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High-Dose Methotrexate: Preliminary Evaluation of a Pharmacokinetic Approach

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Summary. *Clinical pharmacologic studies have been carried out in patients with head and neck tumors following 36-h continuous infusions of high-dose MTX (1.5 g/m²). The results indicated considerable variation in the amount of MTX in the blood of individual patients. To control these variations, a modified protocol was set up to try to attain the same MTX blood level in all subjects. The protocol has a pharmacokinetic basis and involves determination of the MTX kinetics in* each *patient. The information thus obtained allows us to compute a 36-h infusion dose so that the MTX plasma levels never exceed a threshold beyond which there is a risk of toxicity to the host.*

The computation is validated by taking a blood sample 6 h after the beginning of the infusion. If the MTX concentration is higher than its expected value, the infusion rate can then be immediately reduced. Analytical methods that will allow such a computation, the results of the clinical application of this pharmacokinetic approach, and some implications of such a method are discussed.

Introduction

High-dose methotrexate with leucovorin rescue is used in the treatment of certain cancers, and particularly of osteosarcoma and head and neck tumors [4, 5, 8, 9]. The toxicity of this antimetabolite provokes adverse reactions, often rendering the use of this chemotherapy difficult. The most frequent adverse reactions are the appearance of changes in the digestive mucosa, which can create intestinal troubles, nephrotoxicity due to the weak solubility of methotrexate in an acid medium, hepatoxicity, and myelosuppression. Various authors [4, 5, 7, 10, 11] have already written in detail about these toxic phenomena. The development of these side effects must be imputed not only to the dose regimen (i.e., the dosage and the rhythm of administration) but also to the duration of the exposure to high concentrations of MTX.

The pharmacokinetic work presented here is part of a clinical study of the treatment of head and neck tumor, involving the administration of high-dose methotrexate by a continuous constant-rate infusion (1.5 g/m² over 36 h) followed by a leucovorin rescue. On the basis of pharmacokinetic data obtained from a single IV bolus injection of MTX, the dose to be administered by continuous infusion will then be calculated to give similar plasma levels of the drug in all patients treated with this protocol.

Patients and Methods

1. Patients

All individuals used in these studies were clinically diagnosed as patients with head and neck carcinoma. Ages varied from 46 to 74 years (mean age 55).

2. MTX Assay

The particularities of this administration protocol for high-dose methotrexate with individualization of dosage made it necessary to use two methods of quantitative determination.

The first method, being a radiocompetitive assay [16], is sensitive enough (2.5×10^{-9} *M*) for a purely pharmacokinetic study and for the purpose of preliminary identification of patients.

The other one, an enzymatic assay [2] with a quick response time, ensures that therapeutic control monitoring takes place in the best conditions.

We verified that these two methods correlate fairly well.

MTX Protocols

1. Protocol without Pharmacokinetic Identification

Figure 1 outlines the first protocol used for the treatment of patients with 36-h continuous infusions of MTX. About 12 h after alkaliniza-

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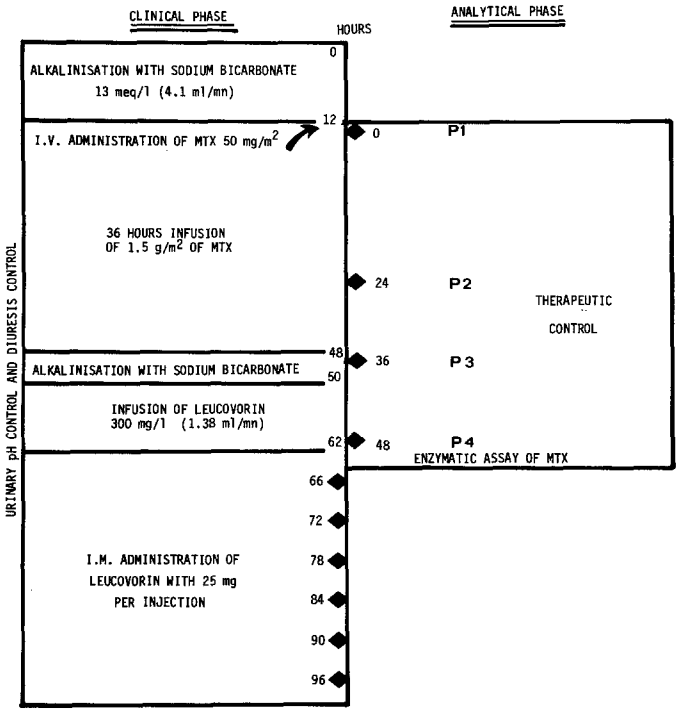

