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PHARMACOKINETICS OF HIGH DOSE METHOTREXATE IN THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): A LITERATURE REVIEW

Syarifah Nurul Maulidah[1]* and Yulistiani[2]

[1]Student of Master Clinical Pharmacy, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.

[2]Department of Clinical Pharmacy, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.

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***Corresponding Author**
Syarifah Nurul Maulidah
Student of Master Clinical Pharmacy, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.

ABSTRACT

Acute lymphoblastic leukemia (ALL) occurs in a large number of children in every year and requires long period chemotherapy treatments. Methotrexate is an antifolate most widely used as anticancer therapy. In pediatric oncology, methotrexate (MTX) is commonly used in the treatment of acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, osteosarcoma, and brain tumors. This success is due to the selection of effective chemotherapy. To assess the effectiveness of methotrexate, it is necessary to examine the pharmacokinetic aspect, although there is a large variability between patients. MTX is a nonlinear Michaelis-Menten pharmacokinetics or dose-dependent. The basic parameters are clearance, distribution volume, and half-life elimination can vary based on kidney function, liver function, age, and other factors; including emesis and drug use along with administration of methotrexate. This phenomenon occur because one or more of the kinetic processes (absorption, distribution, and/or elimination) of MTX which occur through a mechanism that is not in the first order of kinetics. Therefore, the relationship between AUC or plasma concentration at the time of administration under steady state conditions and the dose given is not linear.

KEYWORDS: Pharmacokinetics, High dose Methotrexate, Acute Lymphoblastic Leukemia.

A. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a hematologic malignancy commonly found in children and adults, which usually involves various anticancer drugs (Hunger & Mullighan, 2015) (Schmiegelow, et al., 2016) (Cheng, Lu, Liu, Zou, & Pang, 2018). ALL in children has a good prognosis with almost 80% is able to survive for 5 years with the current intensive protocol (Csordas, et al., 2013) (Pui, et al., 2015) (Foster, Bernhardt, Thompson, Smith, & Schafer, 2017). Methotrexate (MTX) is an anticancer drug widely used in the treatment of malignancies in children (Csordas, et al., 2013) (Umerez, Camino, Maldonado, Guerrero, & Orad, 2017) (Liu, et al., 2017). MTX is one of the drugs for extramedular leukemia in ALL, non-Hodgkin's lymphoma, and osteosarcoma protocol, which has led to a significant increase in long-term survival in patients (Forster, et al., 2016) (Hearps, et al., 2017). Genetic polymorphisms associated with MTX metabolism can significantly affect long-term endurance in patients (Ylinen, Jahnukainen, Pihkala, & Jahnukainen, 2014).

The other hand antineoplastic drugs, the cytotoxic effects of MTX can be antagonistic to folic acid (leucovorin), which is in high doses of intravenous MTX. However, this is susceptible to causing damage to certain organs in the body resulting in a toxicity reaction. The occurrence of this toxicity reaction due to MTX has non-linear Michaelis-Menten pharmacokinetic characteristics (V_{max} and K_m), so it remains an important problem even now, which includes nephrotoxicity, hepatotoxicity, gastrointestinal mucositis, bone marrow suppression, and neurotoxicity (Yang, Zhao, Song, Shen, & Xu, 2015) (Cooper & Brown, 2015). Toxicity is even more severe in patients with delayed MTX elimination (Yang, Zhao, Song, Shen, & Xu, 2015) (Yeoh, et al., 2017).

High-dose methotrexate chemotherapy (HDMTX) with leucovorin (LV) rescue is a main treatment to prevent extramedular infiltration in acute childhood lymphoblastic (ALL) leukemia. Apart from this statement that HDMTX is a very effective treatment, MTX plasma concentrations can be too high in a small portion of the patient population, resulting in toxicity and delays in elimination. Delays in elimination can cause kidney dysfunction and/or liver dysfunction, bone marrow suppression, oral mucosal lesions, secondary infections, and delays in subsequent chemotherapy. Kidney toxicity is a special problem. Conversely, concentrations lower than optimal concentrations of MTX can result in a greater risk of recurrence. Therefore, optimizing the dose of HDMTX through therapeutic monitoring is

very important to maximize therapeutic benefit, reduce the risk of potential hazards, and indeed reduce treatment costs (Xu, Zhang, & Chen, 2014).

The method used in this review is PubMed searches from published journals only done on humans, using the following terms: ("acute lymphoblastic leukemia" AND "pharmacokinetic" AND "methotrexate"). Articles are selected based on relevance and additional articles are obtained from the reference list.

