Clin Pharmacokinet 2003; 42 (2): 139-151
0312-5963/03/0002-0139/$30.00/0

Pharmacokinetics and Pharmacodynamics of Methotrexate in Non-Neoplastic Diseases

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Contents

Abstract

Low dose pulse methotrexate (LDMTX) therapy has become effective in the treatment of autoimmune and lymphoproliferative diseases. The pharmacokinetics of LDMTX is individually highly variable, resulting in a different systemic exposure to the drug and a variable therapeutic/toxic effect in patients. The improvements and exacerbations of disease activity in relation to the introductions and discontinuations of LDMTX therapy suggest the possible immunosuppresive and anti-inflammatory properties of the drug. Because of a strong correlation between the drug pharmacokinetics and the therapeutic outcomes (pharmacodynamics), it seems to be possible to individualise the LDMTX therapy according to the results of pharmacokinetic/pharmacodynamic analysis. In the case of psoriasis, pharmacokinetic/pharmacodynamic analysis in our local study revealed a highly significant inverse relationship between PASI (expressed as a percent of the initial value) and a steady-state AUC_{MTX} (area under the curve of methotrexate plasma concentrations; $r_8 = -0.65$, $p < 0.001$). The considerable inter-individual variability and low intra-individual variability in MTX pharmacokinetics, supports a role for therapeutic monitoring and dose individualisation at the start of pharmacotherapy. The results of this study suggest that a steady-state AUC_{MTX}

value of 700 nmol • h/L and higher are associated with a significantly better success rate of antipsoriatic therapy than lower values. The preliminary results in our follow-up study suggest the statistically higher incidence of unwanted effects depending on maximum plasma concentration of the drug. Moreover, statistically significant correlation was found between the toxic effects and exposure to the drug regarding methotrexate plasma concentrations and intracellular storage in erythrocytes. However, the data are still in the process of being completed and are not yet published.

Methotrexate is an antifolate, and has been used as high-dose pulse therapy (HDMTX) for the treatment of malignancies since 1947. The favourable anti-inflammatory effect of low dose pulse methotrexate (LDMTX), given as 7.5–30mg (approximately 0.3 mg/kg) once weekly orally, subcutaneously or intramuscularly, was first reported in the 1950s in patients with psoriasis and psoriatic arthritis. The drug has been commonly used in the therapy of recalcitrant psoriasis since the 1960s. Its use in the treatment of rheumatoid arthritis began during the 1980s.[1]

At present, methotrexate is one of the most frequently used of the disease-modifying antirheumatic drugs (DMARDs), also called slow-acting or symptom-modifying drugs. In the treatment of rheumatoid arthritis, methotrexate has proved to be more effective and less toxic than auranofin and azathioprine, and as effective as but less toxic than sulfasalazine.[1,2] The continuation rate of LDMTX therapy in patients with rheumatoid arthritis has been reported as 70% after 1 year of therapy, 54% after 3 years and 50% after 6 years.[3] These percentages compare favourably with the overall probability of less than 20% for three other DMARDs after 5 years: 19% for sulfasalazine, 17% for penicillamine and 8% for parenteral gold.[4]

LDMTX therapy was also shown in placebo-controlled randomised trials to be efficacious in children with juvenile rheumatoid arthritis (especially the polyarticular form) and systemic onset juvenile rheumatoid arthritis (Still's disease). Generally, children tolerate higher doses of the drug than adults, up to 0.6 mg/kg. Long-term LDMTX therapy does not induce osteopenia in children, which has been described after HDMTX.[5] Parenteral LDMTX therapy also has a beneficial effect on numerous other inflammatory disorders, including corticosteroid-dependent chronic active Crohn's disease,[6-8] antimalarial-resistant lupus arthritis, cutaneous lupus erythematosus,[9] systemic lupus erythematosus,[10] polymyositis, polymyalgia rheumatica, Reiter's syndrome, sarcoidosis, primary biliary cirrhosis, primary sclerosing cholangitis, scleroderma, graft-versus-host disease and organ allograft rejection.[7,11]

