Pharmacokinetics of Subcutaneous Methotrexate

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The pharmacokinetics of subcutaneously administered methotrexate was studied as a parenteral alternative to oral administration. An initial feasibility study was performed in Rhesus monkeys comparing the subcutaneous route to intravenous (IV) injection and oral administration. The subcutaneous dose was completely absorbed and a sustained-release effect was observed when compared with the IV dose. No local or systemic toxicities resulted from subcutaneous methotrexate in the animals. Twelve children with acute lymphoblastic leukemia on maintenance therapy protocols prescribing either 7.5 mg/m^2 biweekly or 40 mg/m^2 weekly were also monitored after both a subcutaneous and an oral dose of methotrexate. Four children at the higher dosage level were also studied after an equal IV dose. The subcutaneous dose was again completely absorbed in these children at both dose levels, whereas the oral dose, which produced comparable plasma drug concentrations at the lower dosage level, resulted in a total drug exposure (area under the plasma concentration-time curve) that was one third that of the equal subcutaneous dose at the higher dosage level. No local or systemic toxicity was attributed to the subcutaneous methotrexate. Subcutaneous administration of methotrexate is well tolerated and well absorbed and appears to overcome the problems associated with oral administration, including variable absorption and saturation of the absorption mechanism with increasing doses.

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INTERMITTENT low-dose methotrexate has become standard therapy for the maintenance of remission in children with acute lymphoblastic leukemia (ALL) and is also used as an immunosuppressant in the treatment of a variety of other conditions, including psoriasis, rheumatoid arthritis, and asthma. At doses of 30 mg/m^2 and less, methotrexate is routinely administered by the oral route. However, pharmacokinetic studies have demonstrated that plasma methotrexate concentrations following oral administration are highly variable as a result of interpatient differences in the rate and extent of absorption.[1-4] In one study, peak levels occurred from 0.5 to five hours after the dose, and the fraction of the dose absorbed ranged from 0.23 to 0.97.[2] This variability in plasma drug concentration may have accounted for the higher relapse rate seen in one study in which children with ALL treated with oral methotrexate were compared with those treated intramuscularly.[5] In addition, methotrexate absorption appears to be saturable, so that as the dose is increased, the fraction absorbed declines.[6-9] Therefore, in patients with low plasma drug levels, simply increasing the dose may not overcome poor bioavailability.

The intramuscular route has been the primary alternative to oral methotrexate in leukemia studies. Intramuscular methotrexate is rapidly and completely absorbed resulting in higher serum drug concentrations than following oral methotrexate.[7,9,10] However, intramuscular injections must be administered by a health professional and may be difficult in chronically ill children with minimal muscle mass. In the present study, the subcutaneous route was evaluated as a parenteral alternative to oral administration in children with ALL. The potential advantages for this route of administration include slow release of drug resulting in more prolonged exposure to methotrexate (a critical determi-

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nant of cytotoxicity), ease of administration, and less variable, more complete absorption than that observed with oral administration. The feasibility of this approach was first studied in Rhesus monkeys and then in children with ALL at two different dose levels.

MATERIALS AND METHODS

Drug

Methotrexate was obtained from commercial sources (Lederle, Pearl River, NY). The standard intravenous (IV) preparation was used for both the IV doses and subcutaneous doses in the animals and patients, and for the oral doses in monkeys. Standard 2.5 mg tablets were used in patients studied with an oral dose.

Animals

Five adult male Rhesus monkeys (*Maccaca mulatta*) ranging in weight from 4.8 to 10.1 kg (median, 8.8 kg) were studied. The animals were housed individually and received water and food ad libitum (animals were fasted overnight before the oral dose). Each animal was treated with methotrexate at a dose of 1 mg/kg by three routes, orally, subcutaneously, and by IV bolus, with the order of administration determined randomly. In addition, three of the animals received a 60-minute infusion of 1 mg/kg of methotrexate. Animals were given a minimum of 2 weeks to recover before the next dose was administered. Blood samples were drawn from a saphenous or femoral venous catheter, contralateral to the site of injection in the case of the IV doses. The heparinized specimens were obtained before the dose and 5, 15, 30, 45, 60, and 90 minutes and 2, 3, 4, 6, 8, and 12 hours after the dose. Plasma was separated immediately by centrifugation and frozen at −20°C until assayed.