B. Methotrexate

Metotrexate (MTX) is also known as 4-amino-N-methyl folic acid analogues classified into folate antagonists (Singh, et al., 2017). MTX is a competitive inhibitor of the enzyme dihydrofolate reductase (Figure 1) and blocks the conversion of dihydrofolate into its active form, chemically decreases its tetrahydrofolate form, thereby depleting the intracellular tubules of tetrahydrofolate, which is required by cofactors (single carbon donors) for methionine, thymidine and purine (Ramsey, et al., 2018). Tetrahydrofolate is ultimately involved in the synthesis of purines and pyrimidines, each mediated by MTHFR and TYMS enzymes (Zgheib, et al., 2014). MTX is an intracellular polyglutamates, and in this form (MTXPGs) can also directly inhibit enzymes in the purine / pyrimidine synthetic pathways (Korell, et al., 2013) (Ramsey, et al., 2018). Thymidylate synthesis is the only enzyme that requires folate to oxidize the tetrahydrofolate cofactor to dihydrofolate, and active thymidine synthesis is needed for MTX to deplete intracellular tetrahydrofolate (Umerez, Camino, Maldonado, Guerrero, & Orad, 2017) (Ramsey, et al., 2018). MTX levels in the blood have not been proven to be reliable in predicting a disease. However, accumulation of MTX active metabolites, such as MTX polyglutamates (MTXPGs), has been associated with anti-leukemia activity (Mei, et al., 2015).

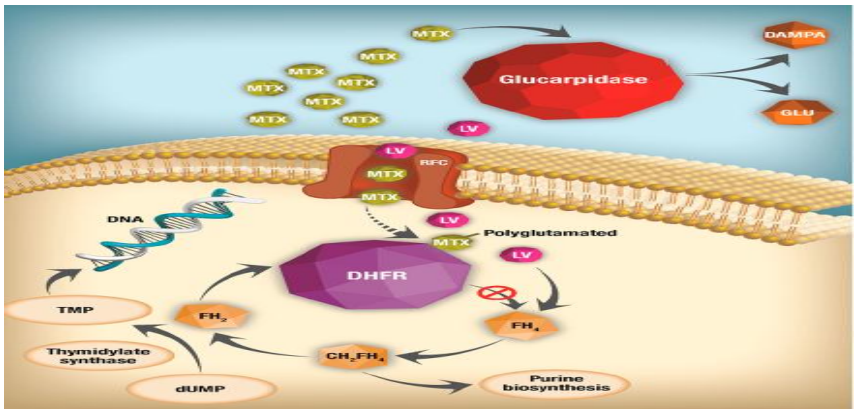

Figure 1: Mechanism and site of action MTX and strategies for eliminating delayed MTX (Ramsey, et al., 2018).

MTX is an antimetabolite that disrupts folic acid metabolism (Csordas, et al., 2014). After entering the cell, methotrexate is polyglutamated, binds dihydrofolate reductase (DHFR) with an affinity 1000 times greater than folate, and competitively inhibits the conversion of dihydrofolate to tetrahydrofolate (Fig. 1). Tetrahydrofolate is very important for the biosynthesis of thymidine and purines, which are needed for DNA synthesis. The blockade of tetrahydrofolate synthesis by methotrexate causes the inability of cells to divide and produce protein. MTX is an essential component of therapy for acute lymphoblastic leukemia (ALL) and is active against many types of cancer (Mei, et al., 2015) (Howard, McCormick, Pui, Buddington, & Harvey, 2016).

