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Bioavailability of oral vs. subcutaneous low-dose methotrexate in patients with Crohn's disease

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SUMMARY

Background: Oral methotrexate and folic acid are partly absorbed by a common intestinal transporter.

Aim: To determine the relative bioavailability of oral low-dose methotrexate administered with and without concomitant folic acid vs. subcutaneous administration in patients with stable Crohn's disease.

Methods: Ten patients were randomized to receive their regular maintenance dose of methotrexate (15–25 mg) for three consecutive weeks: orally, orally with 5 mg folic acid or subcutaneously. Blood samples were drawn at specified intervals during 24 h, and methotrexate levels were determined by fluorescence immunoassay. Areas under the curve extrapolated to infinity (AUC_{∞}) were compared between the three routes.

Results: The geometric mean AUC_{∞} values (95% confidence intervals) were 360 nmol.h/L (301–430 nmol.h/L), 261 nmol.h/L (214–318 nmol.h/L) and 281 nmol.h/L (209–377 nmol.h/L) per milligram of methotrexate administered for subcutaneous, oral and oral with folic acid administration, respectively ($P < 0.05$ and $P < 0.01$ for oral with folic acid and oral vs. subcutaneous administration, respectively). The geometric mean relative bioavailabilities (95% confidence intervals) were 0.73 (0.62–0.86) and 0.77 (0.60–0.99) for oral and oral with folic acid administration, respectively (difference not significant).

Conclusions: In patients with stable Crohn's disease, the oral bioavailability of methotrexate is highly variable and averages 73% of that of subcutaneous administration. Concomitant folic acid has no significant effect on the bioavailability. Dose adjustments based on individual pharmacokinetic assessment should be considered when switching patients from parenteral to oral therapy.

INTRODUCTION

Since the first report of its use in inflammatory bowel disease,[1] a number of controlled[2, 3] and uncontrolled[4–6] studies (recently summarized in a literature review[7]) have shown parenteral low-dose methotrexate (15–25 mg once weekly) to be effective in inducing and maintaining remission in patients suffering from steroid-dependent or steroid-refractory Crohn's disease. However, there is conflicting evidence on the efficacy of the convenient oral administration route,[8, 9] precluding a consensus on whether, how or when to recommend the oral administration of methotrexate in clinical practice. The discrepancy in efficacy between oral and parenteral administration may reflect the incomplete and variable oral bioavailability of methotrexate. In patient populations without intestinal disease, low-dose methotrexate has a mean oral bioavailability of 67–106%.[10–12] To date, no methotrexate bioavailability study has been performed in patients with Crohn's disease.

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In patients with rheumatoid arthritis, daily folate supplements reduce the toxicity of weekly low-dose methotrexate therapy[13] and, accordingly, have been recommended in methotrexate-treated patients with inflammatory bowel disease.[14] Folic acid and methotrexate are structurally similar, and the absorption of both substances is at least partly mediated by a common membrane transporter in the proximal intestinal mucosa.[15] Thus, folic acid could theoretically compromise methotrexate absorption by competitive inhibition of these intestinal carriers.

We therefore designed a randomized, open-label, cross-over study to determine the oral bioavailability of low-dose methotrexate (12.5–25 mg) relative to subcutaneous administration in patients with Crohn's disease, and the influence of simultaneous folic acid administration on the absorption of methotrexate.

METHODS

Patients

Patients were recruited from the referral-based gastroenterology out-patient clinic of a university-affiliated teaching hospital (Chaim Sheba Medical Center, Tel Hashomer, Israel) during the period October 2001 to June 2002. Patients were eligible if they suffered from Crohn's disease (based on clinical and endoscopic, histological or radiographic findings) and had been treated with chronic low-dose methotrexate (at constant doses of 10–25 mg once weekly, orally or subcutaneously, for at least 3 months) and attained significant improvement or remission. Patients were excluded if the disease activity was unstable during the month preceding the study, as assessed by changes in the Crohn's disease activity index of more than 70 points,[16] or if they had taken antibiotics, non-steroidal anti-inflammatory drugs or any other intermittent medication during the 2 weeks preceding the study or during the study period itself. The study protocol was approved by the local institutional review board, and all subjects gave written informed consent.

