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Effect of Combination Chemotherapy, Duration of Methotrexate Administration, and Patient's Age on Methotrexate Pharmacokinetics

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Summary. *The kinetics of methotrexate were studied in a group of 59 patients after infusion of high doses. From the triexponential analysis of the serum concentration curve, rate constants and relative concentrations in non-circulating pools were calculated, using a linear 'mammillary' three-compartment model. No significant variation as a function of dose (300–6,000 mg/m²) was observed. In circumstances the rapidly exchanging pool was a reflection of the circulating pool, suggesting that it is an extravascular, protein-bound pool of methotrexate. Considerable variations of the relative concentration and rate constants of the slowly exchanging pool were observed to be a function of the duration of methotrexate infusion, the presence of other drugs, and the patient's age. If this compartment is assumed to include the target cells for methotrexate, then the large variations observed could be responsible for individual differences in toxicity and/or effectiveness.*

Introduction

High doses of methotrexate (MTX), 500 mg/m² or higher, have been used in the treatment of acute recurring leukemias [18] and of solid tumors [10, 11, 16] for the last decade. The mode of administration proposed by Djerassi et al. [4] is used in most treatment protocols: MTX is infused for 4–6 h and folinic acid rescue is begun several hours after MTX infusion is stopped. Other protocols have, nevertheless, been established, characterized by infusions as long as 20 [14], 24 [1, 3, 6], 36 [13], and even 48 h [7]. The doses of MTX in these schedules vary from 500 mg/m² to more than 10 g/m².

Relatively few detailed comparative pharmacokinetic studies have been performed under these circumstances. The present report employed a three-compartment multiparametric model to measure variations of pharmacokinetic parameters, especially intercompartmental rate constants as a function of administered dose, duration of infusion, patient's age, therapeutic combinations, and repetition of treatments.

It was found that greatest individual variations were due less to concentration and rate of escape from the central compartment than to the concentration and rate constant to an exchangeable compartment. It thus appears to be important to consider pharmacokinetic parameters for analyzing the efficacy, and especially the toxicity, of the treatment.

Materials and Methods

Assay of Serum Methotrexate. A radioimmunoassay (Diagnostic Biochemistry, San Diego, California, USA) was used. This assay exhibits a cross reaction of 0.005% with folic acid and *N*5-methyl tetrahydrofolic acid, and less than 7% with 7-OH-MTX. The cross reaction with 4-deoxy-4-amino-*N*10-methylpteroic acid (DAMPA) is 10% [2], but this was not an important factor in the present study, since it has been shown that this MTX metabolite appears in the serum of treated patients only 48 h after MTX administration [2].

Sampling Schedule. Pharmacokinetics were monitored for 24 h following MTX infusion. In all cases, at least 10 samples were collected: before infusion, immediately afterwards, at 5, 15, and 30 min post-infusion, and at 1, 2, 3, 6, 12, and 24 h afterwards.

Calculation of Pharmacokinetic Parameters. In all cases a surprising triexponential system following the end of the infusion could be calculated from the experimental points. The data were obtained from the equation:

$$\text{MTX concentration (t)} = A\exp\text{-}\lambda 1t + B\exp\text{-}\lambda 2t + C\exp\text{-}\lambda 3t,$$

where A, B, and C were the concentrations determined by extrapolating to zero time (end of infusion) of each corrected exponential curve, $\lambda 1$, $\lambda 2$, and $\lambda 3$ were the slopes of these curves, expressed as h-1. A typical elimination curve of MTX is shown in Fig. 1.