From many clinical studies it is evident that LDMTX treatment is associated with great interindividual variability in the therapeutic response. Regardless of the different immunological characteristics of patients, a significant relationship between pharmacokinetics and pharmacodynamics (i.e. efficacy and toxicity) has been reported.[12-15]

1. Pharmacokinetics

1.1 Absorption

1.1.1 Oral Administration

Methotrexate is a weak dicarboxylic organic acid with a molecular weight of 454 daltons. The molecule is negatively charged at neutral pH (pKa_1 = 4.84, pKa_2 = 5.51), resulting in limited lipid solubility. After oral administration, active absorption of the drug occurs in the proximal jejunum. The process is capacity-limited and decreases nonproportionally with increased oral doses.[16-18] Earlier studies indicated that methotrexate absorption was rapid and complete after oral doses of less than 30 mg/m^2.[16,19] More recent investigations with larger numbers of patients demonstrated that the rate and extent of absorption are highly variable between patients, and that the absolute bioavailability may be less than 50% for doses as low as

10–15 mg/m^2.[15,20,21] The mean absolute bioavailability is about 70–80% and a large interindividual variation from 30–90% has been observed.[15,20,22-24] Conversely, only a moderate intra-individual variability in LDMTX pharmacokinetics was noticed during intermediate-term (13 weeks[15]) and long-term (2 years[21]) treatment in patients with psoriasis and rheumatoid arthritis receiving a single weekly dose of methotrexate 15mg.

Under fasting conditions, maximum plasma concentrations of methotrexate (C_{max}) range between 0.3 and 1.6 μmol/L, and occur at a t_{max} of 0.75–2 hours after administration.[15,21,22,24] Food did not significantly influence the bioavailability of methotrexate, but slightly reduced C_{max} and prolonged t_{max} by about 0.4–0.7 hours as a result of delayed gastric emptying.[24]

1.1.2 Parenteral Administration

LDMTX is given parenterally to ensure effective compliance and, presumably, uniform bioavailability.[25] The drug is absorbed more rapidly and reaches higher serum concentrations after intramuscular or subcutaneous administration compared with the oral route.[26,27] Nevertheless, the mean absolute bioavailability is very similar,[28,29] suggesting that the routes of LDMTX administration are interchangeable.[30,31]

LDMTX may also be injected intra-articularly. The mean synovial methotrexate concentration exceeds the serum concentration by a minimum of 10-fold throughout the whole 24-hour post-dose period and ensures the therapeutic effect.[32] The topical application of LDMTX in cream results in drug absorption and accumulation in keratinocytes of psoriatic plaques, but without any histological change.[33]

1.2 Distribution

The volume of distribution of methotrexate is 0.87–1.43 L/kg, which corresponds to the intracellular distribution of the drug.[26,34] In blood, 30–70% of methotrexate is bound to proteins, almost exclusively to albumin.[23,26,27,34-36] Edno et al. demonstrated a significantly increased drug plasma concentration for 8 hours following methotrexate administration,[34] reflecting the possible enterohepatic cycling of the drug. The concentrations of methotrexate in the synovial fluid are approximately equal to plasma concentrations at 4 and 24 hours after oral or intramuscular administration.[23]

With regard to the intracellular mechanism of action, it is believed that the most important process is transport of methotrexate into cells and its accumulation within cells in the form of polyglutamates. Transport of methotrexate occurs both by passive transmembrane diffusion and by a carrier-mediated active transport system that methotrexate shares with folates.[18,25] A folate surface receptor responsible for the intracellular transport of both reduced folates and methotrexate has been well described in various *in vitro* studies.[37] Once inside the cell, up to six glutamate residues may be progressively added to the drug molecule by the folyl-polyglutamate synthetase enzyme. Polyglutamyl derivatives of methotrexate cannot be transported extracellularly unless they are hydrolysed back to the monoglutamate.[16,18] Intracellular accumulation of methotrexate polyglutamates allows drug administration once weekly as a bolus or divided into three equal subdoses.[38] It was found that the pool of folate polyglutamates in liver cells and erythrocytes is gradually replaced by methotrexate polyglutamates during long-term LDMTX therapy.[39,40]

