Cancer Chemother Pharmacol (1984) 12: 120–124

Cancer Chemotherapy and Pharmacology

Methotrexate-vindesine association in the treatment of head and neck cancer Influence of vindesine on methotrexate's pharmacokinetic behavior

N. Lena, A. M. Imbert, T. Pignon, R. Favre, G. Meyer, J. P. Cano, and Y. Carcassonne

Institut J. Paoli – I. Calmettes, 232 bd de Ste Marguerite, B.P. 156, Marseille Cedex 9, France

Summary. *Determination of methotrexate (MTX) kinetics after an IV bolus (50 mg/m²) allows prediction of the steady-state plasma level of this drug during a constant infusion. This prediction allows high-dose MTX (HD-MTX) therapy without major toxicity.*

Patients with head and neck carcinoma received HD-MTX and vindesine (VDS) infusions concomitantly. The therapeutic survey of these patients showed that the predicted plasma level of MTX was not achieved in the presence of VDS. Moreover, the computed dose of MTX had to be increased by a larger amount if the MTX plasma clearance after the identification IV push was low (< 9 l/h).

In the presence of VDS, the creatinine clearance is lower than when MTX is infused alone, and MTX renal elimination is identical (MTX or MTX + VDS infusions). Thus it seems that the decrease of the MTX plasma level during MTX-VDS infusion could be due to an increase of cellular incorporation.

Introduction

High-dose methotrexate (HD-MTX) with leucovorin rescue is widely used in the treatment of different types of cancer (osteosarcoma, non-Hodgkin's lymphoma, head and neck carcinoma, and other solid tumors) [3, 8, 15], but involves a high risk of severe toxicity (myelosuppression, renal failure, mucosal ulcerations, and skin rashes). To decrease these adverse reactions, on the basis of their knowledge of MTX's pharmacokinetic behavior [2, 18], Monjanel et al. [16] proposed pharmacokinetic monitoring of the therapeutic protocols, which involves: an MTX identification IV push (50 mg/m²) and one or more continuous constant-rate infusions with the MTX dose computed from the MTX plasma clearance after the IV push, makes it possible to obtain similar plasma levels of the drug in all patients [9, 16].

Because of its cytotoxicity in epidermoid head and neck cancer [7] and its lower neurotoxicity than other vinca alkaloids [20], vindesine (deacetyl vinblastine sulfate or VDS) was also given during the MTX infusions (36-h VDS infusions starting concomitantly with MTX).

We report in this paper on MTX's pharmacokinetic behavior in plasma and its urinary elimination when it is associated with vindesine.

Reprint requests should be addressed to Dr. N. Lena (address see above)

Materials and methods

Patient selection: Thirteen patients from the Institut Paoli-Calmettes entered the study. Age, tumor localization, performance status, and pretreatments are noted in Table 1. Patients with serum creatinine < 130 µmol/l, granulocyte count > 1,500/mm³, and platelet count > 100,000/mm³ were eligible to take part.

Therapeutic protocol

Pharmacokinetic monitoring. The pharmacokinetic monitoring which enables the infusion of HD-MTX [16] consists of two successive phases, the first of which is pharmacokinetic identification using an IV bolus (50 mg/m²). Patient samples are taken at 0.25, 0.5, 1, 3, 4, 6, 12, 24, and 30 h after the IV push.

Study of the MTX plasma elimination curve allows definition of the pharmacokinetic parameters for each patient: terminal half-life time and plasma clearance (Cl):

$$Cl = \frac{\text{Infused dose}}{\text{AUC}},$$

where AUC represents the area under the plasma elimination curve. The MTX dose for infusion is computed from the clearance to achieve a predetermined plasma steady-state level (10^{-5} mol/l, 2.5×10^{-5} mol/l, 5×10^{-5} mol/l) according to the equation:

$$\text{Dose} = P \times Cl \times t \text{ [16]},$$

where P is the plasma steady-state level (mol/l), Cl the MTX plasma clearance (l/h), and t the infusion duration (h).

