Comparison of intracellular methotrexate kinetics in red blood cells with the kinetics in other cell types

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

- Red blood cell (RBC) concentrations of methotrexate (MTX) have been suggested to monitor MTX treatment of rheumatoid arthritis.
- No clear link between RBC kinetics of MTX and clinical outcomes could be established so far.
- RBCs are unlikely to be involved in the mechanism of action of MTX.

WHAT THIS STUDY ADDS

- RBC kinetics of MTX differ from the kinetics in other cell types, and might not reflect the kinetics in cell lines that are assumed to be on the causal path of action in rheumatoid arthritis.
- A clear link between RBC MTX concentrations and clinical outcomes might not exist.

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AIM

To assess the similarities in intracellular pharmacokinetics (PK) of methotrexate (MTX) in red blood cells (RBCs) and other cell lines.

METHODS

Three previously published PK models for intracellular MTX and MTX polyglutamate (MTXGlu₂₋₅) concentrations were used: (i) a model for the kinetics in RBCs, (ii) a model for the kinetics in human breast cancer cells (HBCCs) and (iii) a model for the kinetics in various white blood cell (WBC) lines. All three models were used to simulate the response in a typical individual receiving 10 mg oral MTX once weekly and the predicted steady-state concentrations (C_{ss}) and time to C_{ss} (t_{ss}) were compared.

RESULTS

The HBCC model showed a lower C_{ss} for MTXGlu₂ and ₃ and higher C_{ss} for MTXGlu₄ and ₅ compared with the RBC PK model, while t_{ss} and overall intracellular MTX exposure appeared similar. The WBC PK model showed much lower C_{ss} for the parent MTXGlu₁ and of t_{ss} for all MTXGlu_n, as well as a much lower cumulative C_{ss} for MTXGlu₂₋₇ for the majority of the WBC cell lines.

CONCLUSION

RBC kinetics of MTX differ from the kinetics in other cell types such as WBCs and HBCCs to a variable degree. It is possible that similarly diverse profiles may exist across other cell lines, including those on the causal path in rheumatoid arthritis. Hence, there may not necessarily be a clear link between RBC MTX concentrations and disease control in rheumatoid arthritis.

Introduction

The treatment of rheumatoid arthritis (RA) requires rapid and effective disease control to prevent irreversible joint

damage. Low dose methotrexate (MTX) is the gold standard disease modifying anti-rheumatic drug (DMARD) used in the treatment of RA, and rapid disease control is desired to prevent joint damage. However, no method for

therapeutic drug monitoring of MTX is currently available that assists clinicians to predict clinical outcomes and guide MTX dosing. MTX doses required to achieve clinical effect vary between patients, and MTX plasma concentrations do not appear to have a predictable correlation with clinical outcomes [1]. This might be attributed partially to the short half-life of the parent drug in the plasma, as well as the intracellular formation of active metabolites (MTX polyglutamates) which contribute to the overall clinical effects of MTX treatment [2, 3] and which are not present in measurable quantities in the plasma. Measuring the intracellular concentration of the active metabolites might provide better predictors of clinical response, particularly if the cells are located on the pathogenic pathway of RA. A target therapeutic range for intracellular MTX polyglutamates would aid decisions regarding dose increases for MTX monotherapy vs. changing to alternative or combination DMARDs or biological therapies.

Intracellular uptake of MTX takes place via the reduced folate carrier (RFC). MTX polyglutamates are formed inside the cells by the enzyme folypolyglutamate synthetase (FPGS), which adds glutamate moieties to the molecule in a chain-like manner. This results in MTX polyglutamates containing 'n' number of glutamate moieties (MTXGlu_n). The parent drug already contains one glutamate moiety (MTXGlu₁). The enzyme γ -glutamate hydrolase (γ GH) sequentially deglutamates MTXGlu_n back to MTXGlu₁, which can leave the cells via efflux transporters.