Patients

Twelve children (nine males and three females) with ALL in remission being treated on either an initial protocol (n = 6, National Cancer Institute [NCI], Children's Hospital National Medical Center) or a relapse protocol (n = 6, St Jude Children's Research Hospital) participated in this study. The patients ranged in age from 3 to 19 years (median, 8 years). Informed consent was obtained from the patients and their legal guardians before entry onto the study. All patients were in their first or second remission and receiving methotrexate as maintenance therapy on one of two schedules, either 7.5 mg/m^2 orally, twice a week (six patients) or 40 mg/m^2 orally, once a week (six patients). The six patients at the low-dose level (actual mean dose received, 6.5 mg/m^2) were studied following both their standard oral dose and an equal dose administered subcutaneously. At the high-dose level, four patients were monitored after oral, subcutaneous, and IV bolus doses, one patient was studied after only an oral dose, and the sixth patient after only a subcutaneous dose. The order of administration was determined randomly. Patients were fasted overnight before the oral dose. Complete blood counts, liver function tests, and renal function tests were routinely monitored on these patients before and 1 week after each dose and demonstrated normal bone marrow, hepatic, and renal function.

Blood samples were collected in heparinized tubes before the dose and 15, 30, 60, and 90 minutes and 2, 3, 4, 6, and 8 hours after the dose. Plasma was separated by centrifugation and frozen at −20°C until assayed.

Sample Analysis

Methotrexate was measured with the dihydrofolate reductase inhibition assay which is specific for methotrexate and has a lower limit of sensitivity of .001 μmol/L.[11]

Pharmacokinetic Calculations

Area under the plasma concentration-time curve (AUC) was derived using the linear trapezoidal rule and extrapolated to infinity using the elimination rate constant derived from nonlinear regression analysis of the data.[12] For the IV bolus doses the methotrexate concentration at time 0 used in the calculation of the AUC was the sum of the intercepts (A + B) derived from fitting the data to the biexponential equation below (using MLAB[13]):

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t}.$$

Absolute bioavailability (F) at the 40 mg/m^2 dose was calculated from the AUC using the following equation:

$$F = \frac{AUC^{PO \text{ or } SC} \cdot Dose^{IV}}{AUC^{IV} \cdot Dose^{PO \text{ or } SC}}$$

(Abbreviations: PO, oral; SC, subcutaneous)

Clearance was calculated by dividing the dose by the AUC.

RESULTS

Animal Study

The plasma disappearance curves for methotrexate administered subcutaneously, by IV bolus, and by 60-minute IV infusion are shown in Fig 1. The plasma methotrexate concentration following the subcutaneous dose peaked 15 to 30 minutes after the dose, and ranged from 1.0 to 2.3 μmol/L. The sustained-release effect from subcutaneous administration can be appreciated from the curves. The plasma methotrexate concentration remained above 0.1 μmol/L two- to three-fold longer with subcutaneous administration than with IV bolus or infusion doses. Plasma concentrations following the oral dose are not shown because the absorption of methotrexate in these animals was poor (< 2% of the dose) and was not felt to be a representative model for humans.

Table 1 lists the pharmacokinetic parameters for methotrexate administered by the various routes. The AUC for the subcutaneous dose actually exceeded that for the IV bolus dose in all five animals studied. Since the dose was identi-

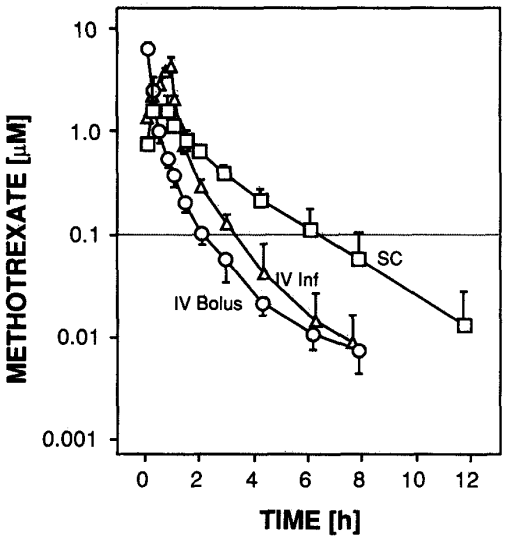

Fig 1. Plasma disappearance curves for methotrexate in Rhesus monkeys following administration of 1 mg/kg by IV bolus (○) (n = 5), 60-minute IV infusion (Inf) (△) (n = 3), and subcutaneously (□) (n = 5). Points and error bars represent the geometric mean and one SD. Plasma concentration of methotrexate following the subcutaneous dose is maintained above 0.1 μmol/L for 6.4 hours compared with 2.2 and 3.3 hours for the IV bolus and infusion doses.

