Methotrexate bioavailability

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Received on July 16, 2010; accepted in revised form on August 2, 2010.

Clin Exp Rheumatol 2010; 28 (Suppl. 61): S27-S32.

Key words:
Methotrexate, bioavailability, rheumatoid arthritis

Competing interests: none declared

ABSTRACT

The clinical relevance 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 a relationship exists between on the one hand efficacy and/or safety and on the other hand concentration of the active compound or its active metabolite(s) in the systemic circulation. Applying these principles to the current knowledge on methotrexate (MTX), it is clear that bioavailability of MTX is an important parameter for optimal dosing. In this manuscript the current knowledge on MTX bioavailability is reviewed.

This review reveals that bioavailability of MTX in higher oral doses is decreased, most probably by limitation of absorption from the gastro-intestinal tract. It is suggested that higher doses can be given either by splitting the oral dose or by parenteral administration. Both will result in improved bioavailability as compared with one higher oral dose. However, larger, prospective studies directly comparing the efficacy and safety of the splitted oral dose strategy and the switch to parenteral MTX are needed.

Bioavailability: definitions and perspectives

Bioavailability is defined as '.. the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action ..' (1). This definition stresses the use of pharmacokinetic factors in a biological matrix such as blood or serum. These factors can modify the release of the drug substance from the drug product into the systemic circulation.

Current guidelines state that bioavailability can be divided into early exposure, peak exposure and total exposure (1). Early exposure can be expressed in partial areas under the plasma/serum/blood concentration-time curve (AUC), for example from time zero to the time at which the maximum concentration in the biological matrix is reached. Peak exposure can be expressed by means of the maximum concentration obtained from pharmacokinetic studies. Lastly, total exposure can be expressed in AUC from time zero to the end of the dose interval under steady state conditions.

Hereafter, we review the current knowledge on bioavailability of methotrexate (MTX). For this review we searched the literature using the following search terms alone or in combination: methotrexate, (pharmaco) kinetic (s/ally), bioavailability, PK-PD, absorption, rheumatoid arthritis, serum-, plasma, blood concentration, interaction, pharmacogenetics, therapeutic drug monitoring. Using the Medline and Embase search engines. The reference lists of the retrieved publications were searched for further relevant publications. Only manuscripts published after peer review during the last two decades in English and revealing original data were used.

Pharmacokinetic characteristics of methotrexate

After oral administration MTX is actively absorbed from the proximal jejunum (Fig. 1). This process of active absorption is capacity-limited and consequently decreases with increased oral doses. The extent of absorption of MTX is highly variable between individuals, with mean absolute bioavailability ranging from 30–90% (2-8). Intra-individual variability in bioavailability is small. The hepatic first-pass effect of MTX to 7-OH-MTX is estimated around 10% (2, 4, 8) but with large interindividual variability from 0.94-13.2% (9). Differences in the activity of aldehyde oxidase and xanthine oxidase, both enzymes possibly involved in MTX catabolism to 7-OH-MTX , may explain this high interindividual variability (9, 10).