The second stage is the infusion protocol: 12 h before the beginning of the MTX infusion, hydration and urine alkalinization are started [9]. The 24- or 36-h HD-MTX and the 36-h VDS (3 mg/m²) infusions start together (Fig. 1). These infusions are followed 36 h later by folinic acid rescue, which is

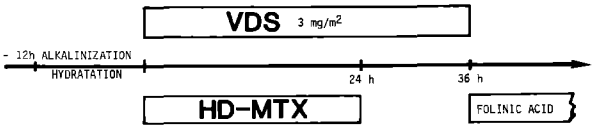

Fig. 1. Therapeutic protocol. The predetermined steady-state levels of MTX were 10^{-5}, 2.5×10^{-5}, or 5×10^{-5} mol/l

Table 1. Patient characteristics

Patient	Age	Sex	Performance status		Tumor localization	Pretreatments
			WHO scale	Karnovsky scale		
Bou . . .	65	M	IV	1	Oropharynx	Radiotherapy, surgery
Oll . . .	39	M	IV	1	Oropharynx	Surgery
Sic . . .	59	M	I	8	Hypopharynx	0
Tes . . .	65	F	IV	1	Lymphosarcoma with head and neck localization	Radiotherapy
Vig . . .	63	M	IV	1	Buccal cavity	Radiotherapy, surgery
Beu . . .	56	M	IV	1	Oropharynx	Radiotherapy
Ron . . .	50	F	III	2	Buccal cavity	0
Dan . . .	56	M	IV	1	Buccal cavity	Radiotherapy, surgery
Zar . . .	57	M	0	9	Buccal cavity	Radiotherapy
Eyr . . .	57	M	III	5	Oropharynx	Radiotherapy
Mad . . .	70	M	0	9	Hypopharynx	0
Deh . . .	74	F	0	9	Buccal cavity	Radiotherapy
Bor . . .	52	M	0	9	Buccal cavity	Radiotherapy

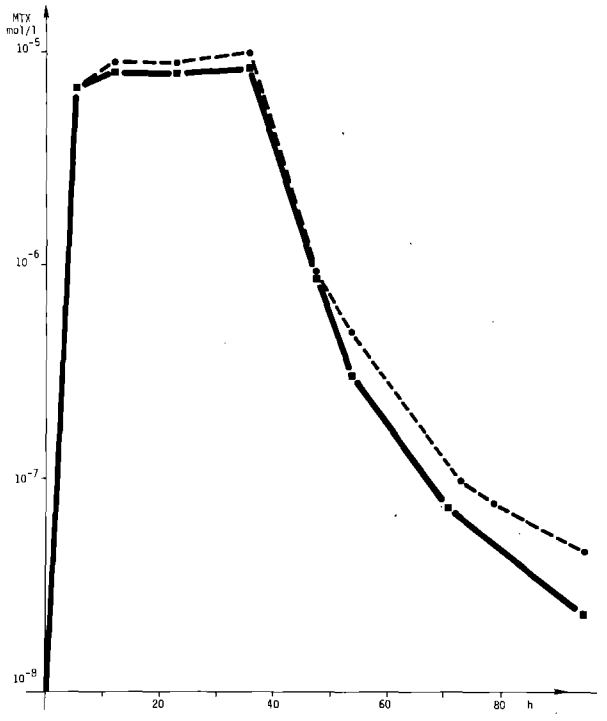

Fig. 2. Steady-state level and elimination achieved with infusion of MTX + VDS (■———■) and of MTX alone (●– – –●). Patient Bor. received the same dose of MTX (1,245 mg) in each infusion and 3 mg VDS/m^2 in the infusion of MTX + VDS

continued until the MTX concentration has fallen below 10^{-7} mol/l.

For this study, all the patients received one or more infusions of MTX and VDS in association. For pharmacokinetic comparison, during the second course of therapy some patients received an infusion of MTX alone followed by a VDS IV push (3 mg/m^2) at the end of the folinic acid rescue.