Fig. 1. Protocol for the treatment of head and neck cancer with a continuous 36-h infusion of high-dose MTX (1.5 g/m²) preceded by a bolus IV loading dose (50 mg/m²). *Arrow* indicates point at which a loading dose of MTX (50 mg/m²) was administered; P, plasma samples

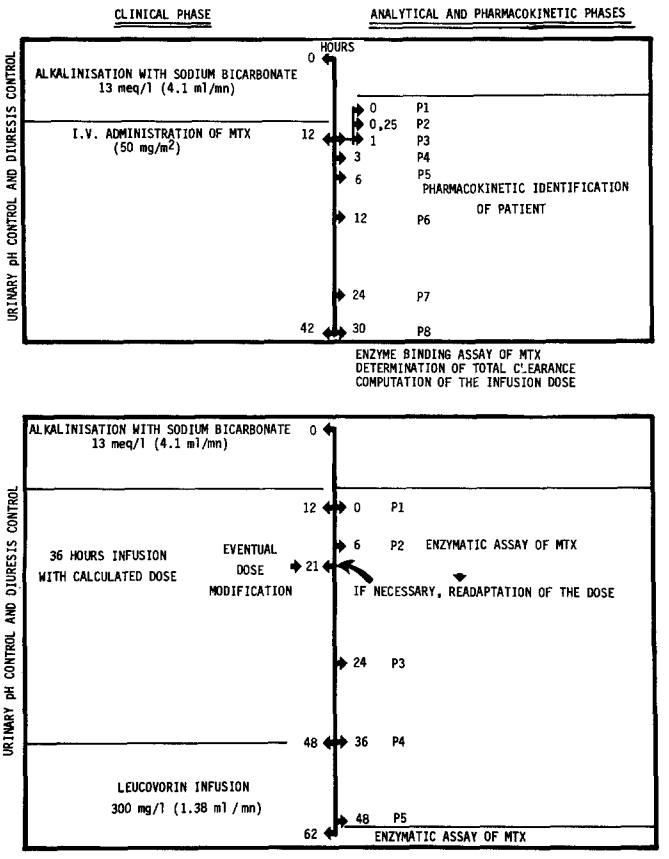

Fig. 2. Protocol for high-dose MTX with individualization of dosage on a pharmacokinetic basis

tion of the patients, a loading dose of MTX (50 mg/m²) was administered just prior to the infusion (1.5 g/m²), and plasma samples were taken on day 0 (prior to initiation of therapy) and on days 1–2 and 3 thereafter. At the end of the 36-h continuous infusion of MTX, leucovorin rescue was initiated. Table 1 summarizes the results obtained in individual patients.

It can be seen that almost all the patients had received one or several previous treatments. The individual variations observed in the plasma concentrations during the infusion can be imputed to the variation of the infusion rate for at least three reasons: the state of the patients' veins, the large number of infusion bottle changes, and, above all, the lack of a pump. To this, we can add that clinical practice tends to place more emphasis on the duration of the infusion than on the regularity of its flow.

The dispersion of the plasma concentrations from one subject to another (interindividual variations) can also be seen clearly in Table 1. It was not possible to establish any notable correlation with biological parameters (e.g., creatinine clearance). Finally, we must indicate that some patients showed undesirable reactions, mostly buccal ulceration.

The analysis of these data has led us to consider a modification of the protocol. Our aim was to help to reduce the frequency and the degree of toxic manifestations. We therefore proposed an individual adaptation of the dose of MTX so that all patients could achieve similar plasma concentrations. We improved the clinical conditions by using an infusion pump whenever possible, and finally, we established a therapeutic monitoring procedure to be applied during the infusion.