Although red blood cells (RBCs) are not located on the postulated pathway of MTX action, measuring intracellular

RBC concentrations of MTXGlu_n has been suggested as a means for monitoring MTX treatment [4]. This is mainly due to the abundance of and relative ease of accessibility to RBCs in comparison with white blood cells (WBCs), which are more likely to be involved in MTX action. RBCs are unique cells that lack a nucleus [5]. They have a long lifespan of several months and a reduced enzyme and transporter capacity. Thus, it is likely that intracellular MTX kinetics observed in RBCs might differ from the kinetics in other cell types which might be expected to have a direct relationship with MTX outcomes and RA pathogenesis, such as WBCs.

Previously, we developed a population pharmacokinetic (PK) model for MTXGlu_n measured in RBCs [6]. The objective of the current work was to assess whether RBC kinetics of MTX are comparable with the kinetics observed in other cell lines such as human breast cancer cells (HBCCs) or WBCs. In this work we have considered all published models of intracellular MTX to get a sense of whether RBCs are similar to other cell lines, even when these cell lines are not related to the activity of MTX in RA (such as HBCCs).

Methods

The previously constructed RBC PK model

Figure 1 shows the RBC PK model for MTXGlu_n developed previously [6]. The plasma PK of MTXGlu₁ is described by a two compartment model. The corresponding population

Figure 1

Structure of the parent-metabolite pharmacokinetic model for MTXGlu_n in RBCs (adapted from [6]). $k_{a,oral}$ and $k_{a,s.c.}$ = absorption rate constants after oral and subcutaneous administration, respectively; F_{oral} and $F_{s.c.}$ = bioavailability after oral and subcutaneous administration, respectively; V_1 = apparent volume of distribution of MTXGlu₁ in the plasma compartment; CL_1 = clearance of MTXGlu₁ from the plasma compartment; k_{12} and k_{21} = intercompartmental transfer rate constants; k_{in} = rate constant of MTXGlu₁ uptake into RBCs; V_{RBCs} = apparent volume of distribution of MTXGlu_n in RBCs; $k_{FPGS1-4}$ = polyglutamation rate constants; $k_{\gamma GH2-5}$ = deglutamation rate constants; CL_{RBCs} = clearance of MTXGlu_n from RBCs

mean parameter values were obtained from articles published by Hoekstra *et al.* [7] and Herman *et al.* [8]. These values were fixed as no plasma data were available during the development of this model. The intracellular RBC PK of MTXGlu_n is described by a series of five compartments, one compartment for each MTXGlu_n species. The uptake of MTXGlu₁ into RBCs was approximated as a first order process for which the rate constant k_{in} was estimated. The apparent volume of distribution inside RBCs (V_{RBCs}) was assumed to be the same for all MTXGlu_n. V_{RBCs} was estimated based on a prior model for the parent drug only and kept fixed in the development of the full model. The polyglutamation rate constants $k_{FPGS1-4}$ were fixed to values obtained from model 1 published by Morrison & Allegra [9], which is described in the next section. The deglutamation rate constants $k_{\gamma GH2-5}$ were estimated. In the final model, a mechanism that allowed all MTXGlu_n to be lost from RBCs was superior to a model where only MTXGlu₁ was lost from the RBCs. In this model all MTXGlu_n were described to be lost according to the same value of clearance (CL_{RBCs}).

For comparison with the two alternative PK models developed for HBCCs and WBCs, we simulated the typical response in an individual receiving 10 mg oral MTX once weekly and calculated the individual RBC concentration of each MTXGlu_n at steady-state (C_{ss}), the ratio of C_{ss} for each metabolite to MTXGlu₁, the time to reach steady-state (t_{ss}) for each MTXGlu_n, as well as the sum of C_{ss} for the metabolites MTXGlu₂₋₅. To keep the model structure equivalent to Morrison & Allegra's model 1, we did not include loss of MTXGlu₂₋₅ from RBCs for this analysis. The same simulations and calculations were conducted using the alternative PK models described below.