The time until maximal plasma concentration (t_{max}) is 0.75-2 hours after oral

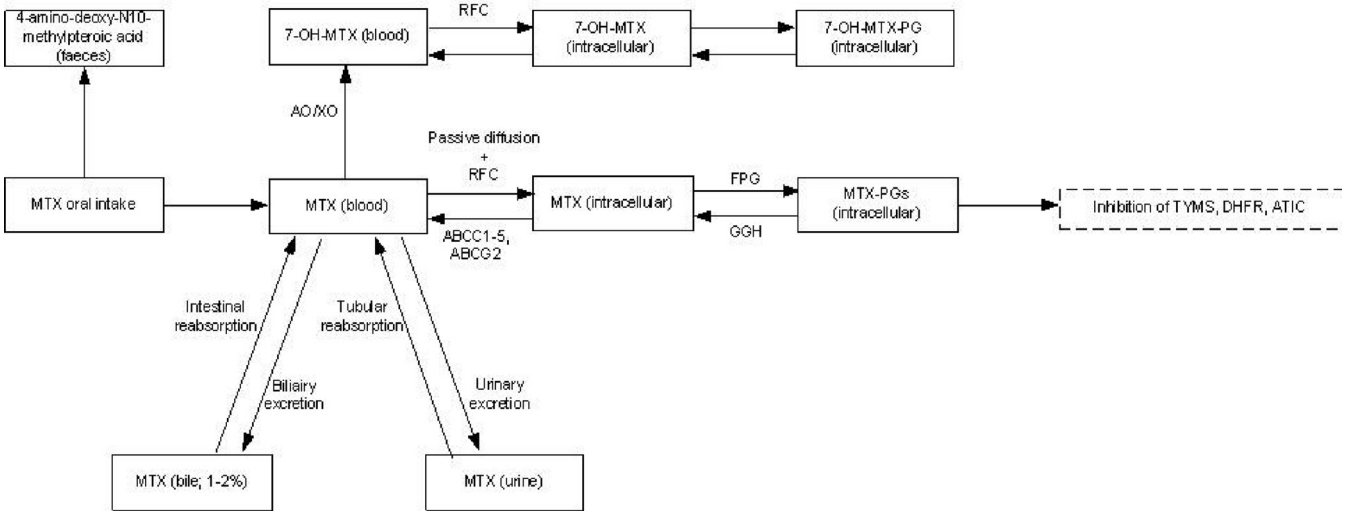

Fig. 1. Metabolic pathways of methotrexate.
ABCC /G: ATP-binding cassette (ABC) transporters; AO: aldehyde oxidase; ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; DHFR: dihydrofolate reductase; FPG: folylpolyglutamate synthase; GGH: gamma-glutamyl hydrolase; MTX: methotrexate; MTX-PG: polyglutamated form of methotrexate; RFC: reduced folate carrier; TYMS: thymidylate synthetase; XO: xanthine oxidase.

administration, reflecting a high rate of absorption (2, 3, 11). This rate of absorption is influenced by food, with a prolonged t_{max} of about 0.4-0.7 hours, due to delayed gastric emptying. However, the extent of the absorption is not altered by food (3, 12). Higher maximum concentrations (c_{max}) are reported to be related to a higher incidence of adverse reactions (13).

Absorption of MTX is not influenced by diurnal variation. Carpentier *et al*. (14) compared intramuscular MTX administration at 10 AM with administration at 6 PM and found no significant differences in MTX pharmacokinetic parameters. Furthermore, Hoekstra *et al*. (6) found no differences in bioavailability of two equal oral doses of MTX given at 9 AM and 5 PM.

MTX has a volume of distribution of 0.7–1.4 L/kg reflecting intracellular distribution. MTX is actively transported into erythrocytes, white blood cells , hepatocytes , and synoviocytes through the reduced folate carrier (RFC). Intracellularly MTX is polyglutamated with up to 5 glutamate moieties by the enzyme folyl-polyglutamate synthetase. This polyglutamated MTX (MTX-PG) cannot be transported extracellularly unless hydrolysed back to MTX-monoglutamate by gamma-glutamylhydrolase. This intracellular accumulation of MTX-PG allows prolonged intracellular presence of MTX following administration once a week.

Besides the intracellular polyglutamation two other metabolic pathways for elimination of MTX are of importance. First, MTX is converted to 7-hydroxy-MTX in the liver. Around 5-7% of the dose of MTX is recovered as 7-OH-MTX in urine (2, 4). Since 7-OH-MTX is less water soluble than MTX it may therefore contribute to the acute nephrotoxicity due to precipitation in acidic urine. Baggott *et al*. (15) report two phenotypes for the catabolism of MTX to 7-OH-MTX, with potential consequences for MTX-efficacy. Increased catabolism to 7-OH-MTX may interfere with polyglutamate formation, decrease MTX retention and lower efficacy *in vivo*. Second, less than 5% of the administered dose of MTX is metabolized by the intestinal flora to 4-amino-deoxy-N10-methylpteroic acid (16).

The major route of elimination of MTX and its metabolites is via urinary excretion. MTX is submitted to glomerular filtration, active tubular secretion and active tubular reabsorption. The capacity of tubular secretion and tubular reabsorption have high interindividual variability and both can be saturated, leading to non-linear pharmacokinetics following the administration of a wide dose range.