The individual adaptation of the MTX dose, i.e., the personalization of the dosage schedule, involves the determination of the kinetics of this antimetabolite in *each* patient. So the new protocol to be described in this paper relies entirely on pharmacokinetic and analytic bases.

2. *Protocol with Pharmacokinetic Identification*

This protocol consists essentially of three successive phases, as outlined in Figure 2: (1) Pharmacokinetic identification of each patient with the aid of an IV bolus of a moderate test dose of MTX (50 mg/m²); (2) Determination of some relevant pharmacokinetic parameters and computation of the dose to ensure a certain plasma level of MTX during the 36-h infusion; (3) Administration of the infusion and therapeutic monitoring.

a) Pharmacokinetic Model of Methotrexate. The entire protocol depends upon the knowledge of a pharmacokinetic model. For this antimetabolite, several authors have proposed a kinetic behavior corresponding to a three-compartment linear mammillary system with central elimination [3, 5, 13, 14, 16, 17]. This kind of model, represented in Figure 3, is characterized by the following properties: (i) There is a central compartment, containing the plasma; (ii) The

Table 1. Clinical status and blood level of MTX achieved in individual patient receiving high dose of MTX at 24, 36, and 48 hours following initiation of the infusion

Patient	Previous treatment[a]	Creatinine clearance (ml/min)	MTX dose		Plasma levels (10^{-8} *M*)		
			Loading dose (mg)	Infusion dose (g)	Blood samples at 24 h	36 h	48 h
LES	R, C	80	0	2.8	1105	875	60
PAY	R, C, S	65	78	2.3	–	1075	–
SEG	R, S	62	77	2.3	2960	2613	65
		45	77	2.3	2733	1480	68
QUE	C, R	103	80	2.4		440	11
		64	77	2.4		220	22
		70	75	2.2		8050	114
FOR	R, C	65	75	2.3	3748	668	20
PIE	R	115	85	2.5	428	351	1612
SOR	C, S	115	85	2.55	2140	1132	–
OUE	R, C	70	80	2.4	1270	1660	69
		70	80	1.4	–	1665	118
PER	R, C, S	34	80	2.4	1980	2126	76
TAR	R	86	80	1.8	6868	1396	100
		110	80	2.4	2332	1254	69
		100	80	2.4	1300	1795	–
		86	80	2.4	1970	1425	138
LAV	–	92	70	2.1	–	155	20
		106	0	2.1	880	550	13
REY	S, R	96	70	2.2	589	655	17
		70	70	2.2	640	644	17
		80	70	2.2	589	655	17
		80	70	2.2	645	–	19

[a] R, radiotherapy; C, chemotherapy; S, surgery

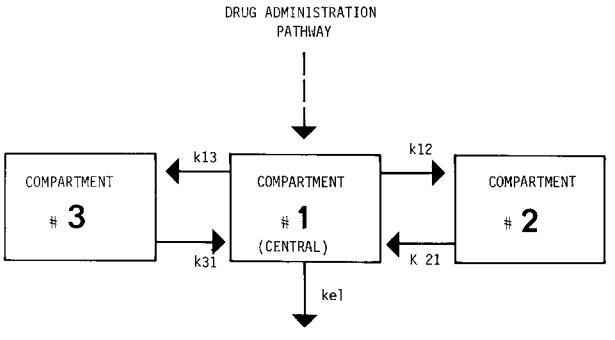

Fig. 3. A three-compartment linear mamillary model with central elimination

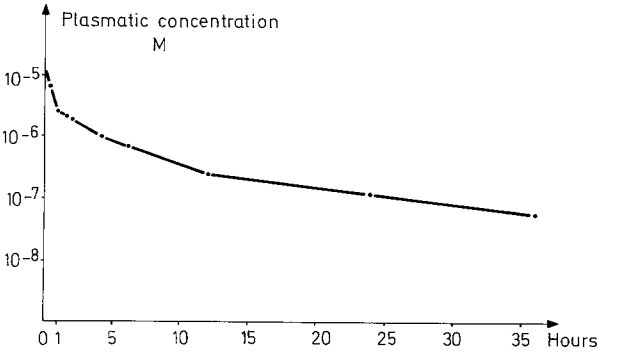

Fig. 4. Time-course of MTX plasma concentration after IV bolus of 100 mg: a classic triphasic curve

quantity of drug transferred from one compartment to another is proportional to the quantity of drug in the source compartment (*hypothesis of linearity*); (iii) The elimination of the drug takes place from the central compartment only.