MTX PK model in HBCC lines

Morrison & Allegra [9] developed a model for MTXGlu_n in HBCCs measured in an *in vitro* experiment. This model has the same basic structure as our RBC PK model. The authors specified three different submodels. However, in this analysis we only consider one of their models (model 1) which includes no efflux of MTXGlu₂₋₅ from HBCCs.

MTX PK model in WBCs

Panetta *et al.* [10] used a two compartment model to describe the intracellular PK of MTXGlu_n in WBCs, where the first compartment describes MTXGlu₁ and the second compartment describes the sum of the concentrations of the polyglutamates MTXGlu₂₋₇ (Figure 2). Uptake of MTXGlu₁ into the cells and the polyglutamation steps were described as active processes (with an additional passive uptake component that was kept fixed), while the overall deglutamation (from $n > 1$ to $n = 1$) was approximated by a single first order process.

We used each of the four sets of parameter values estimated by Panetta *et al.* [10] for different WBC cell lines, to conduct the same simulation of the response in a typical individual as described before. However, we focus the presentation of the results in this report on the response obtained for T-lymphocytes as they appeared to differ the least from the predictions based on the RBC PK model.

Note that the WBC PK model only provides a combined measure for MTXGlu₂₋₇ concentrations. Results for C_{ss} and t_{ss} for the individual polyglutamates cannot be obtained.

Figure 2

Structure of the WBC PK model published by Panetta *et al.* [10], adapted to include the dosing seen in the RBC MTX PK study [6]. Parameters in the plasma PK model are defined as in Figure 1. V_{max} = maximum velocity and K_m = Michaelis constant of the active uptake transport of MTXGlu₁, k_p = rate constant of the passive uptake component (fixed by Panetta *et al.* to 0.4 h^{-1}), k_{eff} = rate constant of MTXGlu₁ efflux, $V_{maxFPGS}$ = maximum velocity and K_{mFPGS} = of the enzymatic polyglutamation, $k_{\gamma GH}$ = deglutamation rate constant, $V_{intracell}$ = apparent intracellular volume of distribution of MTXGlu_n.

Table 1

Comparison of the individual steady-state MTXGlu_n concentrations and their sum for the metabolites MTXGlu₂₋₅ in a typical individual receiving 10 mg oral MTX once weekly as predicted based on simulations from the different models. Results for WBCs are based on the predictions for T-lymphocytes

PK models	MTXGlu ₁	MTXGlu ₂	MTXGlu ₃	MTXGlu ₄	MTXGlu ₅	MTXGlu ₂₋₅
Concentration at steady-state (nmol l ⁻¹)						
RBCs	14.4	14.5	26.2	10.6	5.0	56.3
HBCCs	13.9	3.8	11.9	18.4	21.4	55.6
WBCs*	0.19	–				48.1†
Ratio of steady-state concentrations of MTXGlu ₂₋₅ compared with MTXGlu ₁						
RBCs	1	1.01	1.82	0.73	0.35	–
HBCCs	1	0.28	0.86	1.33	1.55	–
WBCs*	1	–				253.2†
Time to reach steady-state (weeks)						
RBCs	45	60	63	57	70	–
HBCCs	59	69	65	69	73	–
WBCs*	2	–				2†

*Using the parameter values obtained by Panetta *et al.* for T-lymphocytes [10]. †Results for a combined measure of MTXGlu₂₋₇.