Table 2. Variations between predetermined and achieved steady-state levels or computed and infused doses in the presence of VDS

Patient	Steady-state level (10^{-8} mol/l)		Dose (mg)		Percentage of variation	
	Predetermined	Achieved[a]	Computed	Infused	Steady-state level	Dose
Bou . . .	5,000	5,120	4,300	5,485		+ 27.6
Oll . . .	5,000	5,000	4,860	5,460		+ 13
Sic . . .	5,000	5,000	2,310	2,820		+ 22
Tes . . .	5,000	3,520	3,425	3,425	− 29.6	
Vig . . .	5,000	4,320	5,460	5,460	− 13.6	
Beu . . .	5,000	5,280	4,175	4,900		+ 17.4
Ron . . .	5,000	5,000	4,260	5,250		+ 11.5
Dan . . .	5,000	4,700	3,675	4,695		+ 27.7
Zar . . .	2,500	2,500	2,500	2,500	0	0
Eyr . . .	2,500	2,560	1,775	1,950		+ 11.5
Mad . . .	2,500	2,245	2,000	2,000	− 10.2	
Deh . . .	1,000	1,010	460	560		+ 21.7
Bor . . .	1,000	850	1,245	1,245	− 14.9	

[a] Differences < 6% are not significant

Therapeutic survey

Control samples. During the infusions, plasma samples were taken at the following times: 5, 12, 24, 36, 48, and 54 h, and then every 12 h until an MTX plasma level lower than 10^{-7} mol/l was recorded. At 5 h, when MTX plasma concentration were about 80% of the steady-state level, doses were modified if necessary to actually achieve the steady-state level.

MTX assay. The particular nature of HD-MTX administration monitoring makes it necessary to use a technic with a quick response for plasma MTX determination. The enzymatic method proposed by Bertino and Fischer [1] was adapted for

Table 3. Statistical comparison between a population receiving MTX alone and one receiving MTX + VDS by infusion

		Steady-state level (10^{-8} mol/l)		Dose (mg)	
		Predetermined	Achieved	Computed	Infused
MTX alone ($n = 12$)	$\bar{x}$	4,000	4,122	4,015	3,923
	σ	1,809	1,834	2,074	2,059
	t paired	1.099		0.728	
	t ($\nu = 11$, $2\alpha = 0.05$)	2.201			
	Significance	NS		NS	
MTX + VDS ($n = 9$)	$\bar{x}$	4,000	4,019	3,146	3,742
	σ	1,561	1,567	1,460	1,807
	t paired	0.374		4.161	
	t ($\nu = 8$, $2\alpha = 0.05$)	2.306			
	Significance	NS		S	

Table 4. Variations in steady-state level or dose for seven patients receiving infusions of MTX + VDS or MTX alone

		Dose		Steady-state level		Percentage of variation	
		Computed (mg)	Infused (mg)	Predetermined (10^{-8} mol/l)	Achieved (10^{-8} mol/l)	Steady-state level	Dose
MTX + VDS (1st course of therapy)	Beu . . .	4,175	4,900	5,000	5,280		+ 17.4
	Ron . . .	4,260	5,250	5,000	5,000		+ 23.3
	Dan . . .	3,675	4,695	5,000	4,700		+ 27.7
	Eyr . . .	1,775	1,950	2,500	2,560		+ 11.5
	Mad . . .	2,000	2,000	2,500	2,245	− 10.2	
	Deh . . .	460	560	1,000	1,010		+ 21.7
	Bor . . .	1,245	1,245	1,000	850	− 13.6	
MTX alone (2nd course of therapy)	Beu . . .	4,175	4,175	5,000	5,300	0	0
	Ron . . .	4,260	4,260	5,000	5,000	0	0
	Dan . . .	1,840	1,840	2,500	2,580	0	0
	Eyr . . .	1,775	1,775	2,500	2,600	0	0
	Mad . . .	2,000	2,000	2,500	2,500	0	0
	Deh . . .	460	460	1,000	1,060	0	0
	Bor . . .	1,245	1,245	1,000	990	0	0

application with a centrifugal Cobas-Bio analyzer [14], and it was confirmed that VDS concentrations up to 100 µg/ml did not affect the result of the assay.