A three-compartment model calculated after the end of the infusion was used (Fig. 2). The fractional volume of each compartment and the fractional rate constants between compartments were calculated with Laplace transformations. In the case of interpatient comparisons, the MTX volume in the central compartment was assigned a value of 1. Q2 and Q3, the fractional volumes of compartment 2 and 3, in fact represented the evolution with time of the following ratios:

$$\frac{\text{volume compartment 2}}{\text{volume compartment 1}} \text{ and } \frac{\text{volume compartment 3}}{\text{volume compartment 1}}.$$

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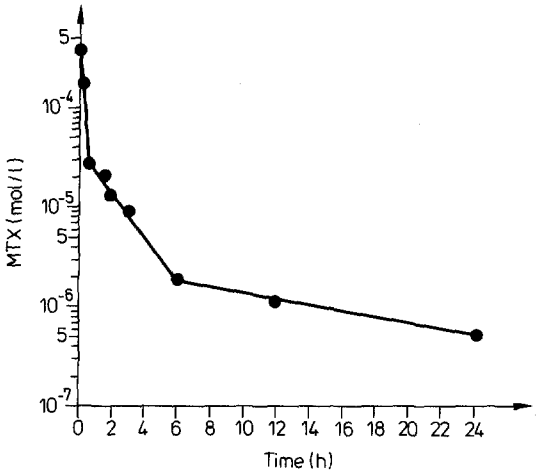

Fig. 1. Typical elimination curve of MTX in the patients' serum

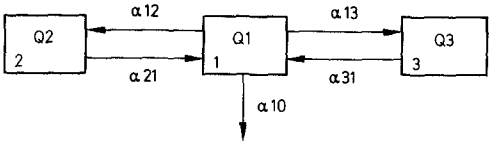

Fig. 2. Pharmacokinetic model used for MTX, where compartment 1 represents the central compartment and compartments 2 and 3 represent the rapidly and slowly exchangeable pools of MTX, respectively

Patients. The study involved 36 patients receiving 59 MTX courses. They were suffering from acute leukemia (12 cases) or metastatic solid tumors (24 cases). MTX was given alone in 13 courses and in combination therapy in 46 courses. Leucovorin rescue (15 mg every 6 h from the time when infusion was stopped up to 24 h post-infusion) was performed in all cases. MTX was given either as short (3-h) infusions at a dose of 300–600 mg/m^2 in 30 courses, short (3-h) infusions at a dose of 1–6 g/m^2 in 13 courses, or long (24-h) infusions at a dose of 300–600 mg/m^2 in 16 courses. In all cases, one-third of the dose was given as an IV bolus and the remainder by continuous infusion. Only patients free of renal disorders (normal creatinine clearance) were included in this study.

In 16 cases, the patients had been evaluated three to four times during similar treatments at 2- to 3-week intervals.

Statistics. Isotopic determination was performed in duplicate with a 1% counting accuracy. Curves were constructed by the least-squares method. The analytical program had been written for a Hewlett Packard 41C desk calculator and could be called up upon request. Data were compared using Student's *t*-test.

In all cases, data were expressed as $\bar{X} \pm SE$.

Results

Slopes and Half-Lives

Maximum concentrations obtained after 300 or 5,000 mg/m^2 and after a 3- for 24-h infusion are shown in Table 1. It can be seen that the C_{max} value is not closely related to the quantity of MTX administered, the 5,000/300 mg/m^2 dose ratio is 17, and the 564/6 C_{max} ratio is 94.

It is also interesting to note that individual variations of C_{max} during a long (24-h) infusion are much smaller than during 3-h infusion. The elimination slopes found in the present study are similar to those reported in the literature for an IV bolus injection of MTX; they are independent of the administration schedule and of administered dose. The values of these slopes are:

$\lambda 1 = 1.51 + 0.50$ h-1;
$\lambda 2 = 0.38 + 0.18$ h-1;
$\lambda 3 = 0.11 + 0.04$ h-1.

Table 1. Maximum concentrations (A + B + C) of MTX after the different schedules of administration

Dose (mg/m^2)	300	300	5,000
Duration of infusion (h)	3	24	3
C_{max} (A + B + C) ± SE (10E−4 M/l)	6.10 ± 1.93	1.02 ± 0.14	564 ± 274