Under these assumptions the time-course of the drug concentration in the central compartment (i.e., in the plasma) after an instantaneous IV bolus of dose q_0 follows Equation (1):

$$c(t) = q_0 (Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}) \quad (1)$$

c(t) is the sum of three exponential terms, and the corresponding curve presents a classic triphasic shape in semilogarithmic coordinates (Fig. 4). One can also remark that in Equation (1), c(t) is proportional to the dose q_0 at any moment. (This is an essential implication of the hypothesis of linearity.)

The most relevant pharmacokinetic parameter for this study is the total plasma clearance Cl. This quantity is defined as the volume of the central compartment from which the drug is removed to be eliminated outside the organism per unit of time. Thus the elimination flow at time t is given by Cl × c(t).

It is well known that Cl is constant in the linear hypothesis and, for any intravascular mode of administration, is given by Equation (2):

$$Cl = \frac{q_0}{AUC} \quad (2)$$

where

$$AUC = \int_0^\infty c(t)\,dt \tag{3}$$

denotes the area under the plasmatic concentration curve. AUC can be estimated either by the trapezoidal rule or, if one has already determined the parameters appearing in Equation (1), by the formula:

$$AUC = q_0\left(\frac{A}{\alpha}+\frac{B}{\beta}+\frac{C}{\gamma}\right). \tag{4}$$

b) Pharmacokinetic Identification of a Patient. For the purpose of pharmacokinetic identification of a patient, an IV bolus of 50 mg MTX/m² is given. Blood samples are taken at the following hours: 0 (just before the injection), 0.25, 1, 3, 6, 12, 24, 30.

Analysis of the unchanged compound in the plasma enables us to give a sufficient approximation of some pharmacokinetic parameters, and specifically of the total plasma clearance, Cl, of MTX, according to the trapezoidal rule.

It is important to note that the administration of this test dose is preceded by a 12-h alkalinization to allow the patient to be in the same condition of diuresis and urinary pH as he will be during the infusion.

c) Infusion Dose Computation. The time-course of plasma concentration during a constant rate infusion of q_0 over time is given for $t \leqslant T$ by:

$$c(t) = \frac{q_0}{T}\left(\frac{A}{\alpha}+\frac{B}{\beta}+\frac{C}{\gamma}\right) - \frac{q_0}{T}\left(\frac{Ae^{-\alpha t}}{\alpha}+\frac{Be^{-\beta t}}{\beta}+\frac{Ce^{-\gamma t}}{\gamma}\right) \tag{5}$$

where A, B, C, α, β, γ are the same coefficients as in Equation (1) (i.e., the parameters of IV bolus response). One can see that c(t) is proportional to the rate of the infusion ($= q_0/T$).

The plasma concentration P at the steady state is derived from Equation (5) (setting t equal to infinity):

$$P = \frac{q_0}{T}\left(\frac{A}{\alpha}+\frac{B}{\beta}+\frac{C}{\gamma}\right) = \frac{q_0}{T}\frac{1}{Cl}. \tag{6}$$

From the values of the pharmacokinetic parameters observed with MTX [5], it can be stated that at 6 h the plasma concentration is less than 10% below the steady state level. Thus we shall consider that the steady state level is achieved from 6 h and persists until the end of the infusion.

So, if one desires to maintain a desired concentration, P, during most of the infusion time, the dose to be infused is given by the formula:

$$q_0 = P \times Cl \times T. \tag{7}$$

If P is expressed in mol/liter, Cl in liters/hour, and T in hours, q_0 will be in moles; to obtain a dose in grams it is necessary to multiply the result by the molecular weight W of MTX (W = 454.45 g/mol).