Results

With the protocol defined, 13 patients received an infusion of MTX + VDS. Only one patient of the 13 reached the predetermined steady-state plasma level with the computed dose. Four patients received this infusion without any modification at 5 h and the steady-state level was lower than the predetermined level (10.2%−29.6%) (Fig. 2). Eight other patients received the treatment with dose readjustment at the 5th h (11.5%−27.6%) and the predetermined plasma level was actually achieved (Table 2).

The statistical comparison (t paired) between predetermined and achieved levels and between computed and infused doses showed that:

In 12 other patients, i.e., not taking part in this study (with other solid tumors) according to the same regimen with HD-MTX alone, the predetermined steady-state levels were actually achieved with the computed doses (Table 3).

In nine patients treated by MTX + VDS protocol with readjustment at 5 h there was no statistically significant difference between predetermined and achieved steady-state levels, but the doses infused were increased (by a mean of 15.9%) (Table 3).

Therefore it seems that the presence of VDS modifies MTX's pharmacokinetic behavior.

To confirm this idea, seven of the 13 patients received an infusion of MTX alone at the computed dose in the second course of treatment (Table 4).

The steady-state plasma levels (10^{-5}, 2.5×10^{-5}, or 5×10^{-5} mol/l) were statistically (t paired) achieved without any dose readjustment (Table 5) during the infusion of MTX alone. But when VDS was associated with MTX the MTX doses had to be increased (+ 17.3%) to achieve the predetermined steady-state level (Fig. 2).

A possible correlation was tested for between the percentage dose increase during MTX + VDS infusions and MTX clearance after the identification IV push (Fig. 3). This correlation was significantly different from 0 (coefficient of correlation −0.708), demonstrating that:

When MTX clearance was lower than 9 l/h, MTX doses had to be substantially increased (by a mean value of 20.8%).

Table 5. Statistical comparison between infusion of MTX alone and of MTX + VDS in the same patients

		Steady-state level (10^{-8} mol/l)		Dose (mg)	
		Predetermined	Achieved	Computed	Infused
MTX + VDS (1st course of therapy) ($n = 5$)	$\bar{x}$	3,700	3,710	2,869	3,471
	σ	1,857	1,852	1,680	2,091
	t paired	0.108		3.059	
	$t(\nu = 4,\ 2\alpha = 0.05)$	2.776			
	Significance	NS		S	
MTX alone (2nd course of therapy) ($n = 5$)	$\bar{x}$	3,200	3,308	2,869	2,869
	σ	1,753	1,797	1,680	1,680
	t paired	2.125		0	
	$t(\nu = 4,\ 2\alpha = 0.05)$	2.776			
	Significance	NS		NS	

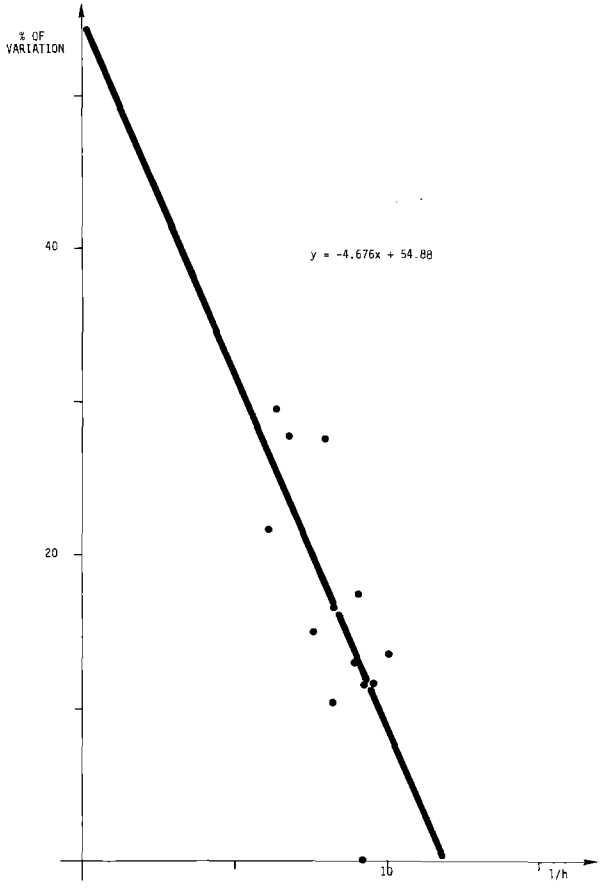

Fig. 3. Correlation between the percentage of dose modification during MTX + VDS infusions and MTX clearance after the MTX identification IV push. The *line* represents the best fit as determined by the unweighted least squares linear regression equation. The coefficient of correlation ($r = -0.708$) was significantly different from 0

When MTX clearance was higher than 9 l/h, the mean MTX dose increase required was only 10.8%.