Table 2. Pharmacokinetic parameters obtained after 3-h infusion of MTX in patients treated either with 300–600 mg/m^2 (n = 30) or with more than 1,000 mg/m^2 (n = 13)

n	α 12	α 21	α 10	α 13	α 31	Q 2	Q 3
30	0.33 ±0.20	0.70 +0.32	0.74 ±0.29	0.16 ±0.20	0.14 ±0.04	0.50 ±0.27	1.11 ±1.03
13	0.29 ±0.09	0.72 ±0.35	0.74 ±0.15	0.21 ±0.15	0.14 ±0.05	0.52 ±0.33	1.47 ±1.14

n number of courses; α ab, h−1

Table 3. Pharmacokinetic parameters obtained in patients after the first (n = 16) and third (n = 16) course of MTX

n	α 12	α 21	α 10	α 13	α 31	Q 2	Q 3
16	0.30 ±0.15	0.93 ±0.31	0.87 ±0.20	0.31 ±0.22	0.18 ±0.05	0.36 ±0.18	1.63 ±0.94
16	0.34 ±0.16	0.78 ±0.23	0.80 ±0.22	0.28 ±0.19	0.17 ±0.05	0.47 ±0.30	1.59 ±0.86

n number of courses; α ab, h−1

Rate Constants and Compartment Fractional Volumes

Linearity of the Model. Linearity was tested by comparing the different transfer rates as a function of administered dose. Similar results where observed for each rate in all groups (Table 2).

Table 2 shows that approximately 60% of the infused drug ($\alpha 21 + \alpha 31/\alpha 12 + \alpha 13 + \alpha 10$) left the central compartment directly at a high rate (about 80% h-1). About 25% of the drug ($\alpha 12/\alpha 12 + \alpha 13 + \alpha 10$) reached a peripheral compartment in a rapid equilibrium with the circulatory pool. The volume ratio between these compartments at equilibrium was about 2/1. Approximately 15% of the infused drug ($\alpha 13/\alpha 12 + \alpha 13 + \alpha 10$) reached a slowly exchangeable pool. The relative volume of each compartment was highly variable from one case to another.

Comparison Between First and Third Course

In the 16 patients studied following similar doses and schedules (Table 3) it was noted that kinetic parameters were not significantly modified after each course, although most of the

Table 4. Pharmacokinetic parameters obtained in patients treated either with MTX alone (n = 17) or by MTX + adriamycin (n = 14)

n	α 12	α 21	α 10	α 31	α 13	Q 2	Q 3
17	0.32 ±0.13	0.71 ±0.32	0.76 ±0.22	0.21 ±0.15 *	0.15 ±0.05	0.54 ±0.31	1.42 ±1.17
14	0.28 ±0.13	0.62 ±0.16	0.68 ±0.29	0.10 ±0.06	0.14 ±0.04	0.49 ±0.26	0.81 ±0.79

n number of courses; α ab, h−1 Student's t-test was performed between the two groups: * $P < 0.02$

Table 5. Pharmacokinetic parameters obtained after 3−h infusion of MTX alone in patients either more (n =14) (mean = 49.3. 25−64) or less (n = 8) (mean = 14.5, 13−19) than 20 years old

n	α 12	α 21	α 10	α 31	α 13	Q 2	Q 3
14	0.30 ±0.14	0.93 ±0.43 *	0.89 ±0.24 **	0.33 ±0.25 *	0.16 ±0.04	0.37 ±0.20 **	2.13 ±1.23 **
8	0.30 ±0.07	0.49 ±0.21	0.56 ±0.17	0.09 ±0.07	0.14 ±0.06	0.70 ±0.32	0.52 ±0.36

n number of courses; α ab, h−1 Student's t-test was performed between the two groups: * $P < 0.02$; ** $P < 0.01$

Table 6. Pharmacokinetic parameters obtained in patients under 20 years old treated with either 3−h (n = 8) or 24-h (n = 16) infusion of MTX but not treated with adriamycin

n	α 12	α 21	α 10	α 31	α 13	Q 2	Q 3
8	0.30 ±0.07	0.49 ±0.21 **	0.56 ±0.17 **	0.09 ±0.07 **	0.14 ±0.06 *	0.70 ±0.32 *	0.62 ±0.32 **
16	0.36 ±0.14	1.01 ±0.22	0.90 ±0.17	0.42 ±0.20	0.20 ±0.05	0.39 ±0.19	2.00 ±0.60

n = number of courses; α ab = h−1 Student's t-test was performed between the two groups: * $P < 0.01$; ** $P < 0.001$

rate constants were seen to be slightly reduced during the third course in comparison with the first.