The pharmacokinetics of methotrexate in erythrocytes have been studied extensively.[39] After a single dose, methotrexate concentrations in plasma and erythrocytes change simultaneously as a consequence of a rapid equilibrium between these compartments. The concentrations of methotrexate both in plasma and erythrocytes usually fall below 10 nmol/L within 24 hours after LDMTX administration. At 3–4 days later, the drug reappears in erythrocytes despite its negligible plasma concentration.[39] Methotrexate polyglutamates in erythrocytes accumulate until a steady-state level is reached after 4–6 weeks of intermittent administration.[15,41] This is a much shorter time than that required for medullar maturation of erythroblasts into erythrocytes (14–18

weeks). The probable explanation is that methotrexate polyglutamates are synthesised mainly in the circulating erythrocytes.[41] The steady-state erythrocyte methotrexate concentration is also highly variable among patients undergoing long-term LDMTX therapy. A range of 10–170 nmol/L erythrocytes was observed following the administration of 7.5–15mg methotrexate once a week.[15,40] No correlation was found between the total cumulative dose of methotrexate and erythrocyte methotrexate concentration.[42]

Although no close relationship was found between folate status and intracellular accumulation of methotrexate, the highest methotrexate concentrations were found in erythrocytes with the lowest folate concentration.[40] Moreover, erythrocyte methotrexate concentration seems to be indicative of hepatic changes that occur during LDMTX therapy. Significantly higher erythrocyte methotrexate concentrations were found in patients with progressive hepatic changes than in patients with no progression. Nevertheless, a critical erythrocyte concentration was not established because of its very large interindividual variability.

Peripheral blood T-lymphocytes also intensively convert methotrexate to polyglutamyl derivatives (tetra- and penta-glutamates). Similarly, methotrexate is highly accumulated in fibroblasts, myeloid precursors in bone marrow and keratinocytes.[1,20,33] Relatively high and equal methotrexate concentrations were found in the synovial membrane, cortical bone and trabecular bone.[43] The intracellular accumulation results in drug-induced apoptosis of T-lymphocytes.[44] On the contrary, the activity of intracellular hydrolases in intestinal epithelium cells is very high, and therefore the accumulation of methotrexate in small intestine mucosa is not significant.[18]

1.3 Elimination

Elimination of methotrexate from plasma was shown to be biphasic or triphasic (dependent on the length of sample collection period) with a mean terminal biological half-life ($t_{½\beta}$) of 6–15 hours.[15,18,22-24,26,27,29,34] Thus, accumulation of methotrexate in plasma cannot occur after intermittent administration once a week. Extensive sampling of plasma over 1 week after methotrexate administration in nine patients with rheumatoid arthritis allowed estimation of a $t_{½\beta}$ of 55 hours, reflecting slow release of methotrexate from its intracellular forms.[29] The longer the sampling, the longer the reported $t_{½\beta}$ of the drug, probably due to intracellular methotrexate storage, polyglutamylation and slow release back to plasma.[16,18] Accumulation of methotrexate in pleural effusion and ascitic fluid is the reason for its slowed elimination in cancer patients receiving HDMTX therapy, but seems to be of no importance after LDMTX.[18]

1.3.1 Metabolism

Three metabolic pathways of methotrexate have been described in humans. First, the drug is metabolised by intestinal bacteria to 4-amino-deoxy-N^{10}-methylpteroic acid. The metabolite usually accounts for less than 5% of the administered dose, and is rarely detectable in human plasma and urine.[18]

Secondly, in the liver, methotrexate is converted to 7-hydroxy-methotrexate. 7-Hydroxy-methotrexate is less water soluble than methotrexate, and it may therefore contribute to acute nephrotoxicity because of its precipitation in acidic urine. The metabolite is a 10-fold less potent inhibitor of dihydrofolate reductase (DHFR), one of the intracellular target enzymes for methotrexate.[16,18,45] The hepatic first-pass effect of methotrexate is low (about 10%), as is its metabolic clearance to 7-hydroxy-methotrexate: 5–7% of the dose was recovered as 7-hydroxy-methotrexate in urine after a broad range of methotrexate doses.[3,15,29] However, due to its slower rate of urinary excretion, plasma concentrations of 7-hydroxy-methotrexate usually exceed those of methotrexate within 8–10 hours after drug administration.[15,29,35] Despite its extensive binding to serum albumin (91–93%), 7-hydroxy-methotrexate does not alter the protein binding of methotrexate.[18,23,26,27,34-36] Both compounds compete for the same membrane carriers, intracellular transporters and, subsequently, for folyl-polyglutamate synthetase.[46]