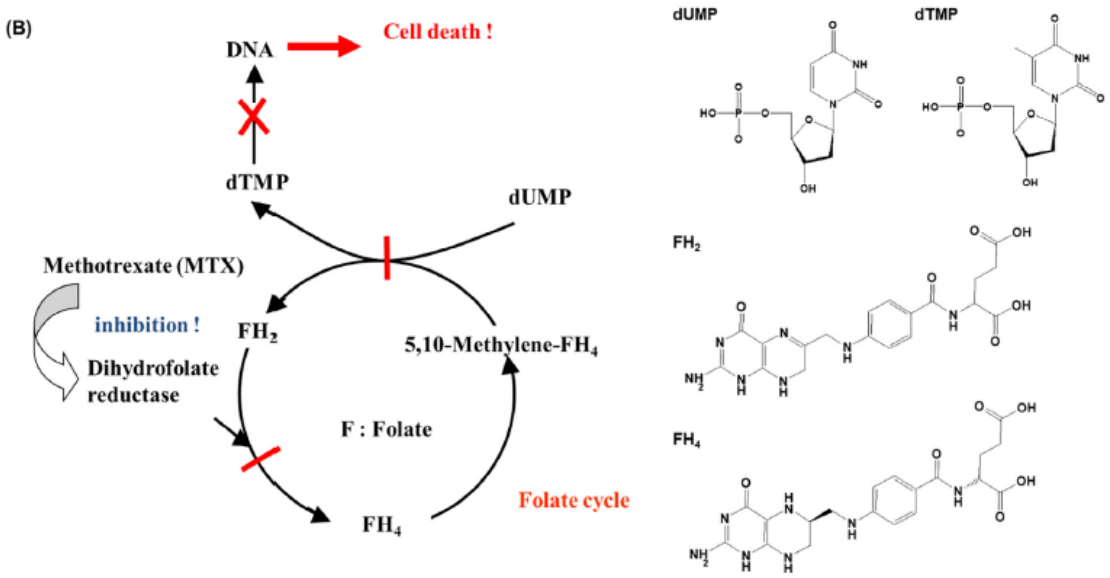

Figure 2: MTX anti-cancer mechanism (Choi, Kim, Oh, & Choy, 2018).

MTX was introduced into clinical practice for the first time in the 1950s and has become one of the drugs commonly used during maintenance therapy for ALL. To understand the MTX function can be changed by the genomic variant, it is necessary to understand the mechanism of action of MTX. MTX is very similar to folic acid or folate in terms of structure (Fig. 3). Thus, MTX acts as a competitive inhibitor of enzymes that utilize folate and has a 1000-fold increase in affinity for this (Rudin, Marable, & Huang, 2016).

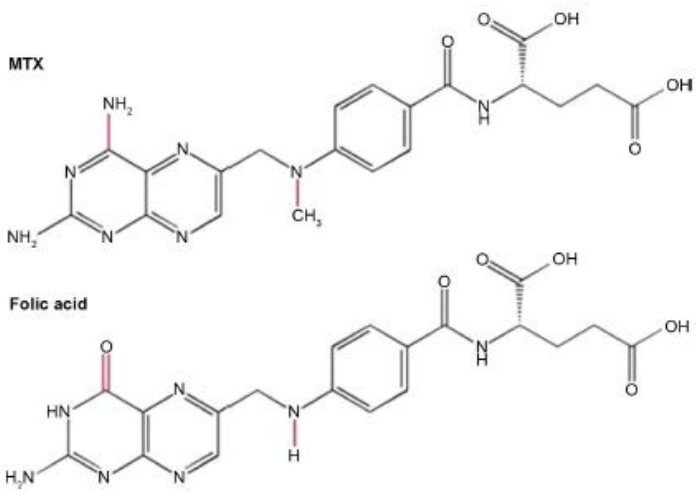

Figure 3: Structure of MTX and folic acid (Rudin, Marable, & Huang, 2016).

Methotrexate is given at doses ranging from 6-12 mg intrathecally and 20 mg/m^2 orally, or intravenously as ALL weekly treatment chemotherapy for a dose of 1000 mg/m^2 with a leucovorin rescue dose of 15 mg/m^2. Administration of intrathecal MTX doses varies according to the age of the pediatric patient, dose of 6 mg for age less than 1 year, dose of 8 mg for children aged 1 year old, dose of 10 mg for 2 years old, and dose of 12 mg for children aged more than 3 years old (BCCA, 2018). The results of a study conducted by Febriansyah showed that at 0 o'clock the administration of high doses of methotrexate, it could be measured in the patient's blood. This is because point 0 in the study was carried out 2 hours after the patient received intrathecal methotrexate (Utomo, Yulistiani, Zairina, & Permono, 2017). Bleyer and Dedrick's research in 1978 showed that intrathecal administration methorexate would provide the highest plasma levels 3 to 12 hours after injection and would decrease with a half-life of 5.5 to 24 hours. Pharmacokinetic analysis of studies shows that the main way of transferring the level of methotrexate from cerebrospinal fluid to the systemic circulation is to absorb the flow of mass containing methotrexate from the cerebrospinal fluid. This is the result of the interaction of convective transport and diffusion between cerebrospinal fluid and extracellular fluid of the brain (Bleyer & Dedrick, 1978). A dose of 500 mg / m2 given intravenously is defined as high-dose MTX (HDMTX) and is used to treat various cancers for adults and children, including ALL, osteosarcoma, and lymphoma. HDMTX therapy can cause significant toxicity, which not only leads to morbidity and mortality but also interferes with cancer treatment, which has the potential to cause cancer therapy results not to be maximized (Maxwell & Cole, 2017). To prevent toxicity,

standardized care and treatment protocols are needed (Table 1) (Howard, McCormick, Pui, Buddington, & Harvey, 2016).

Table 1: HDMTX protocol for ALL patients (Howard, McCormick, Pui, Buddington, & Harvey, 2016).