Influence of Drug Combinations

There was no apparent difference between the results in patients treated with MTX alone and those treated with MTX + cyclophosphamide (without adriamycin). When adriamycin was administered with MTX, however (Table 4), there were slower rate constants between the peripheral and central compartments: α31 was 0.21 ± 0.15 h-1 and 0.10 ± 0.06 h-1, respectively ($P < 0.02$). With this combination, the fractional value of the slowly exchangeable pool was higher.

Influence of Patient's Age

As seen in Table 5, patients receiving a 3-h infusion of the same dose of methotrexate (without adriamycin) showed clear-cut age-related differences in kinetics. The transfer rate between the central compartment and the rapidly exchangeable pool was similar in both groups. In children, the fractional volume of this compartment (2) was higher (0.37 ± 0.20 vs. 0.70 ± 0.32) and return to the central compartment was lower (0.93 ± 0.44 vs. 0.49 ± 0.21).

Definitive efflux was lower by about 30% (0.89 ± 0.24 vs. 0.49 ± 0.21). In particular, a considerably slower transfer rate toward compartment 3 was observed in young patients (α13 = 0.33 ± 0.25 vs. 0.09 ± 0.07 h-1). The fractional volume of the latter compartment in patients younger than 20 was four times lower than in older patients.

Influence of the MTX Infusion Schedule

A substantial increase of the exchange rates was observed in patients who had undergone a 24-h infusion in comparison with those who had received a 3-h infusion (Table 6).

There was heterogeneity in the clinical status of the patients and in the chemotherapies used, and we observed no MTX-related clinical toxicity. As a result of this, kinetic parameters could not be compared with toxicity and/or efficiency. This study is currently under way in a homogenous group of patients.

MTX Pharmacokinetic Studies and Clinical Response

Correlation between the pharmacokinetic parameters and the clinical response was impossible because of the heterogeneity of the patient's diseases and because of the different chemotherapies used.

Discussion

In most published reports, toxicity is compared with both the maximum concentration obtained and the time for which the MTX concentration exceeds a certain threshold [5, 8, 9, 17]. In the present study we attempted to analyze other parameters and to determine whether they varied in a different way from those compared previously.

MTX kinetics were analyzed with a linear three-compartment model, since this is the one most often used in the literature [8, 17]. Certain workers, however, favor a non-linear model with constant rates obeying the Michaelis Menten law [12, 15]. In our study, the zero time used for the mathematical treatment of the results is not a real zero time, because of the infusion duration.

Several important results were recorded in the present study: the ratio of the volume of the rapidly exchangeable compartment (Q2) to that of the central compartment (Ω1) varied relatively little from one case to another and was close to 1/2. Equilibrium was reached within several hours.

The definitive efflux (α10) of MTX is more rapid after slow infusions and in older subjects. This conflicts with the results published by Wang et al. [19]. In these two groups of patients, we noted that overall rate of efflux from the central compartment (α12 + α13 + α10) was much higher after a slow infusion (1.68 ± 0.94 h-1). This implies that with the same infused dose, there is a longer transit time in the central compartment at a higher concentration after rapid infusion and in young patients.

The greatest intergroup differences are observed for the slowly exchanging pool. Its fractional volume is clearly affected by patient's age and by duration of the infusion. In the case of slow infusion to the oldest patients, the fractional volume of the slowly exchanging compartment is multiplied by a factor greater than 3.

MTX kinetics is not modified by the simultaneous administration of cyclophosphamide, but is slowed by the presence of adriamycin, resulting in a 50% decrease of the fractional volume of the slowly exchanging pool. The patient

population in the two groups does not differ in the disease they are suffering from, which does not influence the results.

The use of such a pharmacokinetic model does not allow allocation of physiological function to any dynamic compartment. It is, nevertheless, possible that the slowly exchangeable compartment contains at least some of the MTX that is fixed or penetrates into cells.

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