The desired level P has been chosen according to the clinical observations recorded with the first protocol. Its value at this time is $P = 10^{-5}$ *M*. It is clear that this choice is somewhat arbitrary (see below).

d) Treatment and Therapeutic Control. The infusion is preceded by an alkalinization checked by measuring the urinary pH and the diuresis throughout the therapeutic phase.

To verify that the evolution of the plasma levels of MTX during and after the infusion is consistent with mathematical prediction, blood samples are taken at 6 h, 24 h, 36 h, and 48 h after the beginning of the infusion.

The immediate analysis (within 2–3 h) of the blood sample at 6 h allows a diminution of the infusion rate if the measured value is significantly higher than the expected level ($P = 10^{-5}$ *M*). This correction is, moreover, proportional to the ratio of the theoretical to the experimental value.

The method of administering the antidote remains unchanged.

Clinical Application

This protocol of administration of MTX has been used in more than 20 patients.

1. Example of Application

For illustrative purposes we report in Figures 5 and 6 an example of the protocol's application in one subject.

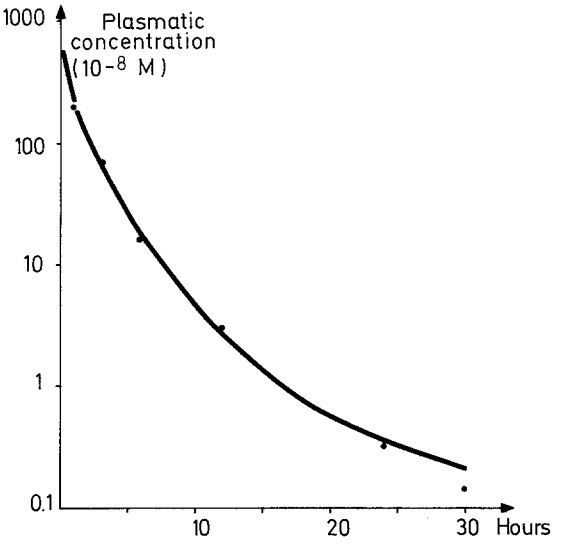

Fig. 5. Application of the protocol: the IV bolus test dose. ●, experimental data; —, computer-predicted values: the computed total plasmatic clearance is 368 ml/min

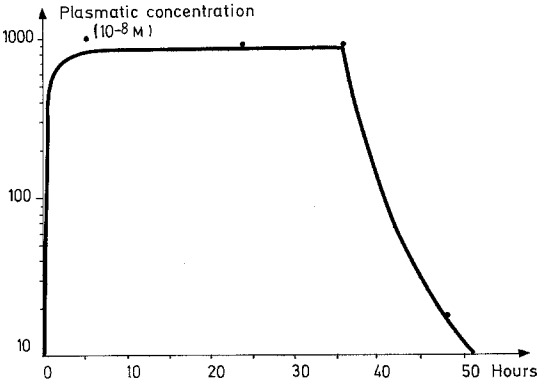

Fig. 6. Application of the protocol: 36-h infusion of the dose computed owing to identification phase. ●, experimental data; —, computer-predicted values: the calculated and administered dose is 3.6 g MTX

Figure 5 shows the result of the identification phase. An IV bolus of 95 mg MTX was administered, blood samples were taken according to the schedule discussed above, and the total plasma clearance was evaluated from the area under the curve, giving a value of 368 ml/min, i.e., 22.08 liters/h. Simultaneously, a complete pharmacokinetic study was made of these data, to allow estimation of the parameters A, B, C, α, β, γ. Such a treatment is absolutely superfluous for the application of the protocol (the area under the curve is the only indispensible parameter). However, it was necessary for simulation purposes and to make this example didactic. The computed curve thought to fit the experimental data is the solid line in Figure 5.

On the basis of the clearance obtained with this IV bolus, a dose was computed according to the formula of Equation (7) and found to be 3.6 g MTX. Figure 6 shows the result of the administration of a continuous 36-h infusion with this dose. The actual plasma concentrations are in keeping with the simulation (solid line) carried out with the parameters calculated by the complete mathematical treatment of the IV bolus.

