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Methotrexate-vindesine association in head and neck cancer: modification of methotrexate's hydroxylation in presence of vindesine*

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Summary. High-dose methotrexate (HD-MTX) infusions associated with vindesine (VDS) have been used in the treatment of head and neck cancer. In a previous study, VDS was shown to increase the apparent plasma clearance of MTX.

We have studied the MTX hydroxylation process in the presence of VDS. Different routes of administration have been tested (IV pushes and 24-h or 36-h infusions). A radioimmunoassay has been developed to measure 7-OH-MTX. The defined protocols enabled us to show that VDS influences not only the pharmacokinetic behavior of methotrexate but also its hydroxylation, which is decreased in presence of VDS.

Introduction

Methotrexate (MTX) is one of the most widely used drugs in the treatment of human malignancies [2, 22]. Different dose schedules have been evaluated, most of them with leucovorin rescue. This use of leucovorin rescue, associated with pharmacokinetic considerations, allowed a significant improvement in MTX regimen in combatting the resistance of many carcinomas, such as head and neck tumors and disseminated breast cancer [20, 23].

Another possibility in the treatment of such carcinoma is the combination of two or more drugs [1, 9, 15, 21]. To improve the therapeutic impact of these combinations it is important to know of any interactions between these drugs that might affect either their transport at the site of biotransformation or their metabolism in tumor cells.

On the basis of previous reports demonstrating a synergism between MTX and the vinca alkaloids [6, 7], a protocol associating MTX with vindesine (VDS) was undertaken. The two drugs were administered simultaneously as 36-h infusions, followed at the 36th hour by leucovorin rescue, which was continued until MTX plasma concentrations decreased below $10^{-7}M$. Routine monitoring of MTX plasma concentrations showed that the administration of VDS during the MTX infusions led to lower MTX plasma levels [19]. So, in this study the metabolism of MTX, especially its hydroxylation into 7-hydroxymethotrexate (7-OH-MTX), and the possibility of an influence of VDS on this metabolism have been investigated.

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Materials and methods

Chemicals

Bovine serum albumin (BSA), dihydrofolate reductase (DHFR), NADPH, and dihydrofolic acid were purchased from Sigma Chemicals Company (St. Louis, Mo, USA); MTX and folinic acid were supplied by Lederle Laboratories Division (American Cyanamid Co., Pearl River, NY); VDS was supplied by Eli Lilly and Co (Indianapolis, Ind); and 7-OH-MTX was obtained by hydroxylation of MTX using a partially purified aldehyde oxidase preparation from rabbit liver [18]. The purity of this metabolite was confirmed by UV absorption and high-pressure liquid chromatography with comparison against an authentic standard kindly supplied by Dr B. Chabner (National Cancer Institute, Bethesda, Md).

MTX and 7-OH-MTX determination

MTX assay. MTX plasma concentrations were measured by an enzymatic assay based on DHFR inhibition [3] which has been adapted for a centrifugal analyzer Cobas-Bio [14]. The useful concentration ranges were between 9×10^{-9} *M* and 1.6×10^{-7} *M* (determined by a coefficient of variation (CV) lower than 4.8%). The 7-OH-MTX interference on MTX measurements was lower than 1% [14].

7-OH-MTX assay. Recently we developed a liquid phase radioimmunoassay [4] in which MTX did not interfere significantly (cross-reactivity factor = 2.5×10^{-4}). Concentrations of unknown samples were calculated by an interpolation linearized logit (B/BO) versus log of concentration standard reference curve. With this procedure 7-OH-MTX was between 8.60×10^{-10} *M* and 2.15×10^{-9} *M* (CV lower than 7.5%). All samples were run in duplicate.