Results

Comparison with the HBCC PK model

Table 1 shows the results for the simulations of the response in a typical individual receiving 10 mg oral MTX once weekly. For the HBCC PK model, C_{ss} and their ratios compared with MTXGlu₁ were lower for MTXGlu₂ and ₃ and higher for MTXGlu₄ and ₅ than predicted based on the RBC PK model. The summed C_{ss} of MTXGlu₂₋₅ were similar in both models (55.6 vs. 56.3 nmol l⁻¹), as were the C_{ss} for MTXGlu₁ (13.9 vs. 14.4 nmol l⁻¹). Although t_{ss} values were marginally longer for all MTXGlu_n based on the HBCC PK model when compared with the RBC PK model, these values were on the whole similar. It needs to be noted, however, that the differences in the modelling approaches used for RBCs and HBCCs and in the nature of the experimental data (*in vivo* vs. *in vitro*) do not allow for a statistical comparison of the uncertainty in the model predictions.

Comparison with the WBC PK model

Simulating the response in a typical individual receiving 10 mg oral MTX once weekly based on the WBC PK model showed a lower C_{ss} for MTXGlu₁ for all four sets of parameter estimates reported by Panetta *et al.* [10] compared with the RBC PK model. Three of these simulations also showed a more than 1000-fold higher C_{ss} for the combined measure of MTXGlu₂₋₇ compared to the sum of the MTXGlu₂₋₅ concentrations predicted based on the RBC model. The magnitude of response for MTXGlu₂₋₇ for the fourth parameter set published for T-lymphocytes was more in line with the RBC model predictions. Table 1 includes the predicted C_{ss} for MTXGlu₁ and MTXGlu₂₋₇, their ratios and t_{ss} based on the T-lymphocyte parameter values. The combined C_{ss} for MTXGlu₂₋₇ is somewhat less than the summed concentration of MTXGlu₂₋₅ in the RBC PK model

(48.1 vs. 56.3 nmol l⁻¹). The t_{ss} is considerably shorter compared with the RBC PK model.

Discussion

This analysis shows that RBC kinetics of MTX are different from the kinetics observed in HBCCs for the individual MTXGlu₂₋₅, as their individual C_{ss} differed between the RBC and HBCC model. However C_{ss} for MTXGlu₁, the summed C_{ss} of MTXGlu₂₋₅ and the t_{ss} for all MTXGlu_n were similar between the models. This suggests that the time course of intracellular MTX accumulation and the overall exposure to MTX are comparable in these cell lines, although the individual exposure to the different metabolites differs.

On the other hand, RBC MTX kinetics differ considerably from the kinetics observed in WBCs. Accumulation of MTX in WBCs seems to occur much faster than in RBCs and the formation of the polyglutamated metabolites also seems to be increased as C_{ss} for MTXGlu₁ was much lower in all four WBC cell lines while C_{ss} for MTXGlu₂₋₇ was much higher for three of the four cell lines compared with the RBC results. For the fourth parameter set Panetta *et al.* published for T-lymphocytes [10] C_{ss} of the metabolites was of similar magnitude to the predicted value based on the RBC PK model. However, the WBC PK model assumes the presence of two more metabolites, MTXGlu₆ and ₇ and the combined C_{ss} for MTXGlu₂₋₅ only is therefore expected to be lower. Notably, t_{ss} is considerably shorter for the WBC model than the RBC and HBCC models. This difference is important with respect to pharmacokinetic–pharmacodynamic modelling, as a pharmacodynamic effect of MTX might still be correlated with RBC PK through a similar time course of change in concentrations even though the absolute concentrations differ between cell lines. Based on these results, RBC

MTXGlu_n kinetics seem unlikely to reflect the intracellular MTX kinetics in those cell types that are believed to be directly involved in the mechanism of action of MTX and in the pathogenesis of RA, namely WBCs. This is in accordance with findings of van Haandel *et al.* [11] who recently measured MTXGlu_n concentrations in peripheral blood mononuclear cells (PBMC) as a potential marker for MTX treatment in juvenile idiopathic arthritis. PBMC cells play an important role in the immune response and autoimmune diseases, including RA. The intracellular MTXGlu_n profiles the authors obtained in PBMC cells differed from the profiles seen in RBCs. Any suggested correlation between RBC MTXGlu_n concentrations and clinical outcomes of MTX therapy are therefore probably not directly attributable to similarities in the MTXGlu_n kinetics in RBCs compared with those cell types that are presumed to be involved in the autoimmune pathology of RA. However, the mechanism of action of MTX in RA is not fully understood. Other cell lines might be involved, for example as effector cells in the disease process, which could have a similar time course of accumulation that RBCs and HBCCs have shown in this analysis.