Study, year [reference]	Methotrexate dose	Duration of methotrexate infusion (hours)	Leucovorin rescue dose	Time from start of methotrexate infusion to first leucovorin dose (hours)
Acute lymphoblastic leukemia				
Takeuchi et al., 2002 [98]	100-mg/m^2 bolus, then 500 mg/m^2 per hour	4	15 mg every 6 hours × 8 doses	28
Linker et al., 2002 [99]	220-mg/m^2 bolus, then 60 mg/m^2 per hour × 36 hours	36	50 mg every 6 hours	36
Hill et al., 2004 [100]	6 g/m^2 (age < 4 yr) 8 g/m^2 (age > 4 yr)	10% bolus, remainder over 23 hours	15 mg/m^2 every 3 hours, then every 6 hours when serum methotrexate < 2 × 10^6 μM	36
Pui et al., 2007 [58]	2 g/m^2	2	10 mg/m^2 every 6 hours	44
Zhang et al., 2014 [101]	3–5 g/m^2	24	15 mg/m^2 every 6 hours, then pharmacokinetically guided to serum methotrexate 0.1 μmol/L	36

When using high dose MTX (HD-MTX) it is necessary to reduce the administration of folinic acid (leucovorin) to reduce drug side effects (Vang, Schimiegelow, Frandsen, Rosthoj, & Nersting, 2015). The most common side effects include hepatotoxicity, nephrotoxicity, myelotoxicity, mucositis, and neurological symptoms. The goal of HDMTX therapy is to keep serum MTX at a high level (10-100 mmol/l) for a prolonged period (12–36 hours). Serum MTX concentrations are monitored routinely to control drug levels, to identify patients at high risk of toxicity, and to free folinic acid (Csordas, et al., 2013) (Nielsen, et al., 2016).

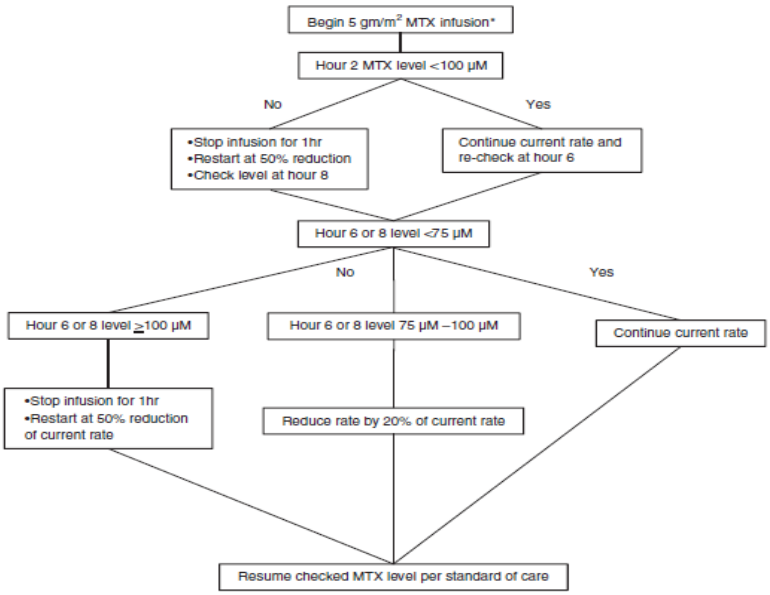

Figure 4: MTX target dose algorithm (Foster, Bernhardt, Thompson, Smith, & Schafer, 2017).

C. The pharmacokinetics of Methotrexate

MTX were absorbed at peak levels of 0.11 to 2.3 micromolar after oral MTX use of 20 mg/m^2 and at the time of peak absorption of MTX oral use in children was 0.67 to 4 hours, with bioavailability as much as 23-95%. Its half-life is about 0.7 to 5.8 hours (in the dose range 6.3 to 30 mg/m^2). The MTX protein binding is 50% with the distribution volume in pediatric patients more than 0.4 to 0.8 L/kg. MTX metabolism occurs in the liver with active metabolites in the form of polyglutamates and 7-hydroxymethotrexate. MTX is eliminated mainly by renal excretion (48-100%), and nearly 10% of each dose is excreted in an unchanged form in bile. Its main metabolite is 7-hydroxy-methotrexate (7-OH-MTX), contributes to MTX activity and can settle in the renal tubules causing acute renal insufficiency (Sahni, Choudhury, & Ahmed, 2009) (Csordas, et al., 2013).

The results of research conducted by Hempel *et al.*, Showed that plasma MTX loss (Figure 5) followed the first order kinetic with a half-life of 150.8 ± 5.6 minutes. Conversion to the main 7-OH-MTX metabolite appears to be effective, plasma concentrations reach an average of 24 hours after starting intravenous administration, about one third of MTX. For this reason, the relationship between MTX and 7-OHMTX changed significantly between 24 and 36 hours after the start of treatment. At 24 hours the proportion of MTX to 7-OH-MTX is around 3–1, even though the 36th hour has switched to 1 to 3 (see inset in Figure 5). There was no correlation between MTX results versus 7-OH-MTX and glomerular function parameters 24 hours after initial therapy or correlation between results for MTX versus 7-OH-MTX 30 and 36 hours after initial therapy and glomerular renal function parameters 48 hours after initial therapy (not shown) (Hempel, et al., 2003).