Clinical data

Ten patients entered the study. Their ages ranged from 43 to 74 years (Table 1). Only patients with serum creatinine $< 130\ \mu M$, granulocyte count $> 1500/mm^3$, and platelet

Table 1. Patient characteristics

Patient	Age	Sex	Performance status		Tumor localization	Pretreatment
			WHO scale	Karnovsky scale		
MAD . . .	70	M	0	9	Hypopharynx	0
BOR . . .	52	M	0	9	Buccal cavity	Radiotherapy
ROC . . .	57	M	I	8	Buccal cavity	Radiotherapy
WOJ . . .	57	M	0	9	Buccal cavity	Radiotherapy
BOU . . .	43	F	0	9	Breast	Surgery, chemotherapy
HAR . . .	50	F	0	9	Breast	Radiotherapy, surgery, chemotherapy
EYR . . .	57	M	III	5	Oropharynx	Radiotherapy
ROM . . .	74	M	II	7	Oropharynx	Radiotherapy
BER . . .	46	M	II	2	Oropharynx	Radiotherapy
SIM . . .	48	M	0	9	Buccal cavity	0

count $>100000/mm^3$ were eligible to take part in this study.

MTX and VDS (3 mg/m^2) were administered simultaneously as a 36-h infusion. The MTX dose to be infused was calculated according to a test dose protocol described previously [20]: the MTX plasma clearance (Cl) was determined for each patient after an IV bolus administration (50 mg/m^2) from the following equation:

$$Cl\ (l/h) = \frac{\text{IV dose}}{AUC_{0 \rightarrow \infty}},$$

where AUC is the area under the MTX plasma elimination curve calculated by the trapezoidal rule [12].

The 36-h infusion dose (qo) to reach a predetermined plasma level (P) of 10^{-5} *M* was given by the formula:

$$qo\ (mg) = P \times Cl \times 36 \times 454.45,$$

where 454.45 is the molecular weight of MTX.

At the 5th hour of the infusion, MTX plasma levels are about 80% of the predetermined steady state level. This allows adjustment of the MTX dose when the MTX concen-