Circadian rhythm in glomerular filtration would suggest variations in MTX clearance during the day. However, Carpentier (14) was unable to demonstrate chronopharmacology in his study in 23 patients with RA. The authors hypothesize that increased tubular secretion compensates for the decreased glomerular filtration rate. This hypothesis has not been confirmed in further studies.

After long-term MTX administration the renal clearance of MTX and the creatinine clearance are decreased (17). This effect of MTX could be explained by an increase in plasma adenosine concentrations in extracellular fluid, the subsequent activation of A1 receptors in renal parenchyma resulting in diminished renal blood flow and salt and water excretion.

Besides urinary excretion approximately <1–30% of MTX is excreted via the bile (18, 19). Biliary excretion is inversely related to the dose. Therefore, biliary excretion is supposed to be a low-capacity, active process. Since only 1–2% of the MTX is excreted in feces, this suggests MTX has extensive enterohepatic circulation (20). This enterohepatic cycling is stereo-selective, only the L-MTX form of MTX is extensively reabsorbed after biliary secretion.

Plasma MTX concentrations fall rapidly after intravenous administration (21). The rapid intracellular uptake and the short plasma half-life of MTX mean that plasma concentrations cannot be used for therapeutic drug monitoring

(22). Plasma-elimination of MTX is bi- or triphasic with a terminal half life of 6-15 hours (18). However, the terminal half life is strongly dependent on the period of sampling, due to intracellular storage of MTX, polyglutamation, deglutamation and slow redistribution to the plasma.

Bioavailability and route of administration

Pharmacokinetic studies show inconsistent results on the relative bioavailability of oral versus parenteral administration of MTX for RA (Table I). In these studies the parenteral route for MTX administration is either intravenous, subcutaneous or intramuscular. From the studies mentioned in Table I, a dose dependency of the bioavailability of oral MTX emerges, higher oral doses lead to decreased bioavailability compared to parenteral administration. Explanations for the difference in bioavailability after oral or parenteral administration of MTX can be found in either the absorption limitation or a first-pass effect. The inverse relationship between oral dose and bioavailability suggest an important role for absorption limitation.

Hoekstra *et al*. (26) studied the role of absorption limitation by comparing the bioavailability of a divided higher (25-35 mg weekly) oral dose of MTX in comparison to a single dose in 10 adult patients with RA. The bioavailability of the split dose was 28% higher compared to the single dose group with a median weekly oral dose of 30 mg MTX. Compared with a historical subcutaneous control group the relative bioavailability was 0.76 and 0.9 for the single dose and the split dose group, respectively. The authors conclude that when higher MTX doses are needed, splitting the oral dose is an option and a suitable alternative for subcutaneous administration. Their findings support the hypothesis that absorption limitation reduces bioavailability with higher oral doses of MTX.

Studies on the association of the route of administration and the bioavailability are too small to draw definite conclusions on the dose dependency of this association. However, Hoekstra *et al*. (6) in their study found no relationship between the AUC after oral administration and the dose of MTX, in contrast to the positive relationship between the AUC and subcutaneous administration and the dose of MTX. Hamilton et al (24) also found an association between decreasing bioavailability with an increasing oral dose.

Several studies report that MTX characteristics of individual patients from the study population deviate remarkably outside the 95% confidence interval of the mean pharmacokinetic point estimates. Herman *et al*. (11) report two patients within the population with an oral MTX bioavailability more than 2.6 standard deviations higher than the mean for the whole study population (n=41). Jundt *et al*. (25) report on one patient out of twelve with characteristics that are significantly different from the rest of the population. Finally, data from Hoekstra *et al*. (6) show 1 patient out of twelve with oral bioavailability <2.0 standard deviations under the population mean. Pharmacokinetic studies are in general characterised by large interindividual differences between study subjects. Despite this, these findings lead to intriguing questions as to whether there is a subpopulation on low-dose MTX with different pharmacokinetic profiles and what this means in terms of optimal route of MTX administration and dosing. No studies have addressed this issue thus far.