Thirdly, the intracellular conversion of methotrexate to polyglutamates represents the most important metabolic pathway regarding efficacy.[16,18] Polyglutamyl derivatives of methotrexate also exhibit more efficient inhibitory properties towards intracellular metabolism of pyrimidines and purines than does the parent drug.[18,25] Alteration of the intracellular folate cycle results in intracellular accumulation of homocysteine and depletion of polyamines such as spermine and spermidine. Polyamines have proinflammatory properties.[47]

1.3.2 Excretion

Renal excretion constitutes the major elimination route for methotrexate. The drug is filtered in renal glomeruli and, additionally, undergoes bidirectional transport across the renal tubules, i.e. active secretion, utilising the general transport mechanism for organic acids, and active reabsorption unaffected by acidic compounds from the distal tubule. At serum concentrations from 0.1–0.4 μmol/L, tubular secretion prevails over reabsorption, which reaches saturation.[16,41] Accordingly, renal clearance (CL_R) of LDMTX usually exceeds creatinine clearance (CL_{CR}) by about 2–28%.[26,48] At methotrexate plasma concentrations of 0.6–1 μmol/L, CL_R equals CL_{CR} (i.e. 80–120 ml/min), reflecting the saturation of methotrexate active tubular secretion. There is a considerable interindividual variation in the saturation point of both secretion and reabsorption in tubules. Both kinetic processes can occasionally be saturated even at low methotrexate plasma concentrations within the range of 0.1–1 μmol/L. Thus, nonlinear elimination may result following the administration of 7.5–30mg of methotrexate and contribute to the interindividual variability in methotrexate concentrations.[40]

After 6 months of LDMTX therapy, CL_R of methotrexate decreased by a mean of 23.8 ml/min and CL_{CR} by 8.6 ml/min.[48] A decrease in glomerular filtration rate has also been reported in rheumatoid arthritis patients taking LDMTX, usually over a period of 2–4 weeks.[29] This important effect of methotrexate has also been observed in HDMTX.[48] It could be explained by an increase in plasma adenosine concentration in extracellular fluid and by subsequent activation of A_1 receptors in renal parenchyma, diminishing renal blood flow and salt and water excretion.[47]

In addition, a variable amount of methotrexate is eliminated by active biliary excretion, responsible for 10–30% of methotrexate clearance.[26,36,41,48] However, only about 1–2% of the drug is excreted in faeces, suggesting extensive enterohepatic circulation of methotrexate.[40,45] Biliary excretion can become more important in patients with renal insufficiency.[17,20] Enterohepatic cycling can be interrupted by cholestyramine or charcoal, which can be administered to attenuate potentially life-threatening toxicity of LDMTX in patients with renal insufficiency or after methotrexate poisoning.[49]

1.4 Therapeutic Drug Monitoring

Recently, to provide therapeutic drug monitoring effectively and more practically, limited sampling methods to estimate methotrexate pharmacokinetics using a Bayesian approach and population data modelling programs have been implemented.[50-52]

2. Pharmacodynamics

Methotrexate is an analogue of folic acid that was originally designed to inhibit the activity of the enzyme DHFR. This enzyme converts dihydrofolates to tetrahydrofolates, which are involved in single carbon atom transfers in crucial intracellular metabolic pathways such as *de novo* synthesis of purines, pyrimidines and polyamines and transmethylation of phospholipids and proteins. In oncology, the rationale for the use of HDMTX is that malignant cells become starved of the purine and pyrimidine precursors required for DNA and RNA synthesis, proliferation and cell division. As a result of their inability to synthesise DNA and RNA, the number of malignant cells rapidly falls under such therapeutic conditions.[53]

LDMTX has immunosuppressive and anti-inflammatory properties. Concerning immuno-

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