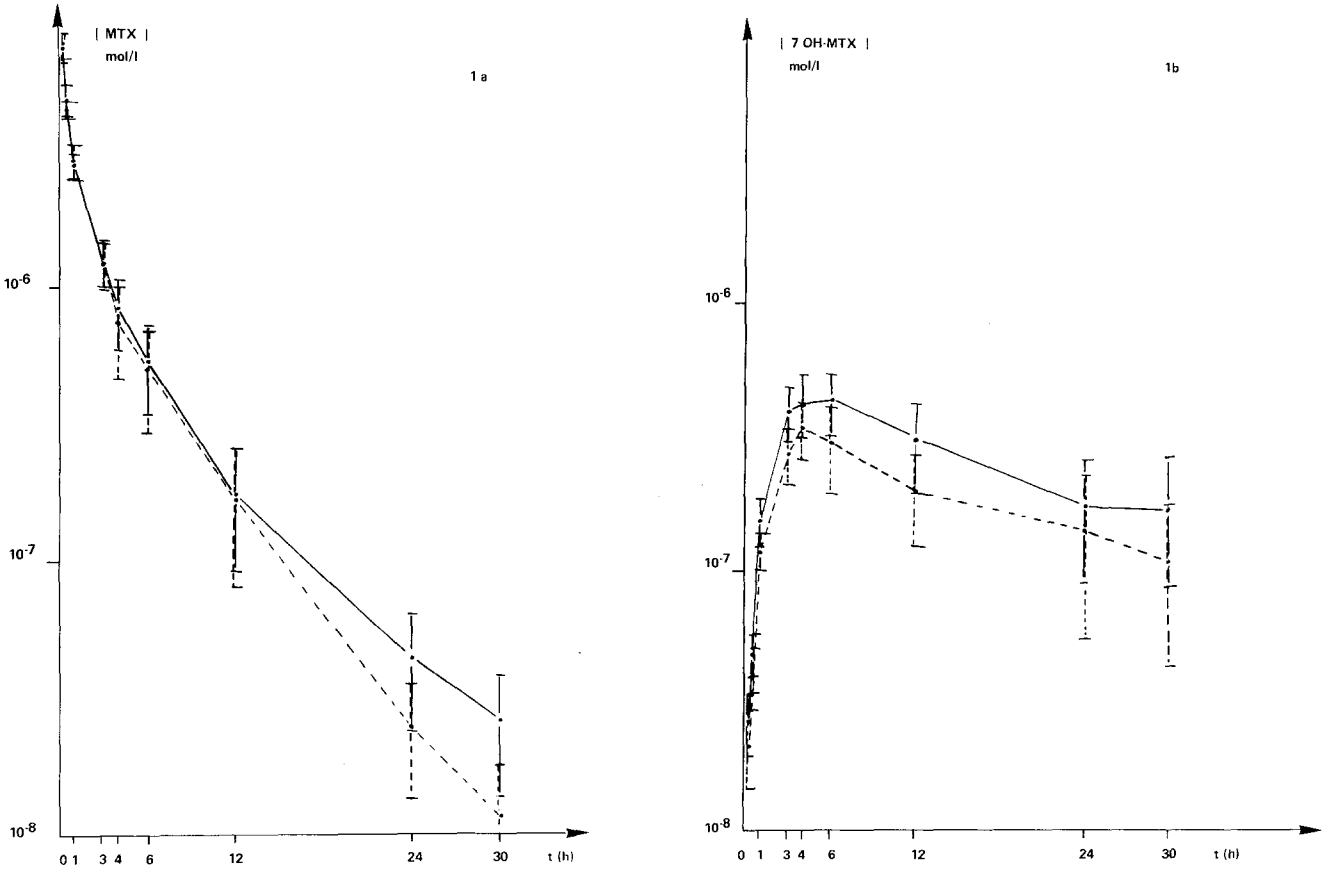

Fig. a, b. Plasma concentrations of MTX **(a)** and 7-OH-MTX **(b)** in six patients after 50 mg/m^2 MTX (●———●) and 50 mg/m^2 MTX +3 mg/m^2 of VDS (●-------●). The plasma values plotted are mean value for these six patients and *bars* indicate the standard deviation

Table 2. MTX pharmacokinetic parameters and statistical comparison[a] between MTX alone and MTX + VDS IV bolus

MTX pharmacokinetic parameters

Patients	MTX dose (mg)	MTX IV bolus		MTX + VDS IV bolus	
		AUC (10^{-8} Mxh)	Cl (l/h)	AUC (10^{-8} Mxh)	Cl (l/h)
MAD . . .	90	3528	5.6	3349	5.9
BOR . . .	90	1282	15.4	1406	14.1
BER . . .	90	1748	11.3	1406	14.1
WOJ . . .	80	1263	13.9	1507	11.7
ROC . . .	75	1210	13.6	1339	12.3
SIM . . .	80	1101	16.0	975	18.1

[a] Statistical analysis

	AUC after MTX IV bolus	AUC after MTX + VDS IV bolus
$\bar{x}$	1689	1664
σ	928	846
t-paired	0.27	
t ($\nu = 5$; $2\alpha = 0.05$)	2.571	
Significance	No	

tration in patient sample is outside the expected range at this time.

Study of VDS interaction on MTX pharmacokinetics

To study the interaction of VDS on MTX pharmacokinetics, six patients received two IV boluses (with and without VDS) and eight patients received at least two infusions (one of them without VDS). Four of these patients received the entire protocol (two IV bolus and two infusions with and without VDS). The administration of MTX alone protocol (by IV bolus or infusion) before or after MTX associated with VDS was randomized.

Results

IV Push

Six patients each received two IV boluses. MTX doses for a MTX IV push ranged between 75 and 90 mg (50 mg/m^2). For MTX + VDS IV push the MTX doses were identical and VDS (3 mg/m^2) was injected at the same time. Blood samples were collected at the following times after the IV push: 0, 0.25, 0.50, 1, 3, 4, 6, 12, 24, and 30 h. The plasma concentration curves for MTX and 7-OH-MTX are shown in Fig. 1.