Visser *et al*. (27) in their systematic review conclude that in patients with longstanding RA, after failure of oral MTX a switch to intramuscular MTX with subsequent dose escalation did not result in increased efficacy. In contrast, other studies and case series reports show better clinical efficacy of parenteral MTX compared to oral dosing, especially at higher doses (28-30). The results of these studies may be explained by the pharmacokinetic findings as presented above.

Drug-drug interactions and MTX bioavailability

Disease-modifying anti-rheumatic drugs have a number of clinically relevant

Table I. Studies on the relative bioavailability of MTX after oral and parenteral administration.

Ref	Indication, Population	Group size	Comparison	Dose range	Result
(3)	RA, adults	10	PO vs IV MTX	15 mg single dose	BA PO/BA IV 0.67
(6)	RA, adults	15	PO vs SC MTX	25-40 mg weekly	BA PO/BA SC 0.64; higher coefficient of variation of BA PO vs BA SC: 32% vs 23%
(8)	RA, adults	9	PO vs SC, IM MTX	15 mg single dose	No difference in $AUC_{0\text{-}170h}$ for PO, SC and IM administration
(11)	RA, adults	41	PO vs IV MTX	10 mg/m^2	BA PO/BA IV 0.70
(23)	JIA, children	17	PO vs SC MTX	0.19-0.94 mg/kg	Non-linear pharmacokinetics (C_{max}, $AUC_{0\text{-}4h}$ vs dose and C_{max}/dose, $AUC_{0\text{-}4h}$/dose vs dose)
(24)	RA, adults	21	PO vs IM MTX; 7.5 mg start dose vs full dose	7.5 mg start dose Full dose (mean [SD]17 [3.8] mg MTX weekly)	Difference AUC start dose PO vs IM: 0% (NS) Difference AUC full dose PO vs IM: -11% (NS)
(25)	RA, adults	12	PO vs IM MTX		BA PO/BA IM 0.85 (oral solution) and 0.87 (tablet)

AUC: area under the plasma concentration versus time curve; BA: bioavailability; c_{max}: maximal plasma concentration; IM: intramuscular; JIA: juvenile idiopathic arthritis; PO: per os/oral; RA: rheumatoid arthritis; SC: subcutaneous.

drug-drug interactions with drugs not primarily prescribed for rheumatic diseases (31). Drug-drug interactions can alter pharmacodynamics, but also pharmacokinetic characteristics and bioavailability of one of the interacting drugs.

The drug-drug interaction between non-steroidal anti-inflammatory drugs (NSAIDs) and MTX is well known. Since MTX is primarily excreted into urine in the unchanged form, alteration of the glomerular filtration or tubular reabsorption may alter MTX bioavailability through altered elimination. It has been shown that the mechanism of the drug-drug interaction between MTX and NSAIDs cannot be fully explained by the inhibition of the basolateral MTX uptake in the renal proximal tubules (32-34). El-Sheikh *et al*. (35) report that the inhibition by NSAID's of renal MTX efflux via multidrug resistance protein (MRP) 2 and 4 may offer additional mechanisms to explain this drug-drug interaction. Structured assessment of the clinical relevance of drug-drug interactions (36) determined the drug-drug interaction between MTX and NSAIDs to be clinically relevant for MTX-doses used in RA. Evidence on the pharmacokinetic effects of this interaction show a decrease of renal and total MTX clearance with 40% (117 mL/min to 70 mL/min) respectively 22% (168 mL/min to 131 mL/min) in combination of MTX with ibuprofen (37). Standardised monitoring of renal function, serum liver enzyme activities and white blood cell count for early recognition of MTX-toxicity is advised. Since in most current RA treatment protocols this monitoring is already advised in situations in which NSAIDs and MTX are not combined, the potential effects of this interaction are taken care of in routine clinical monitoring.

