Methotrexate bioavailability after oral and subcutaneous administration in children with juvenile idiopathic arthritis

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Abstract

Objective

To compare the bioavailability of oral and subcutaneous methotrexate (MTX) in children with juvenile idiopathic arthritis (JIA).

Methods

Seventeen JIA patients were administered oral (6.1–22.5 mg/m^2) or subcutaneous (8.8–28.6 mg/m^2) MTX. Blood samples were drawn pre-dose, and at 1, 2, and 4 hours after administration. Plasma MTX was determined by high-performance liquid chromatography. Non-compartmental pharmacokinetic analysis included the maximum concentration of plasma MTX (C_{max}) and the area under the plasma concentration-time curve in the interval of 0–4h (AUC_{0-4h}).

Results

The slopes of the regression lines of the dose-corrected parameters C_{max} and AUC_{0-4h} plotted against the dose were negative for oral administration indicating non-linearity in pharmacokinetics, while they did not differ from zero for subcutaneous MTX. In two groups dosed orally with ≤10 or >10 mg/m^2 (the average doses: 7.8 vs. 13.8 mg/m^2, $p<0.002$), the C_{max} and AUC_{0-4h} were comparable ($p\geq0.32$). In four patients switched from oral to subcutaneous administration of the same dose, the bioavailability of oral MTX tended to be 11–15% lower when compared to subcutaneous route.

Conclusion

The differences in the pharmacokinetic measures of early systemic exposure between oral and subcutaneous routes support the view that lower and saturable intestinal absorption of oral MTX limits its bioavailability and efficacy within the range of standard doses used to treat children with JIA. In light of this evidence it can be recommended to use parenteral route of administration when MTX dose around and above 10–15 mg/m^2 is needed to achieve sufficient response.

Key words

Methotrexate, juvenile idiopathic arthritis, bioavailability, oral, subcutaneous.

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Introduction

Over the last two decades, low-dose weekly methotrexate (MTX) has been commonly used as a second-line treatment of juvenile idiopathic arthritis (JIA) (1). Its efficacy and safety in children and adolescents with JIA have been documented in multiple clinical trials (2, 3). The therapeutic dose of MTX is highly individual and often needs to be titrated according to clinical effect. It usually ranges between 7.5–15 mg/m^2/week (4).

MTX shares mechanisms for active, carrier-mediated, saturable intestinal absorption with folic acid and reduced folates. Numerous studies have described the pharmacokinetics of MTX in the dose range of 5–40 mg administered to adult patients with rheumatoid arthritis (5-12). Considerable inter-individual variability in the rate and extent of absorption and reduced bioavailability of MTX after higher doses (≥25 mg) may limit clinical use of oral MTX and justify switch to parenteral routes of administration (subcutaneous or intramuscular) in patients with inadequate clinical response. In contrary to adults, only few studies have examined pharmacokinetics of oral MTX in children with JIA (13-16). In general, higher MTX doses per weight or body surface unit have been used in children when compared to adults. To our knowledge no pharmacokinetic study directly comparing oral and subcutaneous administrations in children with JIA has been published.

In the present study we aimed to evaluate the pharmacokinetics of MTX after oral and intramuscular administration to children with JIA presenting for scheduled visits to an outpatient department. MTX in plasma collected over 4 h after administration was assayed using a thoroughly validated high-performance liquid chromatography method and bioavailability of oral and subcutaneous MTX was compared using pharmacokinetic measures of early systemic exposure, *i.e.* the maximum concentration of plasma MTX (C_{max}) and the area under the concentration-time curve of plasma MTX in the interval of 0-4h (AUC_{0-4h}).

Materials and methods

Patients

Study patients were recruited from the paediatric rheumatology out-patient clinic population of the Department of Paediatrics and Adolescent Medicine, 1st Medical School, Charles University in Prague. The study was approved by the Local Research Ethics Committee and informed consent was obtained from the patients and/or their legal guardians according to the Declaration of Helsinki (Fifth revision, 2000, Edinburgh, Scotland). Seventeen patients qualified for study entry. All JIA patients met proposed ILAR (International League of Associations for Rheumatology) criteria (17,18) with the following onset subtype distribution: oligoarthritis (extended n=5, persistent n=6), polyarthritis RF-negative (n=3), systemic-onset arthritis (n=1), psoriatic arthritis (n=2). Patients were prospectively followed in rheumatology clinic according to the usual practice with one to three-monthly visits. Routinely recorded parameters included the core set outcome measures to assess treatment efficacy (19).