MTX clearances (Cl) and areas under plasma concentration curves (AUC) have been computed as described previously [20] and are reported in Table 2. MTX parameters are not significantly modified by the presence of VDS. The MTX clearances ranged between 5.6 and 16 l/h (mean value: 12.6 l/h; SD: 1.6 l/h) for MTX IV push and between 5.9 and 18.1 l/h (mean value: 12.7 l/h; SD: 1.6 l/h) for MTX + VDS IV push. Similarly, AUC are not statistically different [*t* paired = 0.26; t ($\nu = 5$; $2\alpha = 0.05$) = 2.571].

As noted in Table 3, within 3 to 6 h (T max) plasma levels of 7-OH-MTX reached a maximum value (C max), ranging between 1.73 and 8.54×10^{-7} *M* after MTX administration and between 8.8×10^{-8} and 7.89×10^{-7} *M* when VDS has been added. The C max values decreased in the presence of VDS for each patient. This difference is statistically significant [t paired = 2.40; *t* ($\nu = 5$; $2\alpha = 0.1$) = 2.01]. Similarly, areas under the 7-OH-MTX plasma concentration curves are significantly decreased (14%–60%).

Infusions over 36-h

Eight patients received two infusions (with and without VDS). Blood samples were taken at the following times: 5, 12, 24, 36 h during the infusion and then every 12 h until a MTX plasma level lower than 10^{-7} *M* was achieved. Figure 2 shows the mean values of the MTX and 7-OH-MTX levels in plasma over 72 h for these eight patients. When MTX was given alone (Fig. 2a) the mean values of MTX

Table 3. 7-OH-MTX pharmacokinetic parameters and statistical comparison[a] between MTX alone and MTX + VDS as IV bolus

7-OH-MTX pharmacokinetic parameters

Patients	MTX IV bolus			MTX + VDS IV bolus		
	C_{max} (10^{-8} *M*)	T_{max} (h)	AUC (10^{-8} *M*xh)	C_{max} (10^{-8} *M*)	T_{max} (h)	AUC (10^{-8} *M*xh)
MAD . . .	85.4	6 h	2916	78.9	6 h	2422
BOR . . .	95.5	4 h 05	1743	48.2	4 h 05	1027
BER . . .	34.5	3 h 15	502	22.0	3 h	258
WOJ . . .	30.4	4 h 35	449	19.3	4 h 05	180
ROC . . .	44.8	4 h 05	683	38.0	4 h 10	587
SIM . . .	17.3	3 h	116	8.8	3 h	54

[a] Statistical analysis

	AUC after MTX IV bolus	AUC after MTX + VDS IV bolus
$\bar{x}$	1068	755
σ	1061	889
t-paired	3.07	
t ($\nu = 5$; $2\alpha = 0.05$)	2.571	
Significance	Yes	