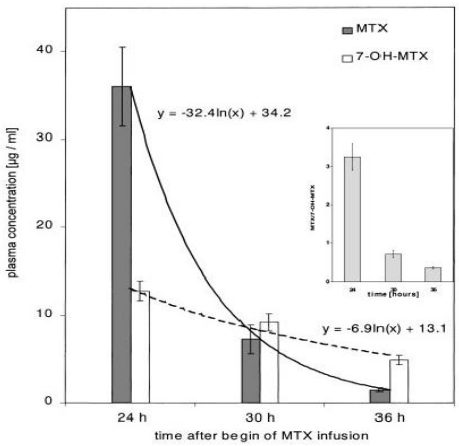

Figure 5: Plasma concentration curves of MTX and 7-OH-MTX, and estimated decreasing plasma curves (Hempel, et al., 2003).

MTX pharmacokinetics after intravenous injection are quite complex, which shows a rapid decline in initial concentration in reflecting distribution in the central compartment. Furthermore, the drug is largely eliminated through the kidney in the form of a drug that does not change with a half-life of α for 1 to 3 hours. β elimination phase reflects the redistribution of MTX to the central compartment of the deeper compartment (for example, the gastrointestinal system). Metabolism to 7-hydroxy-MTX contributes to a lower level of cleansing of drugs from organisms. However, metabolites can compete with MTX for transporters, for example, and are thought to contribute to toxicity. MTX is polyglutamylated in cells for MTX- (Glu) derivatives which are intracellularly trapped and add significantly to MTX action (Rau, Erney, Eschenhagen, & Beck, 2006).

Delayed MTX elimination has been shown to correlate with nephrotoxicity, but serum MTX levels do not show correlation with creatinine levels. 7-OH-MTX has shown a significant correlation with alanine minotransferase (ALT) levels, but there is no correlation with creatinine levels (Csordas, et al., 2013). The occurrence of this toxicity reaction is due to MTX which has michael-mentent non-linear pharmacokinetic characteristics (V_{max} and K_m). MTX which is a nonlinear or dose-dependent pharmacokinetics, the underlying parameters are clearance, distribution volume, and elimination half-life which can vary by age, gender, interactions with other drugs, kidney function, liver function, hydration insufficiency in each patients, and other factors, including emesis and drug use along with methotrexate administration (Rau, Erney, Eschenhagen, & Beck, 2006) (Utomo, Yulistiani, Zairina, & Permono, 2017). This is because one or more of the kinetics processes (absorption, distribution and/or elimination) of MTX occur through a mechanism not in the first order of kinetics. Therefore, the relationship between AUC or plasma concentration at the time of administration in steady state conditions and the dose given is not linear (Csordas, et al., 2014).

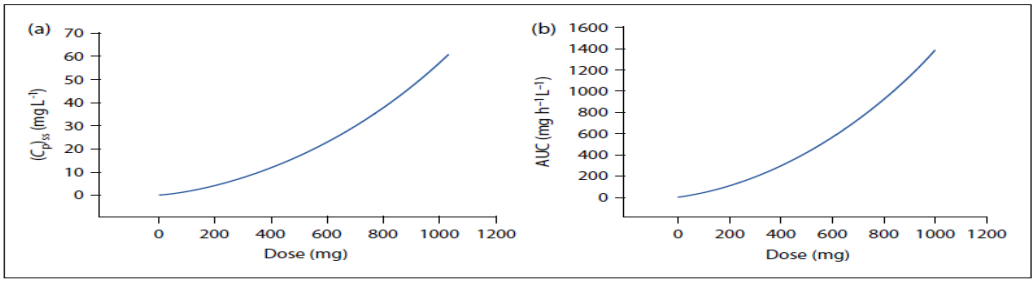

Figure 6: (a) The relationship between Cp at steady state conditions and AUC; (b) administration of drugs that are classified as dose-dependent (Csordas, et al., 2014).

The number of methotrexate measurements performed in one patient compared to other patients is not the same, so it is necessary to estimate the pharmacokinetic parameters individually for methotrexate in ALL patients in Indonesia, thereby reducing the amount of blood taken to monitor blood levels of methotrexate (Utomo, Yulistiani, Zairina, & Permono, 2017). Other assumptions about kinetic variability after high doses of methotrexate may also be due to the function of multispecialty organic anionic transporters in canaluli, also known as Adenosine triphosphate-Binding Cassette Class Transporters (ABCC2), or also known as Multidrug Resistance Protein 2 (MRP2). These transporters are located in luminal/apical hepatocyte membranes, small intestinal epithelial cells, proximal renal tubular cells, and luminal endothelial surfaces of cells in the brain. This transporter has a strong affinity for methotrexate, so it has an important role in the elimination of methotrexate. Polymorphism in MRP2 is one of the genetic factors that can cause pharmacokinetic variability in patients receiving high doses of methotrexate. On the other hand, the overexpression of transporters can cause methotrexate resistance (Alldredge, et al., 2013) (Kliegman, Stanton, & Geme, 2016).