Study design

Patients received their regular once-weekly doses of methotrexate according to three administration schedules over three consecutive weeks in a randomized, three-way, cross-over block design. In schedule A, methotrexate was administered by subcutaneous injection (Amp. Abitrexate 50 mg/2 mL, Pharmachemie, Teva Group, Netanya, Israel). In schedule B, methotrexate was administered as oral tablets (Tab. Methotrexate 2.5 mg, Lederle, Wolfratshausen, Germany) after discontinuing oral folic acid for 24 h. In schedule C, methotrexate was administered as oral tablets simultaneously with a tablet of folic acid (5 mg, Rekah, Azor, Israel). On each treatment day, methotrexate was administered in the morning (08.00–09.00 h) after an overnight fast, and food was allowed only 2 h after drug administration. Other chronic concomitant medication (steroids, infliximab) was continued unchanged throughout the study. All patients continued to receive chronic daily folic acid supplements (5 mg/day), except on the day of administration of schedule B.

Data collection

On each treatment day, blood samples for the determination of methotrexate serum levels were drawn through an indwelling intravenous catheter immediately before and at pre-defined time intervals (15, 30, 45, 60, 90, 120, 150 min and 4, 6, 8 and 24 h) after the administration of methotrexate. Demographic data and details on the type, duration, extent (endoscopic/radiological) and activity of disease (as assessed by the Crohn's disease activity index) were collected from patient files and a structured interview on the days of treatment. Body weight was measured, and routine blood tests (blood count, biochemistry and serum folate levels) were performed on each treatment day.

Measurements

Blood samples were centrifuged and the serum was stored at − 20 °C until assay, which was performed in batches within 4 weeks of collection. Methotrexate concentrations were measured using a fluorescence polarization immunoassay technique (Abbott TDx Methotrexate Reagent Kit, Abbott Diagnostics, North Chicago, IL, USA).[17] The lowest limit of detection reported by the manufacturer was 0.01 μmol/L, and the inter- and intra-day coefficients of variation were 4% and 5%, respectively. For each patient, samples from the three treatment schedules were analysed in the same batch.

Data analysis

Methotrexate concentration–time profiles were analysed using the WinNonlin software package (Pharsight, Mountain View, CA, USA). Non-compartmental analysis was used to estimate the area under the concentration–time curve extrapolated to infinity (AUC_∞) (based on the trapezoidal rule and the terminal phase elimination constant λ_z).[18] The relative bioavailabilities F_o (oral vs. subcutaneous administration) and F_{o+f} (oral + folic acid vs. subcutaneous administration) were defined as the ratio of the respective AUC_∞ value to the subcutaneous AUC_∞ value. For subcutaneous administration, the relative clearance (Cl/F) was obtained by dividing the dose by the AUC_∞ value, and the relative terminal phase volume of distribution (V_z/F) was obtained by dividing the dose by the AUC_∞ value and λ_z.[18] The maximal concentration c_{max} and time to the maximal concentration t_{max} were determined directly from the observed data. Creatinine clearance was calculated according to the Cockcroft–Gault formula.[19]

Statistical analysis

Data are summarized and presented as the geometric mean (for c_{max} and AUC) or arithmetic mean (for all other data) and 95% confidence intervals (95% CI). Pharmacokinetic data (logarithmically transformed and dose normalized for AUC and c_{max}) were compared between the three treatment schedules by one-way repeated measures analysis of variance (ANOVA), followed by a *post hoc* Fisher's least significant difference (LSD) analysis of multiple comparisons. The bioavailabilities for the oral and oral + folic acid schedules were compared by the paired t-test. All tests were two-tailed, and $P < 0.05$ was considered to be significant. All calculations were performed using the GB-STAT statistical software package (Dynamic Microsystems Inc., Silver Spring, MD, USA).

Sample size calculation

Defining a difference of 25% between oral and subcutaneous bioavailability as clinically significant (i.e. requiring the addition of at least one methotrexate tablet of 2.5 mg when changing from parenteral to oral administration at the usual maintenance doses), and assuming a standard deviation of 20% for bioavailability, a sample size of 10 patients was calculated to provide a power of 80% ($1 - \beta$) at the usual level of statistical significance ($\alpha = 0.05$).

Table 1. Demographic data and details of the history, treatment and activity of Crohn's disease

Patient	Age (years)	Gender	Weight (kg)	Methotrexate dose (mg)	Year of diagnosis	Extent of disease	Previous intestinal resection	Concomitant medication	Mean CDAI
1	49	Female	52	12.5	1976	Ileum	Terminal ileum (70 cm)	Budesonide (9 mg/day)	207
2	32	Female	89	25	1991	Ileum	—	Prednisone (20 mg/day), infliximab	27
3	62	Male	75	17.5	1982	Colon	—	—	157
4	47	Female	52	15	1998	Colon	—	—	129
5	49	Male	94	12.5	1989	Colon	Partial colectomy (50 cm)	Infliximab	147
6	54	Female	63	12.5	1967	Terminal ileum	Terminal ileum (60 cm)	Prednisone (15 mg/day), infliximab	150
7	46	Male	52	12.5	1981	Terminal ileum	—	—	320
8	33	Male	87	25	1990	Colon	—	Infliximab	35
9	27	Male	100	25	1999	Terminal ileum	—	—	63
10	27	Female	64	20	1992	Ileum, jejunum and colon	Terminal ileum (40 cm)	—	53

Mean CDAI, average Crohn's disease activity index over the study period.