Furthermore, due to the long lifespan of RBCs and the intracellular accumulation of MTXGlu_n over time, RBC MTXGlu_n concentrations potentially provide a measure of cumulative exposure to MTX, similar to measuring the area under the curve of a plasma concentration–time curve. Based on this, RBC MTXGlu_n concentrations might have a predictable relationship with clinical outcomes of MTX treatment. A population pharmacokinetic–pharmacodynamic modelling approach could prove useful in future studies to assess whether such a predictable relationship exists and whether RBC MTXGlu_n concentrations are suitable as markers for predicting clinical outcomes of low dose MTX treatment in RA and other inflammatory autoimmune diseases. In addition, intracellular MTXGlu_n concentrations in cell lines located on the pathogenic pathway of RA, such as WBCs, should be considered as these might ultimately provide better markers of clinical response.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare JK had support from the University of Otago for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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REFERENCES

- 1 Angelis-Stoforidis P, Vajda F, Christophidis N. Methotrexate polyglutamate levels in circulating erythrocytes and polymorphs correlate with clinical efficacy in rheumatoid arthritis. *Clin Exp Rheumatol* 1999; 17: 313–20.
- 2 Allegra C, Chabner B, Drake J, Lutz R, Rodbard D, Jolivet J. Enhanced inhibition of thymidylate synthase by methotrexate polyglutamates. *J Biol Chem* 1985; 260: 9720–6.
- 3 Chabner B, Allegra C, Curt G, Clendeninn N, Baram J, Koizumi S, Drake J, Jolivet J. Polyglutamation of methotrexate. Is methotrexate a prodrug? *J Clin Invest* 1985; 76: 907–12.
- 4 Dervieux T, Furst D, Lein D, Capps R, Smith K, Walsh M, Kremer J. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. *Arthritis Rheum* 2004; 50: 2766–74.
- 5 Greer J, Foerster J, Lukens J, Rodgers G, Paraskevas F, Glader B, eds. *Wintrobe's Clinical Hematology*, 11th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2004.
- 6 Korell J, Stamp L, Barclay M, Dalrymple J, Drake J, Zhang M, Duffull S. A population pharmacokinetic model for low-dose methotrexate and its polyglutamated metabolites in red blood cells. *Clin Pharmacokinet* 2013; 52: 475–85.
- 7 Hoekstra M, Haagsma C, Neef C, Proost J, Knuif A, van de Laar M. Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis. *J Rheumatol* 2004; 31: 645–8.
- 8 Herman R, Veng Pedersen P, Hoffman J, Koehnke R, Furst D. Pharmacokinetics of low dose methotrexate in rheumatoid arthritis patients. *J Pharm Sci* 1989; 78: 165–71.
- 9 Morrison P, Allegra C. The kinetics of methotrexate polyglutamation in human breast cancer cells. *Arch Biochem Biophys* 1987; 254: 597–610.
- 10 Panetta J, Yanishevski Y, Pui C, Sandlund J, Rubnitz J, Rivera G, Ribeiro R, Evans W, Relling M. A mathematical model of *in vivo* methotrexate accumulation in acute lymphoblastic leukemia. *Cancer Chemother Pharmacol* 2002; 50: 419–28.
- 11 van Haandel L, Leeder J, Becker M. Measurement of methotrexate metabolites in peripheral blood mononuclear cells in juvenile idiopathic arthritis: a more relevant cellular biomarker for drug response? *Arthritis Rheum* 2011; 63: S95.