Capacity-limited metabolism is also called saturable metabolism, Michaelis-Menten kinetic, or mixed order kinetics. The enzymatic metabolic processes of drugs can be explained by the relationships described below.
Enzyme + Substrate (drug) → Enzyme-drug complex → Enzyme + Metabolite.

First, drugs interact with enzymes to produce intermediate enzymes. Then the intermediate complex is further processed to produce metabolites with enzyme release. The enzyme released is recycled again to react with more drug molecules (Csordas, et al., 2014).

According to the Michaelis-Menten kinetics principle, the rate of drug metabolism changes to the function of drug concentration, as illustrated in the following figure.

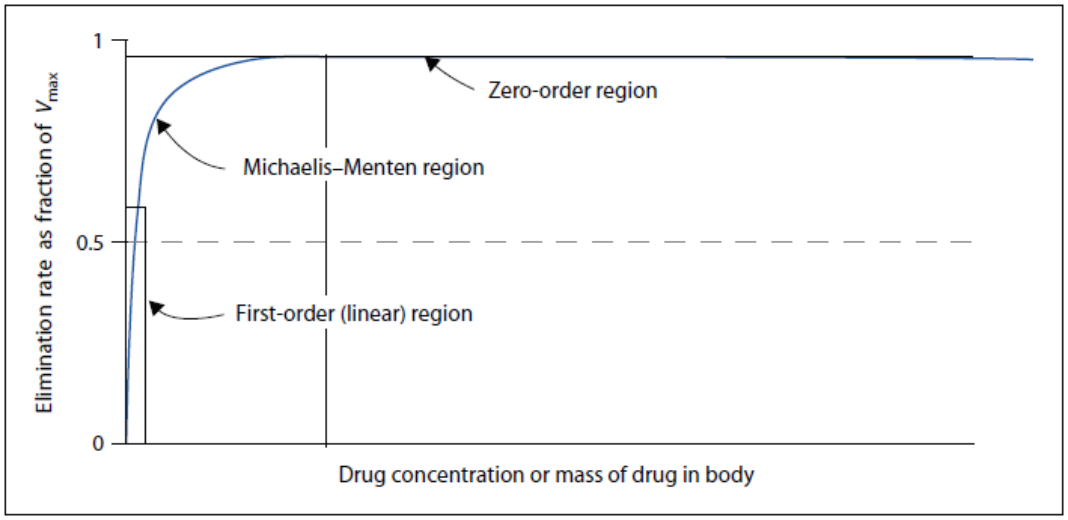

Figure 7: Relationship between elimination rate and dose-dependent plasma concentration of pharmacokinetics (Csordas, et al., 2014).

MTX pharmacokinetics is a Michaelis-Menten nonlinear pharmacokinetics model described as follows.

$$\text{Metabolism rate} = \frac{Vmax\ C}{Km + C}$$

V_{max} is the maximum rate of metabolism (mg/h); K_m is the Michaelis-Menten constant (mg/L); and C is the MTX drug concentration (mg/L).

Based on research conducted by Borsi *et al.*, in 1987, methotrexate occurred in the phenomenon of dose-dependent pharmacokinetic profiles. The higher doses of intravenous methotrexate given will cause a shorter half-life and decreased clearance of methotrexate. In addition, based on this study, also found phenomena in patients aged 1-4 years, methotrexate clearance will increase and consequently plasma steady-state will decrease (Borsi & Moe, 1987).

Renal excretion is the main route for MTX elimination, accounting for around 70-90% of MTX clearance. MTX is a weak acid with limited solubility under acidic conditions (2 mM maximum solubility at pH 5). Alkalinization of urine has a greater impact on the solubility of MTX in urine than hydration of fluid. MTX solubility increases 10-fold by increasing pH from 6 to 7. MTX is secreted and not reabsorbed by the renal tubules, but tubular secretion is saturated at higher plasma concentrations and only plays a small role in eliminating MTX during and immediately after HDMTX infusion. MTX is protein bound by 60%, and glomerular filtration is limited to over-the-counter drugs. Therefore, MTX clearance at high

concentrations is smaller than the glomerular filtration rate (GFR). MTX is also metabolized in the liver by aldehyde oxidase to 7-hydroxymethotrexate, which explains for 5% -10% MTX elimination (Ramsey, et al., 2018).