The pharmacokinetics of MTX was investigated on a single occasion in 12 patients: 9 receiving oral and 3 subcutaneous MTX. Moreover, five patients with persistent disease activity on oral therapy were switched to subcutaneous MTX and pharmacokinetics were examined twice. In 4 out of 5 patients, the same MTX dose was administered on both occasions while a higher subcutaneous dose was used in one. Therefore, 22 sets of plasma concentrations were obtained. Patients had been on a stable MTX dose for at least 6-8 weeks prior to the study entry. The same dose of MTX was administered in the morning after overnight fasting and was followed by blood sampling.

Apart from MTX all patients received folic acid supplementation once-weekly in dose ranging from 5–10 mg/week 24–48 hours after the MTX dose and some of them took non-steroidal anti-inflammatory drug, usually ibuprofen (20–30 mg/kg/day). Other concomitant medication (prednisone in three subjects, hydrochloroquine, sulphasalazine) remained unchanged throughout the study.

Plasma concentration of MTX

Children with JIA were presenting for scheduled visits to an outpatient department. Blood sampling was terminated at 4 h after administration with the aim to reduce the total sampling time and to limit uncomfortable procedures. Venous blood samples for the determination of MTX plasma concentrations were collected into standard EDTA tubes before the administration of the weekly MTX dose and at 1, 2, and 4 h thereafter using an indwelling catheter. All plasma samples were centrifuged (10 min, 4°C) within 1 hour after sampling and stored for no longer than 1 month at -20°C until analysis. Plasma MTX was determined by a standard high-performance liquid chromatography (HPLC) method using fluorometric detection after post-column derivatization in a photochemical reactor as described previously (20). Methotrexate calibration standard was kindly provided by Ebewe Arzneimittel (Unterach, Austria). All other chemicals were analytical-reagent grade or best available purity from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA). Analysis of 11 sets of spiked quality control samples at two concentrations (100 and 500 nmol/L) resulted in the percentage coefficients of variations less than 11% and relative errors 7.1 and -2.4%. In the course of the study, six quality control samples from the United Kingdom National External Quality Assessment Scheme for methotrexate in serum were assayed with the mean error and mean absolute error of -3.3% and 5.3%, respectively.

Pharmacokinetic analysis

Due to the total sampling period limited to 4 h, pharmacokinetic measures of early systemic exposure were calculated. The maximum observed concentration of plasma MTX (C_{max}) and the time to maximum concentration (T_{max}) were determined directly from the observed concentrations. The area under the plasma concentration-time curve in the interval of 0-4h (AUC_{0-4h}) was calculated by the linear trapezoidal method.

Statistical analysis

Selection of statistical tests was performed after visual inspection of data and evaluation of assumptions about distribution of variables. Descriptive statistical analysis of baseline characteristics and pharmacokinetic variables was done using the arithmetic mean, standard deviation and range. The relationship between the dose and dose-corrected pharmacokinetic characteristics was evaluated using linear regression analysis. Differences in pharmacokinetic characteristics between patients dosed ≤10 mg/m^2 orally and those with a higher dose were examined by unpaired *t*-test. To evaluate the relative bioavailability of MTX in four patients switched from oral to subcutaneous administration of the same dose, the geometric mean ratios (90% confidence intervals) of pharmacokinetic characteristics of AUC_{0-4h} and C_{max} were calculated. Analyses were carried out using Statistica 7.0 package (Statsoft, Inc., Tulsa, USA).