2. *Summary of the Results*

Table 2 summarizes the results obtained after IV bolus identification. Several remarks can be made:

(i) The total clearance of MTX was substantially higher than the creatinine clearance, suggesting either a significant hepatic clearance or a tubular secretion (or both).

(ii) Significant interindividual variations in the clearance rate of MTX (the relative standard deviation is over 50%) can be observed though there is no obvious correlation with biological parameters.

(iii) This new protocol leads to infusion doses similar to those of the previous protocol only for patients with Cl in the range 200–300 ml/min.

Table 3 shows the experimental results obtained with the infusions for the patients who had undergone the IV bolus administration. Three remarks may be relevant to this study:

(i) Compared with the first protocol (Table 1), both intraindividual and interindividual variations are largely reduced. The first point is due to the larger number of

Table 2. Calculated MTX infusion dose on the basis of the clearance obtained after an initial test dose of the drug

Patient	IV dose	Clearances (ml/min)		Doses (g)	
		MTX	Creatinine	Computed dose	Dose on the basis of the 1st protocol (1.5 g/m²)
ALB	70	124	65	1.2	2.43
ALE	75	260	78	2.5	2.25
ANC	75	118	88	1.2	2.4
CHA	75	62	30	0.6	1.9
D'AU	75	135	85	1.3	2.55
FER	50	57	42	0.6	1.95
FOR	75	126	–	1.21	2.55
LES	90	266	100	2.6	2.85
LOM	75	104	78	1	2
MAR	75	220	65	2.10	2.4
MEN	75	43	35	0.4	2
PAY	75	216	70	2	2.34
SEG	75	213	62	2.12	2.32
SET	75	91	100	0.875	1.86
PRE	85	137	56	1.35	2.25
CAS	80	125	65	1.2	2.4
GAL	95	128	120	1.5	2.85
BER	65	190	83	1.8	1.95
BOS	95	368	120	3.6	2.85
HER	80	270	95	2.8	2.4
Mean	–	162.75	75.63	–	–
Standard deviation	–	87.70	25.40	–	–

Table 3. Actual levels of MTX achieved during 36 h continuous infusion with a dose calculated on the basis of individual drug clearance

Patient	Previous treatments	Dose (g)		Plasma concentration (10^{-8} *M*)			
		Proposed dose	Infused dose	6 h	24 h	36 h	48 h
ALB	R	1.2	1.2	1490	1360	1405	87.8
				2200[a]	460	760	103.6
ALE	SR	2.5	2.5	1165	1195	960	47
		2.5	2.1	1535	1885	1605	83.2
			1.56	1115	1005	1055	87.7
ANC	RCS	1.2	1.2	636	980	550	–
LES	RC	2.6	2.6	2020	1020	–	–
MAR	SRS	2.10	2.10	975	1005	985	–
				1115	1195	1390	–
				3700[a]	1050	490	50
PAY	RCS	2	1.5	2915[a]	4215	550	–
SEG	RS	2.1	2.1	1275	–	860	32.9
BER	SCR	1.8	1.8	1020	860	1125	51.6
BOS	–	3.6	3.6	1215	1390	2250	34.8
		3.6	2.88	1377	635	1065	23.6
		3.6	3.6	1000	1050	910	17.8
		2.8	2.8	945	890	960	–
				1520[a]	965	1300	35.6
				1045	860	1000	12.8
				1152	915	900	47.6
				800	867	802	59
				830	1250	1210	18.1

[a] Decrease in urinary pH

infusions given with the aid of a pump, and the second was, of course, one of the main goals of the new protocol.

(ii) Taking into account the large number of causes of error, unavoidable inaccuracies, and neglected factors, one can estimate that there is good agreement between these experimental results and the mathematical forecasts. In particular, during infusion, the plasma levels of MTX are near the desired value of 10^{-5} *M*.

(iii) Most instances of this value's being exceeded can be attributed to diuresis problems or chiefly to the decrease of the urinary pH below 6; in cases where the MTX concentration was far too high the dose was reduced immediately, owing to observation of the plasma level at 6 h.