MTX pharmacokinetics affects supportive levels of care and monitoring after treatment. After administering an MTX infusion with a fixed dose and duration, plasma concentrations can vary greatly between patients in different cycles. Plasma protein binding, effusion, kidney function, and liver function can affect peak concentrations after infusion. Methotrexate concentration series is obtained by focusing on values that easily enter the leucovorin nomogram period (i.e 42 hours and after) (Figure 8) (Howard, McCormick, Pui, Buddington, & Harvey, 2016).

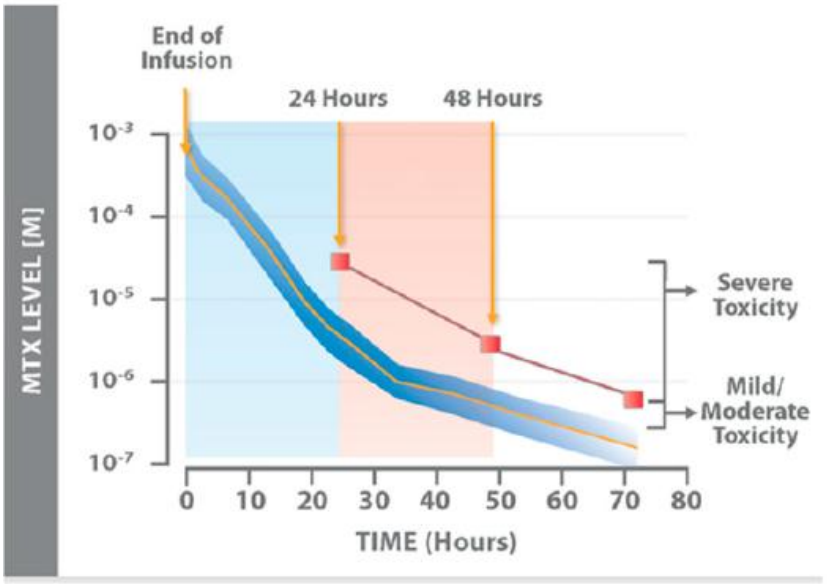

Figure 8: Nomogram for expected time-dependent decrease in serum MTX after MTX infusion. The expected nomogram for serum MTX after 6 hours of MTX infusion. The blue area represents ± 2 SD from the mean (orange line). Values above the red line indicate the toxicity that will occur (Howard, McCormick, Pui, Buddington, & Harvey, 2016).

Intravenous (IV) application of a high dose of MTX (HDMTX) 5 mg/m^2/24 hours infusion, achieves an effective therapeutic concentration, i.e MTX concentration in a steady state of 1x10-6 mol/l. Changes in the dose or duration of HDMTX infusion are the focus of strategies to overcome individual variability in MTX accumulation, because the cytotoxic effects of MTX depend on the concentration and length of exposure to the drug. The most frequently

described side effects of MTX therapy are acute liver toxicity myelosuppression, nephrotoxicity, mucositis, and neurotoxicity (Popovic, et al., 2015).

When an HDMTX infusion is given, therapeutic drug monitoring (TDM) is a standard practice for rescue guidelines related to leucovorine, especially in patients with MTX clearance or other risks associated with prolonged cytotoxic concentrations (kidney or liver damage, fluid collection) (Figure 9) (Popovic, et al., 2015).

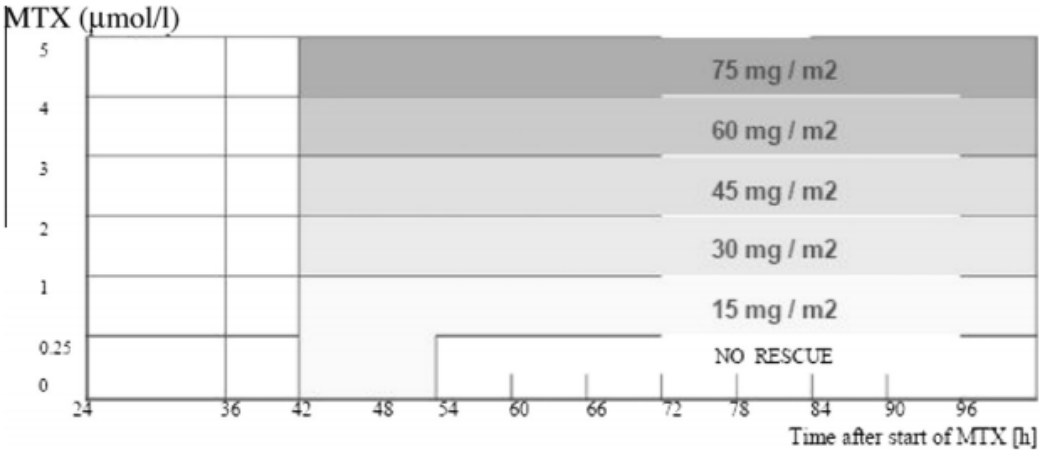

Figure 9: Leucovorin dose diagram at MTX dose level (Popovic, et al., 2015).