Results

Characteristics of patients are shown in Table I. Two children with JIA had inactive disease according to the published definition (no active arthritis; no fever, no rash, serositis, splenomegaly, or generalised lymphadenopathy attributable to JIA; no active uveitis; normal ESR or CRP level; and a physician's global assessment of disease activity indicating clinical disease quiescence) (21). Remaining patients had active disease and majority of them required the switch to parenteral route of administration. At the time of sampling, the mean (range) of doses was 10.8 (6.1–22.5) mg/m^2 (oral MTX) and 15.6 (8.8 to 28.6) mg/m^2 (subcutaneous MTX). There was no relationship between the duration of therapy and the actual dose of the drug (Spearman`s coefficient of correlation 0.38, p=0.14).

Absorption of MTX was rapid. The highest plasma concentration was observed at 1 h in 12 patients after oral and in 7 patients after subcutaneous dosing, respectively, and at 2 h in remaining subjects. After oral and subcutaneous administration, the mean (range) C_{max} of plasma MTX achieved 0.775 (0.436–1.42) mmol/L and 1.93 (0.600–4.55) mmol/L, respectively. The scatter plots of the C_{max} of plasma MTX against dose are shown in Figure 1A. In order to evaluate dose-proportionality of the C_{max}, the dose-normalised values were plotted against dose and linear regression analysis was performed separately for oral and subcutaneous route. The slope of the regression line did not differ significantly from zero for subcutaneous MTX (0.0018, 95% CI: -0.0070 to 0.011) while it was negative for oral MTX (-0.0045, 95-% CI: -0.0074 to -0.0015) (Fig. 1B). The mean (range) values of the truncated area under the curve AUC_{0-4h} were 1.83 (1.27–2.76) h.mmol/L and 4.00 (1.69–7.37) h.mmol/L for oral and subcutaneous MTX, respectively. The scatter plots of the AUC_{0-4h} *vs*. dose are shown in Figure 2A. On the dose-normalised AUC_{0-4h} *vs*. dose plots, the slope of the regression line for subcutaneous route was 0.0024 (95-% CI: -0.0094 to 0.014) and did not differ from zero. The regression line had negative slope for oral MTX (-0.011, 95-% CI: -0.016 to -0.0064) (Fig. 2B).

Non-linearity in pharmacokinetics of orally administered MTX was further examined by separating the patients into two dosing groups (≤10 mg/m^2 and >10 mg/m^2). Results of between-group comparison of pharmacokinetic characteristics are given in Table II. The average dose differed almost two-fold but the mean values for AUC_{0-4h} and C_{max} were comparable. The mean (range) duration of MTX therapy was similar in the two groups: 1.9 (0.2–7.4) years *vs*. 1.9 (0.2–3.5) years, p=0.98.

Figure 3 shows the concentration-time profiles of plasma MTX in four patients

Table I. Patient characteristics.

N.	17
Boys/girls	9/8
Age (yr)	8.6 ± 4.2
BSA (m^2)	1.09 ± 0.37
Disease duration (yr)	2.23 ± 2.0
MTX therapy duration (yr)	1.97 ± 1.92
C-reactive protein (mg/L)	17.1 ± 18.5
ESR (mm/h)	26.3 ± 15.9
No. of active joints	4.2 ± 5.7
No. of joints with limited motion	4.5 ± 6.3
Active disease/inactive	15/2

Data are expressed as arithmetic mean ± standard deviation.
BSA: body surface area; ESR: erythrocyte sedimentation rate.

switched from oral to subcutaneous administration of the same MTX dose ranging 8.8 - 14.5 mg/m^2. The point (90-% CI) estimates for the percent ratio (oral to subcutaneous administration) of the pharmacokinetic characteristics C_{max} and AUC_{0-4h} achieved 89 % (52–153) and 85 % (60–119), respectively.