Discussion and Perspectives

1. Validity of the Pharmacokinetic Model

The protocol proposed here relies on two fundamental theoretical assumptions: (a) The kinetics of unchanged MTX can be accurately represented by a three-compartment linear model (at least for the range of concentrations considered in this study). The hypothesis of linearity is essential; owing to it, not only is the prediction from IV bolus to infusion possible, but in addition it can be achieved in a rather straightforward way. With a nonlinear model, this would not be so easy [6, 15]; (b) The kinetic model is *stationary*: this means that the pharmacokinetic parameters of a given patient (and especially his total clearance to MTX) remain constant throughout the treatment.

The results obtained with the clinical application of this protocol constitute a good a posteriori justification of these two assumptions.

The question as to whether the model is stationary can be extended by asking for how long the identification phase is valid, i.e., whether it is possible to use the same computed dose for several successive infusions without repeating the test dose. Unfortunately, as we are not in command of all the factors capable of modifying the kinetics of MTX, we are unable to give an accurate answer to this question or to propose a rationale for deciding when the test dose has to be repeated. A prag-

matic approach would be to repeat the identification phase whenever an infusion has gone wrong (i.e., whenever the observed values are not close to the expected ones).

2. *Choice of the Test Dose*

We must question whether the rather low test dose (50 mg/m^2) allows an accurate enough prediction of the kinetic behavior during the infusion of higher doses. Here again, the first results allow us to think that the answer is positive. In fact, it is noticeable that though the doses might appear very different, the concentrations achieved with the two modes of administration are in more or less the same range.

3. *Choice of the Blood-Sampling Schedules*

a) Identification Phase. According to previous research and to our own experience, it seems that the chosen method of blood sampling allows a good estimation of the first two phases of distribution. However, limiting the identification to the first 30 h, generally prevents observation of the last phase (apparent elimination). To take this phenomenon into account, we therefore complete the kinetic curve by assuming that this elimination phase has a half-life of 10 h after 30 h. The results indicate that the incidence of this correction factor does not appear to be very important (less than 10% on the calculated doses).

b) Treatment Phase. The timing of the first sample during the infusion itself must satisfy two contradictory constraints: it has to be representative of the steady state and the earlier it takes place, the sooner the infusion rate can be modified, if need be. At this time, there is no reason to reject the choice of 6 h. The other samples are taken mainly to confirm the kinetic behavior during infusion. They are, in general, too late to be used for readjusting the infusion rate.

4. *Choice of the Plasma Level During the Infusion*

We have selected the MTX level to be maintained (10^{-5} *M*) in the plasma during most of the infusion rather arbitrarily. Certainly our first results tend to show a clear regression of the adverse reactions to MTX. But only the extension of this high dose protocol will permit us to validate or modify this value so that the best possible efficiency may be attained.

5. *Implications of the Use of the Protocol for Services*

It is clear that this protocol is very demanding and requires the cooperation and participation of both patients and medical staff. Special devices (such as infusion pumps) and specific training of the nurses are required. The formation of a specialized staff and the creation of a multidisciplinary unit for clinical pharmacokinetics would be highly desirable for the development of such studies.

6. *Simplification of the Protocol and Future Progress Expected*

We instigated this protocol because we could not find any reliable criteria by which to predict the wide interindividual variations observed in MTX kinetics, except the kinetics of MTX itself in each patient. To modify the protocol, it is necessary to find extrapharmacokinetic parameters (e.g., biological ones), to which the MTX clearance should be correlated. Studies are under way to determine the elimination mechanisms of MTX and evaluate the importance of metabolic clearance versus renal clearance. This could give an explanation for the interindividual variations and also some criteria by which the risk of nephrotocicity could be evaluated.

Conclusion

This new protocol attains the very restricted goal for which it was instigated, i.e., the use of pharmacokinetics to monitor plasma levels in a true clinical situation. The results obtained in this study indicate that it is now necessary to resume pharmacokinetic and metabolic studies of MTX to achieve a better understanding of the kinetics of this drug and to try to simplify this approach.

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