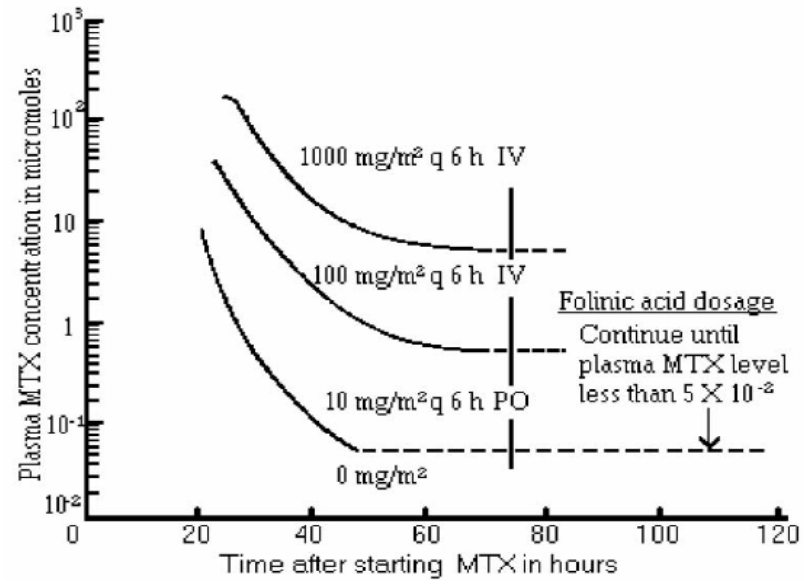

Figure 10: Dosage for Leucovorin giving to MTX (Lanzkowsky, Lipton, & Fish, 2016).

In the administration of methotrexate with a dose of >500 mg/m^2, leucovorin rescue therapy is needed to control the duration of methotrexate exposure in sensitive cells so that the side effects caused by methotrexate can be minimized. Leucovorin is an active metabolite of folic

acid so that the process of nucleic acid synthesis can continue even though there is still methotrexate in the body. Leucovorin can compete with methotrexate in the same transport process into cells. Leucovorin is usually given 24 hours after methotrexate so that leucovorin does not interfere with the therapeutic effect of methotrexate. However, when a methotrexate overdose occurs, this leucovorin must be given immediately (Popovic, et al., 2015).

Leucovorin doses given range from 10-25 mg/m^2 every 6 hours as much as 8-10 doses, starting 24 hours after starting high-dose methotrexate. Modification of leucovorin dose based on methotrexate levels is conducted at 36-48 hours after the start of high doses of methotrexate. Methotrexate levels were measured daily and leucovorin dose adjustments were made based on the above nomogram (Figure 9.). Leucovorin is still given to the level of methotrexate in the blood of 0.05 µmol/L. Leucovorin with a dose of>25 mg must be given intravenously (BCCA, 2018).

Traditionally, therapeutic protocols for ALL patients define risk groups according to age, sex and number of leukocytes. Current risk evaluations include also the characteristics of leukemia, which is obtained from immunophenotypic, cytogenetics, and molecular diagnoses. However, other factors are also known to contribute to the large variability in MTX accumulation, namely fertility, molecular subtype, genome expression of folate cycles. The relationship between low urine pH and high risk MTX concentrations has been well documented. The standard dose of MTX introduces up to 7 times the range of drug concentrations in different patients with the same urine pH. In addition it has also been reported that kidney function and liver affect MTX pharmacokinetics. MTX is eliminated by renal excretion. MTX elimination is extended in patients with renal impairment. MTX is metabolized to metabolites that are less active by the liver. Liver dysfunction can lead to MTX metabolic disorders. Then, kidney and liver function will be considered in patients with reduced elimination (Popovic, et al., 2015).

CONCLUSION

It is important to monitor pharmacokinetic parameters after the administration of high doses of methotrexate in ALL pediatric patients, including clearance, distribution volume, and elimination. Therefore methotrexate is a dose-dependent pharmacokinetic profile, so the higher dose of intravenous methotrexate given will cause a shorter half-life and decreased clearance of methotrexate. This event allows a delay in the elimination of methotrexate which has been shown to have a correlation with the occurrence of toxicity in patients with ALL

children. Therefore, leucovorin rescue therapy is needed to control the duration of methotrexate exposure in sensitive cells so that the side effects caused by methotrexate can be minimized. This finding encourages and suggests monitoring plasma MTX concentrations during administration of high doses of MTX until residual MTX levels in the blood are possible to show no signs of patients experiencing toxicity.

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