RESULTS

Ten subjects were enrolled. One patient did not complete the study for personal reasons, and the data on treatment schedule C are therefore incomplete. Demographic data and information on the extent and activity of Crohn's disease are presented in Table 1. The mean methotrexate dose was 17.8 mg (95% CI, 13.8–21.7 mg; range, 12.5–25 mg). The mean creatinine clearance was 90.3 mL/min (95% CI, 69.8–110.8 mL/min; range, 65–142 mL/min) and the mean folate level was 11.9 ng/dL (95% CI, 8.3–15.5 ng/dL; range, 3.1–17.6 ng/dL). One patient with low baseline folate levels at the beginning of the study admitted low compliance to folate supplements, but his folate levels normalized during the study with regular folic acid administration. The disease activity, as assessed by the Crohn's disease activity index, did not change significantly (> 70) during the study period in any of the patients. Concomitant medication was continued unchanged throughout the study. None of the four patients treated with infliximab (administered at a dose of 5 mg/kg every 2 months) received the drug during the 3-week study period. No adverse effects of methotrexate were recorded.

The results of the pharmacokinetic analyses are presented in Table 2. Oral administration, both with and without folic acid, resulted in significantly lower $AUC_{0-\infty}$ values when compared with the subcutaneous route ($P < 0.05$ and $P < 0.01$, respectively). The mean relative bioavailability was similar for the oral administration schedules, both without and with folic acid, with a non-significant trend towards a larger between-patient variability for the latter (coefficients of variation for F_o and F_{o+f} of 0.23 and 0.33, respectively) (Table 2 and Figure 1). There was a non-significant trend towards lower bioavailability in the five patients taking a higher dose (20–25 mg) when compared with the four patients taking a lower dose (12.5–15 mg) [0.62 (95% CI, 0.38–0.97) vs. 0.80 (95% CI, 0.65–1.0), respectively].

t_{max} was significantly longer in the oral regimens and longest when methotrexate was administered with folic acid [mean t_{max} (95% CI): 1.53 h (1.08–1.97 h) and 1.71 h (1.19–2.24 h) for oral administration without and with folic acid, respectively, vs. 0.91 h (0.72–1.09 h) for subcutaneous administration; $P < 0.05$ and $P < 0.01$, respectively], although the difference between the two oral regimens was not significant. The mean dose-corrected c_{max} values were similar in all the administration schedules [geometric mean (95% CI) of dose-corrected c_{max}: 50 nmol/L (41–61 nmol/L), 48 nmol/L (33–71 nmol/L) and 51 nmol/L (34–75 nmol/L) per milligram dose for subcutaneous, oral and oral + folic acid administration, respectively]. Other pharmacokinetic parameters [for subcutaneous schedule: V_z/F·kg, 0.49 L/kg (95% CI, 0.38–0.63 L/kg); Cl/F·kg, 1.45 mL/min.kg (95% CI, 1.12–1.86 mL/min.kg);

Table 2. Comparison of pharmacokinetic parameters by administration route in individual patients

	Subcutaneous	Oral		Oral + folic acid	
Patient	$AUC_{0-\infty}$ per mg Mtx (nmol.h/L)	$AUC_{0-\infty}$ per mg Mtx (nmol.h/L)	F_o	$AUC_{0-\infty}$ per mg Mtx (nmol.h/L)	F_{o+f}
1	405	297	0.73	279	0.69
2	245	232	0.94	148	0.60
3	376	281	0.75	414	1.10
4	394	255	0.65	260	0.66
5	417	390	0.94	540	1.30
6	368	366	0.99	312	0.85
7	309	232	0.75	287	0.93
8	604	293	0.49	268	0.45
9	280	153	0.55	186	0.67
10	308	200	0.65	NA	NA
Geometric mean (95% CI)	360 (301–430)	261† (214–318)	0.73† (0.62–0.86)	281* (209–377)	0.77* (0.60–0.99)

$AUC_{0-\infty}$, area under the curve from zero time to infinity; CI, confidence interval; F, relative bioavailability; Mtx, methotrexate; NA, not assessed. * $P < 0.05$, † $P < 0.01$ for comparison with subcutaneous administration using repeated measures analysis of variance (ANOVA) with Fisher's least significant difference for multiple comparisons (performed on logarithmically transformed data for *AUC*s). One patient (10) did not complete the study.