It is reported that folic acid may alter the bioavailability of MTX. Baggot *et al*. (9) studied the effect of folic and folinic acid on the catabolism of MTX to 7-OH-MTX. Folic acid, but not folinic acid, was found to competitively inhibit aldehyde oxidase, the enzyme responsible for formation of 7-OH-MTX. The authors conclude that patients on the combination of MTX and folic acid would have less MTX-catabolism to 7-OH-MTX. Since 7-OH-MTX and MTX are both polyglutamated, a higher concentration of 7-OH-MTX may displace MTX from the active site of polyglutamyl synthetase, increase urinary MTX excretion and decrease intracellular MTX retention. This hypothesis is not supported by the results of the study of Chlàdek *et al*. (38) in patients with moderate-to-severe plaque psoriasis on oral MTX. They report that patients on a combination therapy of oral MTX and 20 mg folic acid weekly had comparable MTX-polyglutamate erythrocyte concentrations. However, clinical efficacy was significantly in favor of the MTX monotherapy arm. This finding is supported by the results of the MTX-folic acid studies of Van Ede *et al*. (39) and Hartman (40).

Drug-drug interactions between MTX in doses used in oncology are described for piperacillin/tazobactam (41), ciprofloxacin (42) and proton pump inhibitors (43). For these interactions decreased renal elimination (piperacillin and ciprofloxacin) or inhibition of MTX transport via breast cancer resistance protein (proton pump inhibitors) are suggested as explanatory mechanisms. Although not established in anti-rheumatic MTX doses these interactions may be relevant for patients with RA.

No influence on the pharmacokinetic parameters of oral MTX were found for ferrous sulphate (44) or naproxen/lansoprazole (45).

Bioavailability of MTX-PG

Since the definition of bioavailability includes the rate and extent to which the compound becomes available at the site of action, considerations on the bioavailability of MTX-PG are of relevance within the scope of this review. However, the MTX-PG are less suitable indicators of MTX bioavailability because their steady state concentrations are reached only after several months of stable dosing, assuming patient compliance to MTX intake (46). Furthermore, recent studies show inconsistent associations between MTX-PG concentrations in erythrocytes and disease control (47). The pharmacology of MTX-PG is further discussed in the contribution of Dervieux *et al*. in this issue.

Pharmacogenetics and MTX bioavailability

Pharmacogenetics is the study of variability in drug response due to heredity. Genetic variation may alter aspects of the pharmacokinetic characteristics of MTX, thereby altering bioavailability. Genetic variation relevant to MTX bioavailability can be found in intracellular metabolism and transporter function. These topics will be further elaborated on in this issue by Ranganathan *et al*.

Discussion

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.

In translating these principles to the current knowledge on MTX, it becomes clear that bioavailability of MTX is an important parameter for optimal dosing. The measurement of the active component at the site of action is not possible at the moment. For instance for MTX, its metabolites or PG-forms a validated measurement at the intracellular site of action has not been found. MTX and MTX-PG measurements show inconsistent associations between intracellular MTX-PG concentration and disease activity. On the other hand some evidence exists on the relationship between MTX dose and efficacy and MTX dose and safety. Recent studies offer evidence that improving bioavailability has associations with efficacy and safety.

Although not directly translatable to RA, the treatment of psoriasis with MTX offers interesting information. In the treatment of psoriasis with MTX it is suggested that a target value for bioavailability of MTX is associated with clinical efficacy. Hroch *et al*. (48) state that for a drop of at least 50% in the Psoriasis Area and Severity Index (PASI) an AUC0-8h of 1800 nmol.h/L has to be the target bioavailability parameter. An AUC target value has not been proposed or studied for RA.

Further studies will be needed to elucidate which, if any, metabolite or MTX-PG form will have a useful association with efficacy/safety of MTX therapy. A prospective, long-term study, to obtain steady state concentrations of the long chain MTX-PGs, and containing data on disease activity and pharmacokinetic parameters may elucidate which pharmacokinetic parameter offer optimal and clinically relevant associations with treatment outcome.

Bioavailability of MTX is decreased after oral administration in higher doses, most probably by limitation of absorption from the gastro-intestinal tract. After parenteral administration, lowering of MTX bioavailability is not seen. One study shows that splitting of the oral dose results in improved bioavailability. Larger, prospective studies directly comparing the efficacy and safety of the (split) oral dose strategy and the switch to parenteral MTX are needed. For example to study whether the observed, improved response seen with subcutaneous administration occurs at an earlier moment compared with (split) oral dose regimes.

Acknowledgement

The authors like to thank J. Collins, PhD for his final corrections to the manuscript.

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