state MTX clearance ($P = 0.030$) between younger infants (0–6 months) and older infants (7–12 months). The difference between the youngest infants enrolled in the protocol (0–3 months) compared to the older infants (7–12 months) is also significant ($P = 0.026$).

Prior studies of MTX pharmacokinetics in infants are conflicting as shown in Table 5. McLeod et al. evaluated MTX disposition in patients with ALL treated at St. Jude Children's Hospital [16]. The four infants in their review had a mean clearance of 80 ml/min/m^2 compared to a mean clearance of 103 ml/min/m^2 ($P = 0.01$) in 108 older children [16]. These results are similar to those we report. Additionally, the authors found no difference in toxicity for infants compared to older children.

In contrast, in a retrospective pharmacokinetic analysis of 122 Italian children receiving MTX (5 g/m^2 over 24 h), Donelli et al. found no significant differences between infants and older children [9]. For seven patients in the age range 3 months to 1 year, MTX clearance (mean ± standard deviation) was 178 ± 83 ml/min/m^2. MTX clearance for patients age 1–3 years ($n = 26$) was 160 ± 72 ml/min/m^2, and for patients age 3–10 years ($n = 68$) clearance was 158 ± 63 ml/min/m^2. However, for the oldest children, age 10–15 years ($n = 21$), there was a significant decrease in clearance, 110 ± 63 ml/min/m^2. The cause of the discrepancy between the very high values of MTX clearance reported by Donelli for all age groups compared to our results and McLeod's is not known.

Of the 61 infants in our study 8 (13.1%) had end of infusion MTX levels >90 μM. These eight infants also experienced a significant incidence of adverse events during induction/intensification that are commonly associated with MTX. Five of the eight had Grade 3 or 4 stomatitis, three had Grade 3 or 4 liver toxicity, and one had Grade 2 renal toxicity. However, because the study database groups toxicity across the first 6 weeks of therapy, it is not possible directly establish that all the cases of toxicity were a direct result of high levels of MTX.

Overall, infants in the POG 9407 study experienced significant mucous membrane toxicity. In infants, mucous membrane toxicity is a significant cause of morbidity and frequently necessitates the use of parenteral nutrition [10]. However, because the study database groups toxicity across induction/intensification, it is not clear if the high incidence of stomatitis was a direct result of MTX therapy. However, it is clear that infants being treated on POG 9407 experienced mucous membrane toxicity at a high rate. The incidence of mucositis in older children receiving intermediate dose MTX (1 g/m^2) has been reported to be <10% [7].

In comparing renal toxicity between younger and older infants, a difference was noted between the 0–3- and 7–12-month age groups. It is possible that the higher rate of renal toxicity could be attributable to an effect of MTX on immature kidneys. It should be emphasized, however, that other factors that could not be controlled, such as concomitant nephrotoxic medications, could contribute to the difference in toxicity and were not analyzed in this study.

In conclusion, this study suggests that the pharmacokinetics of young infants (0–6 months) receiving MTX is modestly different from older infants and children and may affect toxicity. These observations may in part be due to the renal immaturity that is present in the first 6 months of life. Future studies to further characterize the pharmacokinetics and toxicity of MTX in infants would be beneficial and could lead to improved treatment protocols for infants with leukemia.

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