Discussion

In the present study we have for the first time shown the non-linear pharmacokinetics of oral MTX administered to children with JIA in the dose range of 6.1–22.5 mg/m^2 (0.19–0.94 mg/kg). This non-linearity was detected by linear regression analysis as negative slopes of the relationships between the dose-corrected pharmacokinetic measures of early systemic exposure (AUC_{0-4h} and C_{max}) and the dose. In the case of linear pharmacokinetics, the dose-corrected characteristics (*i.e.* their values divided by the dose) would remain constant across the entire range of doses. Moreover, the mean values of non-corrected characteristics AUC_{0-4h} and C_{max} were quite comparable in children separated into two dosing groups (≤10 and >10 mg/m^2 orally) despite almost two-fold difference in the mean oral dose. In patients who were administered the same dose orally and subcutaneously, the AUC_{0-4h} and C_{max} after oral MTX showed a trend towards 11–15% lower values.

Non-linearity of the relationship between the oral dose and the pharmacokinetic characteristics of plasma MTX observed in this study can most probably be ascribed to reduced bioavailability of MTX due to its decreased intestinal absorption after higher oral doses. Bioavailability reflects the rate and extent (amount) to which a drug reaches the general circulation. It is measured by comparing pharmacokinetic characteristics after extravascular and intravenous administration (absolute bioavailability) or two extravascular administrations (relative bioavailability), *e.g.* oral and subcutaneous. Other processes like MTX metabolism, storage in cells and excretion influence the concentration-time profiles. However, their contribution to the non-linearity of pharmacokinetics of oral

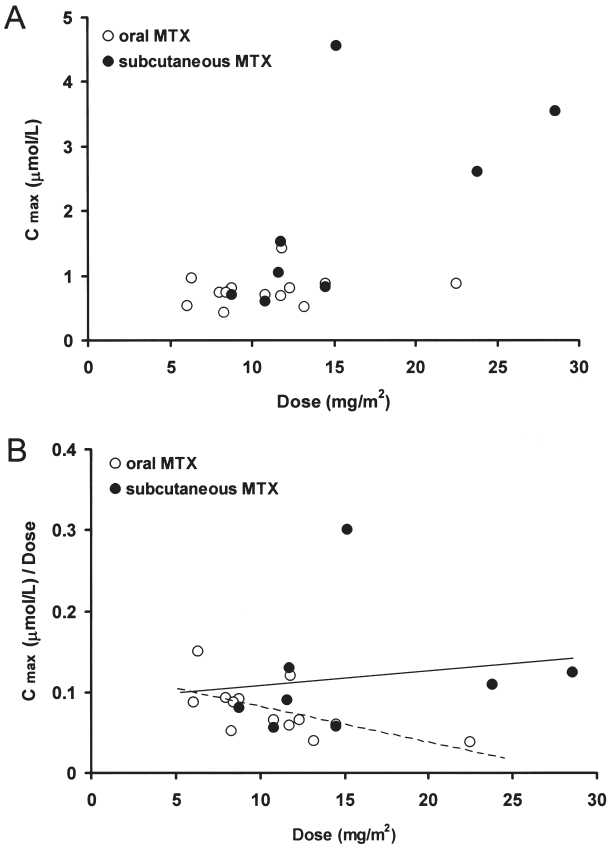

Fig. 1. The relationship between MTX dose administered orally and subcutaneously and the maximum observed concentration of plasma MTX (**A**) and the dose-normalised maximum concentration of plasma MTX (**B**). The results of linear regression analysis are indicated by broken (oral MTX, slope = -0.0045±0.0017, p<0.02) and solid lines (subcutaneous MTX, slope = 0.0018±0.0046, p=0.65).