There is a statistically significant difference between these two means. Therefore it seems that VDS increased the MTX plasma clearance, especially when plasma MTX elimination was very slow after the IV push. To verify that the MTX

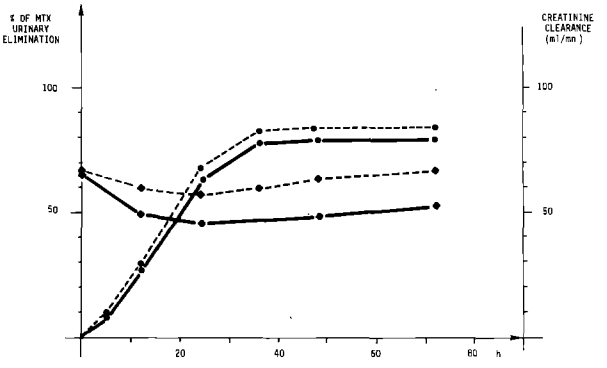

Fig. 4. Creatinine clearance and urinary excretion of MTX over 72 h with infusions of MTX + VDS (◆———◆, creatinine clearance; ●———●, urinary excretion) and of MTX alone (◆– – –◆, creatinine clearance; ● - - - - ●, urinary excretion)

plasma level modification in the presence of VDS was not due to a greater MTX renal clearance, we tested creatinine clearance and urinary MTX excretion in three patients of the 13 during infusions of MTX + VDS and MTX alone.

Beginning with the start of alkalinization, urine was serially collected over 12-h periods until folinic acid infusion was stopped. The creatinine clearance was lower in the presence of VDS, and renal MTX excretion was identical in the two courses of infusions (Fig. 4).

Discussion

The results obtained show that the pharmacokinetic behavior of MTX is substantially modified by the presence of VDS: to achieve the predetermined steady-state levels the computed doses had to be increased (by 11%–28%) for 12 patients of 13. The dose increase required is more pronounced when MTX plasma clearance after the IV push is < 9 l/h. This value (9 l/h) can be compared to the results obtained by Favre et al. [9], who found that an MTX plasma clearance < 9 l/h allowed prediction of a good therapeutic response. In the presence of VDS, the creatinine clearance is lower than and MTX renal excretion identical with that seen in the case of infusion of MTX alone. So the decrease of MTX plasma level during MTX

+ VDS infusions is not due to a greater renal excretion but could result from greater cellular incorporation.

In vitro studies have demonstrated that vinblastine and vincristine enhance MTX transport by 49% and 66%, respectively [21], and that the intracellular exchangeable MTX level is increased by its association with vincristine [13]. Vincristine also synergizes with MTX in vivo [5, 21] and furthermore, Chello and Sirotnak [6] have shown that the synergic interaction between MTX and vinca alkaloids is schedule-dependent in vitro and in vivo.

The results obtained in this study therefore show that vindesine seems to offer the same action as vincristine and vinblastine, which increases the intracellular MTX level necessary for dihydrofolate reductase inhibition with no danger of higher toxicity.

These results seem to be important for the HD-MTX chemotherapy associated with VDS, considering that the cytotoxic activity of MTX in vivo is strictly correlated with the presence of a sufficient free MTX level inside the cell [4, 17, 19] which is limited by an asymmetrical transport carrier system [12]. Vinca alkaloids block the exit mechanism [21] by the inhibition of cellular energy metabolism [10] and by modification of the membrane transport process [11].