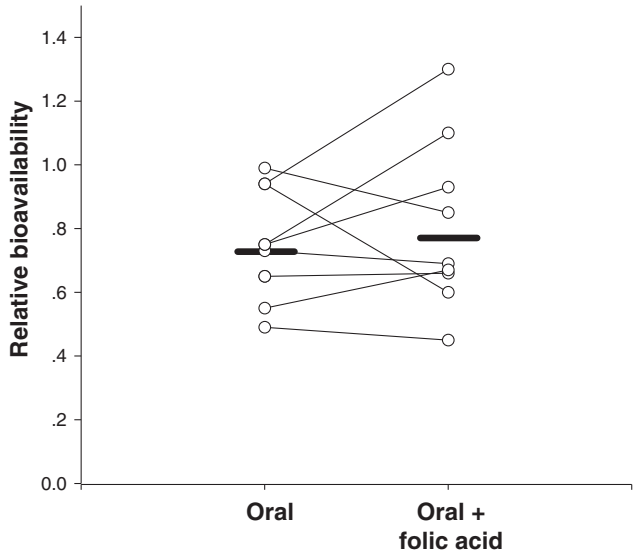

Figure 1. Relative oral methotrexate bioavailability after oral administration with and without folic acid. Data are shown for nine patients who completed both administration schedules. The bold horizontal lines represent the geometric means.

terminal phase half-life, 3.88 h (95% CI, 3.56–4.23 h)] did not differ significantly between the three schedules when corrected for *F* (data not shown), and were all within the previously published range.[20]

DISCUSSION

Our study is the first to assess the oral bioavailability of methotrexate in patients with Crohn's disease. We chose to examine the relative oral bioavailability compared with subcutaneous administration, the standard of care for parenteral low-dose methotrexate, thus addressing the need for dose adjustment when switching from parenteral to oral treatment. The relative oral bioavailability compared with subcutaneous administration for doses between 12.5 and 25 mg was 0.73 (95% CI, 0.62–0.86). As both subcutaneous and intramuscular administration have an absolute systemic bioavailability of 0.90–1.0 when compared with intravenous administration,[11, 21, 22] the calculated mean absolute oral bioavailability in our patients was 0.66–0.73.

Our results in Crohn's disease patients largely concur with those derived from patients without small bowel affection, e.g. with rheumatoid arthritis, psoriasis and asthma. In these patients, the mean relative oral bioavailability was in the range 0.93–1.06 at doses up to 10 mg[12, 23, 24] and 0.67–0.92 at doses up to 25 mg.[10–12, 25, 26] However, differences in the study protocols (e.g. methotrexate assay, dose, parenteral reference route) render direct comparisons of these studies problematic. Our study was not designed to compare methotrexate bioavailability in different patient populations and, in view of the wide between-patient variability, a larger comparative study is required to rule out meaningful differences. Only one small Israeli study has directly compared *AUC* values up to 2 h (AUC_{0-2}) after the oral administration of low-dose methotrexate (12.5 mg) in five patients with Crohn's disease, four patients with ulcerative colitis and six patients with rheumatoid arthritis.[27] As there was no parenteral reference, the bioavailability could not be assessed, but the peak concentrations and AUC_{0-2} values did not differ significantly between the patient groups. However, this study was underpowered to detect differences in *AUC* values as large as 30%, and the short observation period of 2 h (just beyond the mean time to the peak concentration) may not have adequately reflected systemic drug exposure.

An inverse relation between dose and oral bioavailability, as described previously for intermediate and high doses,[28] also seems to exist within the low-dose range (7.5–25 mg).[10–12, 23–26] We noted an overall non-significant trend towards lower bioavailability in patients taking higher (20–25 mg) rather than lower (12.5–15 mg) doses, but this study was not designed or powered to address this question.

The range (0.49–0.99) and between-patient variability (coefficient of variation, 0.23) of the relative oral bioavailability in our study were similar to those derived in other studies. In individual patients, the relative oral bioavailability of low-dose methotrexate ranges from 0.28 to 1.5, with coefficients of variation between 0.15% and 0.31%.[10–12, 23, 25] Differences in age, fasting state, dose, drug interactions (non-steroidal anti-inflammatory drugs, ciprofloxacin) and, possibly, the expression of cellular drug efflux transporters (multi-drug resistance proteins MRP1 and MRP3) may account for such inter-individual differences.[20, 29] This two- to five-fold between-patient variability precludes precise forecasts for the individual patient. In contrast, within-patient variability is comparatively low (< 20%).[30, 31] Thus, the routine assessment of individual bioavailability has been suggested for the individualization of oral methotrexate therapy. For this purpose, *AUC* values can be estimated

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