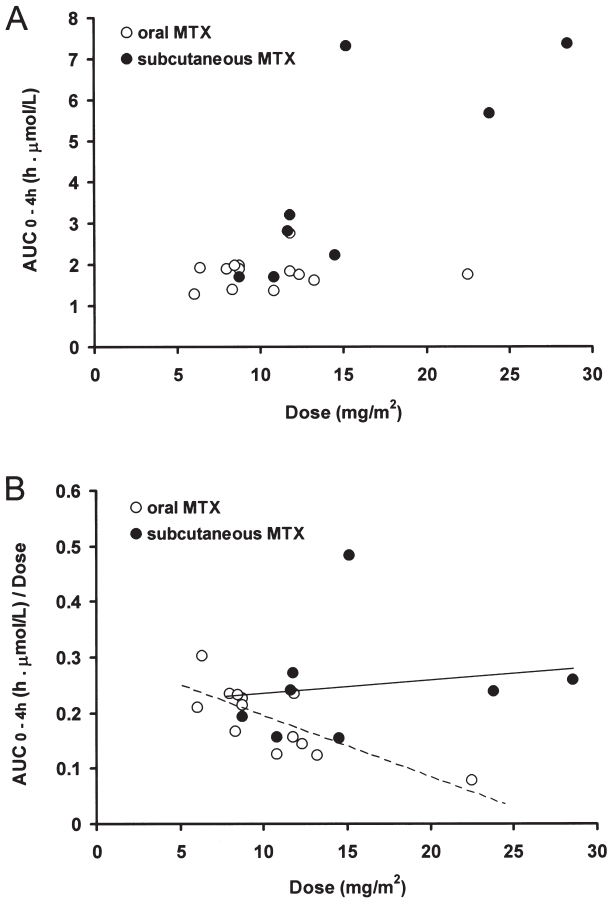

Fig. 2. The relationship between MTX dose administered orally and subcutaneously and the area under the curve of MTX plasma concentrations in the interval 0–4 h (**A**) and the dose-normalised area under the curve of MTX plasma concentrations in the interval 0–4 h (**B**). The results of linear regression analysis are indicated by broken (oral MTX, slope = -0.011±0.0026, p<0.001) and solid lines (subcutaneous MTX, slope = 0.0024±0.0061, p=0.42).

Table II. Pharmacokinetic characteristics of plasma MTX in patients with different oral doses.

	Dose ≤10 mg/m^2	Dose >10 mg/m^2	*p*-value
N.	7	7	–
Dose (mg/m^2)	7.81 (1.13)	13.8 (4.00)	<0.005
C_{max} (μmol/L)	0.711 (0.173)	0.840 (0.283)	0.32
AUC_{0-4h} (h.μmol/L)	1.75 (0.296)	1.90 (0.459)	0.48

Data are expressed as arithmetic mean (standard deviation).
AUC_{0-4h}: the area under the plasma concentration-time curve in the interval of 0-4 hours;
C_{max}: the maximum observed concentration of plasma MTX.

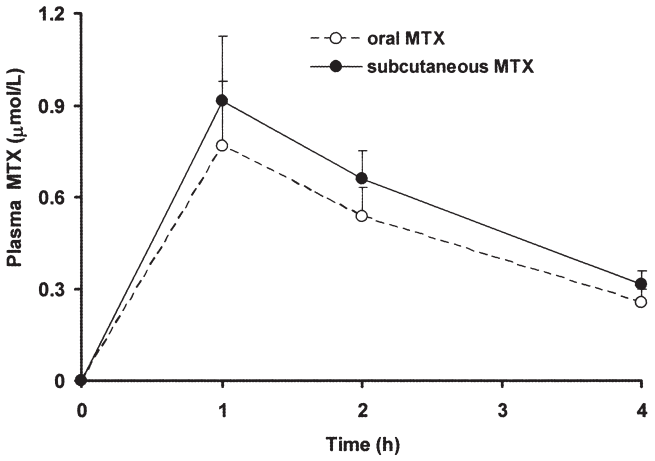

Fig. 3. Mean (SEM) concentration *vs*. time profiles for plasma MTX in four patients after the same MTX dose administered orally and subcutaneously.

MTX seems to be unlikely because this would occur also after subcutaneous dosing. In children with acute lymphoblastic leukemia (ALL), bioavailability of MTX was comparable after subcutaneous and intravenous dosing of 40 mg/m^2 (22). Duration of pharmacotherapy most probably exerted no effect on the dose-concentration relationship. According to the results of studies performed over intermediate (six months, (23)) and long-term periods (two years, (24)), pharmacokinetics of oral MTX did not change significantly with the duration of therapy. Moreover, there was no relationship between the dose of MTX at the time of investigation and duration of pharmacotherapy in our study.