cal for both routes, this difference may either be due to the more rapid clearance of the IV bolus dose or to an underestimation of the time 0 methotrexate concentration from the curve fitting. As a result of this unexpected finding, three animals received a repeat dose of methotrexate infused IV over one hour to more closely simulate the levels achieved with the subcutaneous dose. In these three animals the bioavailability of the subcutaneous dose was 102% ± 12% with the 60-minute infusion is used as the standard. There was no local or systemic toxicity associated with the subcutaneous injection of methotrexate in Rhesus monkeys.

Patient Study

Figure 2 shows the plasma concentration-time profiles for subcutaneous methotrexate compared with oral administration at the 7.5 mg/m^2 dose level (Fig 2A) and compared with IV and oral administration at the 40 mg/m^2 dose level (Fig 2B). At the lower dose level subcutaneous administration approximates the levels achieved with the oral dose. However, at the higher dose subcutaneous injection results in considerably higher plasma drug concentrations. Peak methotrexate concentration and AUC with the subcutaneous dose were four- and three-fold higher, respectively, than achieved with an identical oral dose (Table 2), and the subcutaneous dose provided exposure to ≥ 1 μmol/L concentrations of methotrexate for up to six hours compared with 2.6 hours with the oral dose. The sustained-release effect with subcutaneous administration observed in the animals was not as evident in these patients when compared with the IV dose. Subcutaneous methotrexate was rapidly absorbed with the

Table 1. Pharmacokinetic Parameters for Methotrexate Administered by Various Routes to Rhesus Monkeys

Animal	Weight (kg)	AUC (μmol/L/h)				Bioavailability of SC MTX (%)		Total Plasma Clearance† (mL/min)
		SC	IV Bolus	IV Inf	PO	IV Bolus Std*	IV Inf Std*	
681P	8.5	3.70	2.88	4.08	.089	128	91	108
687P	8.8	3.52	2.97	–	.153	119	–	107
802F	10.1	4.73	4.03	4.12	.056	117	115	92
631T	4.8	2.77	2.52	–	ND	110	–	70
677J	7.5	4.65	3.18	4.60	.044	146	101	87
Mean		3.87	3.00	4.27		124	102	93
±SD		0.83	0.48	0.28		14	12	16

Abbreviations: IV Inf, intravenous infusion; Std, standard; MTX, methotrexate; ND, not detectable.

*Bioavailability calculated by dividing AUC^{SC} by either $AUC^{IV\ bolus}$ or $AUC^{IV\ inf}$.

†Clearance of the IV bolus dose.

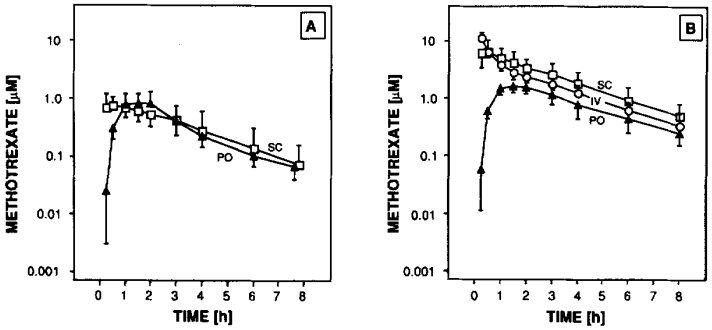

Fig 2. Plasma disappearance curves for methotrexate in children with ALL following administration of (A) 7.5 mg/m² orally (▲) and subcutaneously (□) to six patients; and (B) 40 mg/m² IV (○) (n = 4), orally (▲) (n = 5), and subcutaneously (□) (n = 5).

peak concentration occurring between 15 and 30 minutes.

Pertinent pharmacokinetic parameters are listed in Table 2. The peak plasma concentration and bioavailability at the lower dose are equivalent for oral and subcutaneous methotrexate, but the clear advantage for the subcutaneous dose at the 40 mg/m² level can again be appreciated. The subcutaneous dose was completely absorbed, whereas, only 42% of the oral dose is bioavailable. The advantage for subcutaneous administration can also be appreciated by comparing the relative bioavailability of the subcutaneous and oral 40 mg/m² doses using the lower dose as a standard. The relative bioavailability of the subcutaneous dose is 106% compared with 43% for the oral dose. When injected slowly, subcutaneous administration of methotrexate was well tolerated in these children with ALL, with no evidence of local or systemic toxicity.