References

1. Bertino JR, Fischer GA (1964) Technics for study of resistance to folic acid antagonists. Methods Med Res 10: 297
2. Bischoff KB, Dedrick RL, Zaharko DS, Longstreth JA (1971) Methotrexate pharmacokinetics. J Pharm Sci 60: 1128
3. Cappizi RL, De Conti RC, Marsh JC, Bertino JR (1970) Methotrexate therapy of head and neck cancer: Improvement in therapeutic index by the use of leucovorin "rescue". Cancer Res 30: 1782
4. Chabner BA, Young RC (1973) Threshold methotrexate concentration for in vivo inhibition of DNA synthesis in normal and tumorous target tissues. J Clin Invest 52: 1804
5. Chello PL, Sirotnak FM (1981) Increased schedule-dependent synergism of VDS versus VCR in combination with MTX against L 1210 leukemia. Cancer Treat Rep 65: 1049
6. Chello PL, Sirotnak FM, Dorick DM (1979) Different effects of vincristine on methotrexate uptake by L 1210 cells and mouse intestinal epithelia in vitro and in vivo. Cancer Res 39: 2106
7. Cheng E, Young CW, Wittes RE (1980) Phase II trial of vindesine in advanced head and neck cancer. Cancer Treat Rep 64: 1141
8. Deconti RC, Schoenfeld D (1981) A randomized prospective comparison of intermittent MTX, MTX with leucovorin and a MTX combination in head and neck cancer. Cancer 48: 1061
9. Favre R, Monjanel S, Alfonsi M, Pradoura JP, Bagarry-Liegey D, Clement S, Imbert AM, Lena N, Colonna d'Istria J, Cano JP, Carcassonne Y (1982) High-dose methotrexate: a clinical and pharmacokinetic evaluation. Cancer Chemother Pharmacol 9: 156
10. Fyfe MJ, Goldman ID (1973) Characteristics of the vincristine-induced augmentation of methotrexate uptake in Ehrlich ascites tumor cells. J Biol Chem 248: 5067
11. Fyfe MJ, Lottfield S, Goldman ID (1975) A reduction in energy-dependent amino acid transport by microtubular inhibitors in Ehrlich ascites tumor cells. J Cell Physiol 86: 201
12. Goldman ID (1981) Membrane transport considerations in high-dose methotrexate regimens with leucovorin rescue. Cancer Treat Rep [Suppl 1] 65: 13
13. Goldman ID, Gupta V, White JC, Lottfield S (1976) Exchangeable intracellular MTX levels in the presence and absence of VCR at extracellular drug concentrations relevant to those achieved in high dose MTX folinic acid rescue protocols. Cancer Res 36: 276
14. Imbert AM, Pignon T, Lena N (1983) Methotrexate assay by enzymatic inhibition: comparison between centrifugal analysis (COBAS BIO) and competitive protein-binding assay. Clin Chem (in press)
15. Levitt M, Mosher MB, De Conti RC et al. (1973) Improved therapeutic index of methotrexate with "Leucovorin rescue". Cancer Res 33: 1729
16. Monjanel S, Rigault JP, Cano JP, Carcassonne Y, Favre R (1979) High-dose methotrexate: Preliminary evaluation of a pharmacokinetic approach. Cancer Chemother Pharmacol 3: 189
17. Sirotnak FM, Donsbach RC (1975) Further evidence for a basis of selective activity and relative responsiveness during antifolate therapy of murine tumors. Cancer Res 35: 1737
18. Stoller RG, Jacobs SA, Drake JC, Lutz RJ, Chabner BA (1975) Pharmacokinetic of high-dose methotrexate (NSC-740). Cancer Chemother Rep 6: 19
19. White JC (1981) Recent concepts on the mechanism of action of methotrexate. Cancer Treat Rep [Suppl 1] 65: 3
20. Young CH (1978) Phase II evaluation of vindesine in patients with advanced cancer. Paper presented at the Sixth Vinca Alkaloid Symposium, London, 20 October 1978
21. Zager RF, Frisby SA, Oliviero VT (1973) The effects of antibiotics and cancer chemo-therapeutic agents on the cellular transport and antitumor activity of MTX in L 1210 murine leukemia. Cancer Res 33: 1670

Received October 29, 1982/Accepted October 21, 1983