In adult patients with RA, the mean absolute bioavailability of oral MTX equals 0.7 to 0.8 and no significant changes in absorbed fraction occur with the dose between 7.5 and 25 mg. However, significant inter-patient variation ranging from 0.3 to 0.9 was described (7, 9). The rate of MTX absorption decreased with the increasing oral dose as well as after meal, causing reduced C_{max} and prolonged T_{max} values (11). Jundt *et al*. reported 15% lower bioavailability of oral MTX when compared to intramuscular and subcutaneous administrations in 12 patients with RA taking 5-20 mg of MTX weekly (25). Hoekstra *et al*. studied 15 patients with RA receiving weekly MTX doses of 25–40 mg. At this higher dosage, the AUC was 64–76 % of that after subcutaneous administration (10). Hamilton *et al*. compared bioavailability of oral and intramuscular MTX after administration of a starting dose of 7.5 mg and of a usual maintenance dose (mean 17 mg) to patients with RA. Whereas similar results were obtained after the lower dose, significantly increased AUC was observed when maintenance dose of MTX was given intramuscularly (26).

Results of clinical studies in children with acute lymphoblastic leukemia (ALL) also agree with our findings (22, 27-28). Balis *et al*. described non-linearity of pharmacokinetics of oral MTX used for the maintenance therapy of ALL (22). The authors combined their results with those of several published reports and demonstrated different effects of increasing dose on MTX bioavailability after subcutaneous and oral administration. They observed complete absorption of subcutaneous MTX at doses 7.5 mg/m^2 twice a week and 40 mg/m^2 weekly, whereas absorption of the 40 mg dose given orally was only one third (22). In a large study by a joint children`s cancer group and paediatric oncology branch, the AUC of MTX was monitored in 89 ALL children after 191 oral doses ranging from 2.1 to 36 mg/m^2. The inter-patient variability of the AUC augmented with an increasing dose while its mean value reached a plateau above 15 mg/m^2 (27). Teresi *et al*. also reported decreasing bioavailability of higher doses (13–120 mg/m^2) of oral MTX (28).

According to current guidelines, bioavailability of drugs like MTX administered in immediate-release formulations can generally be evaluated by measurements of the C_{max} and T_{max} as characteristics of the rate of bioavailability and the area under the concetration-time curve from zero to infinity (total AUC) as a measure of the extent of bioavailability (29). In the present study, the limited duration of blood sampling did not allow the total AUC to be estimated. However, the use of the truncated area AUC_{0-t} (the AUC_{0-4h} in this study) is a validated ancillary procedure when the AUC cannot be reliably evaluated such as in bioavailability and bioequivalence trials involving extended-release formulations, endogenous substances, poorly absorbed drugs and drugs possessing very long elimination half-lives (30).

The active polyglutamate metabolites of MTX slowly accumulate in cells and can be assayed in erythrocytes. However, they are less suitable as indicators of MTX bioavailability because their steady state concentrations are attained after several months of unchanged dosing and may be influenced by patient's compliance (31).

Possible limitations of our study: Results may be influenced by a low number of patients treated with higher oral doses of MTX. This situation was caused by clinician's intention to treat in patients who required higher MTX dose and who received it parenterally

from the beginning. This short-term pharmacokinetic study was designed neither to follow the changes in disease activity nor to study the concentration-effect relationship. Several clinical trials in patients with JIA and RA have shown better clinical efficacy of parenteral MTX compared to oral dosing, especially at higher doses (32-34).

Conclusion

Although limited in size, our study is, to our best knowledge, the first one comparing bioavailability of oral *vs*. subcutaneous MTX at standard effective dosage administered to children with JIA. The differences in the pharmacokinetic measures of early systemic exposure between oral and subcutaneous routes observed in this study support the view that lower and saturable intestinal absorption of oral MTX limits its bioavailability and efficacy. General clinical guidelines for MTX therapy in JIA recommend parenteral route of administration when the dose around and above 10–15 mg/m^2 is needed (35, 36). By showing linear pharmacokinetics of parenteral against oral MTX administration our results bring important evidence to support this approach. Such a theoretical background may help to further facilitate effective use of this potent antirheumatic drug and overcome discomfort and higher costs associated with its parenteral administration.

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