DISCUSSION

The results of this study indicate that the subcutaneous route appears to be a feasible route of administration for low-dose methotrexate in patients on an intermittent schedule (most maintenance regimens for ALL include weekly oral methotrexate at a dose of 15 to 20 mg/m²). Subcutaneous methotrexate was rapidly and completely absorbed at both dose levels studied, without evidence of local toxicity at the injection site.

The finding of a greater AUC for methotrexate following subcutaneous administration compared with that resulting from an equal dose administered by IV bolus has two possible explanations. Total plasma clearance could be more rapid after the IV bolus. One could speculate that renal tubular reabsorption is saturated at the initial high plasma methotrexate concentrations or that the rapid injection does not allow time for the complete tissue distribution and therefore less drug is available for slow release at later time points. A more likely explanation is that the methods used to calculate the initial concentration (at time 0) underestimated that value leading to an underestimation of the AUC for the IV bolus dose.

Figure 3 illustrates the advantage for subcutaneous methotrexate over oral administration as the dose is increased. The AUC for a variety of

Table 2. Pharmacokinetic Parameters for Methotrexate Administered by Various Routes and at Two Dose Levels to Children With ALL

Dose (mg/m²)	AUC (μmol/L/h)			Peak Conc. (μmol/L)			Bioavailability (%)	
	SC	PO	IV	SC	PO	IV	SC	PO
7.5	3.38* ±1.65 (n=6)	2.99 ±1.34 (n=6)	–	0.94 ±0.28 (n=6)	0.98 ±0.46 (n=6)	–	–	–
40	22.0 ±9.0 (n=5)	7.93 ±2.88 (n=5)	20.0 ±3.5 (n=4)	7.40 ±3.00 (n=5)	1.73 ±0.35 (n=5)	11.4 ±2.8 (n=4)	126 ±60 (n=4)	42 ±15 (n=4)

*Data presented is the mean ± one SD.

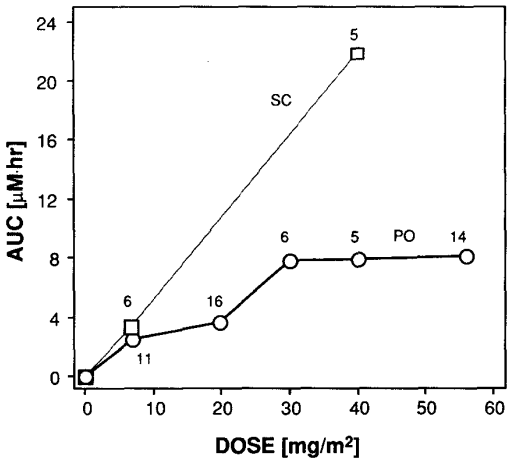

Fig 3. Relationship between the mean AUC and subcutaneous dose administration (□) at low and high levels compared with oral administration (○) at various dose levels. Data from the present study and others[15] are included. The number next to each point is the number of data points averaged to yield the point. The oral dose AUC plateau indicates saturation of the absorptive mechanism.

oral doses both from this study and several other published reports[2,7,14,15] is shown relative to the dose administered. At doses of oral methotrexate of 30 mg/m² and above, the AUC plateaus, presumably as a result of saturation of the absorption process. This saturation of absorption, which has been previously described for orally administered methotrexate,[2,6,9] was not observed with the subcutaneous dose.

Because of its substantially better absorption at doses >20 mg/m², subcutaneous administration appears to have a pharmacokinetic advantage over oral dosing in this dosage range. This bioavailability difference with increasing dose has also been recently observed in a study comparing intramuscular and oral administration.[9] In this study the intramuscular dose was completely absorbed over a dosage range of 13 to 76 mg/m², whereas the percent of the oral dose absorbed fell from 42% at doses of ≤ 40 mg/m² to 18% at dose >40 mg/m².

Subcutaneous methotrexate may also be of use in selected patients on doses <20 mg/m² who absorb the drug poorly from the gastrointestinal (GI) tract.[1-4] Considering published data from one study on the superiority of parenteral (IM) methotrexate in maintaining remissions in ALL,[5] a case could also be made for using intramuscular or subcutaneous methotrexate during maintenance therapy in all children with ALL. Although not studied here, the subcutaneous route may also be useful for long-term, low-dose continuous infusion of methotrexate.

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