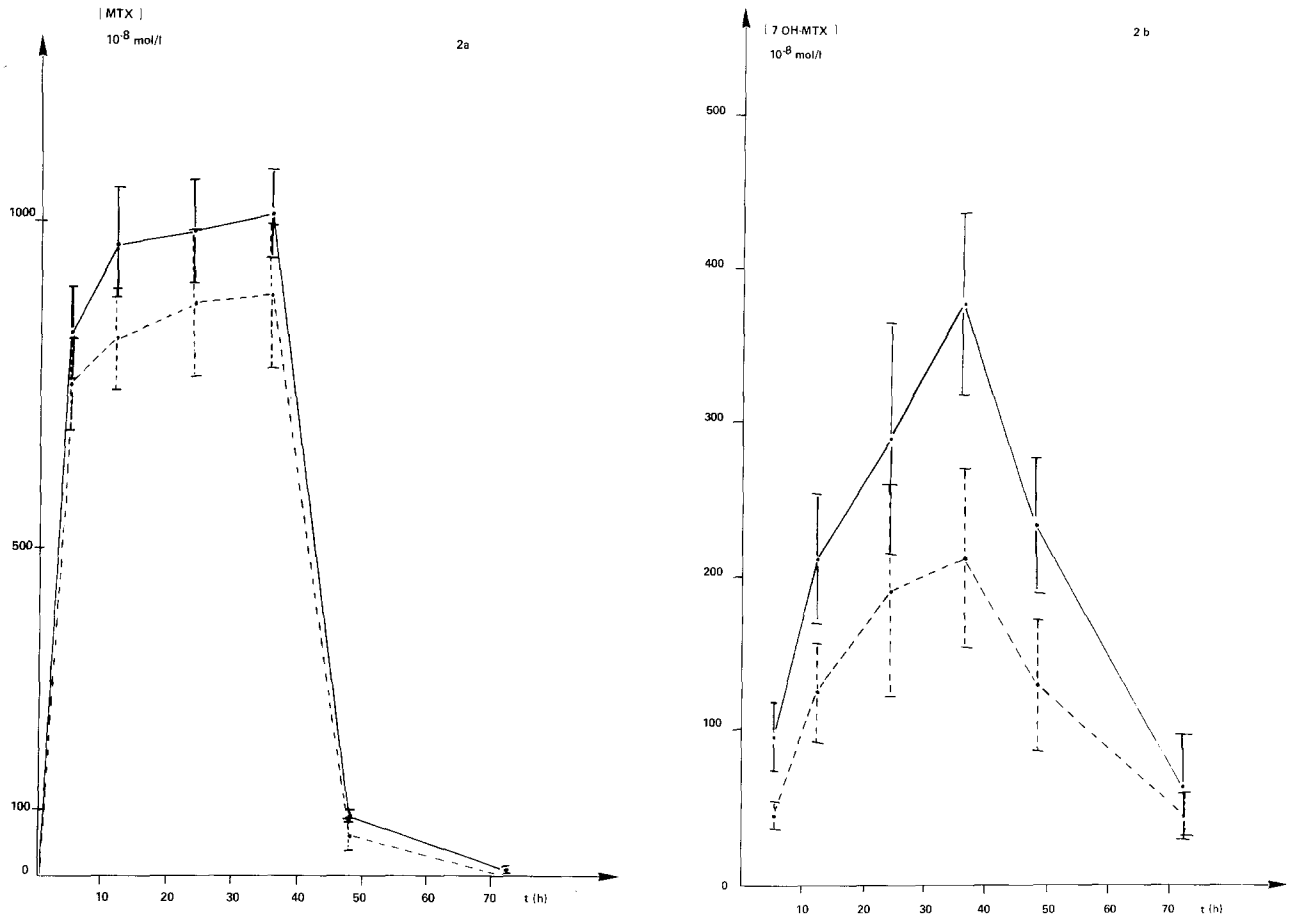

Fig. 2a, b. Effects of 3 mg/m^2 VDS (●- - - -●) administered concomitantly with 36-h HD-MTX infusion in eight patients on MTX **(a)** and 7-OH-MTX **(b)** plasma levels compared with those after infusions of MTX alone (●———●). Plasma values plotted are mean value for eight patients, and *bars* indicate the standard deviation

Table 4. Statistical comparison of the areas under plasma level curves during MTX and MTX + VDS infusions

Patients	AUC[a] MTX		AUC[a] 7OH-MTX	
	MTX alone	MTX + VDS	MTX alone	MTX + VDS
MAD . . .	1.377	0.910	0.590	0.411
BOR . . .	0.842	0.743	0.101	0.028
WOJ . . .	0.770	0.743	0.354	0.261
ROC . . .	0.675	0.642	0.261	0.116
BOU . . .	0.821	0.682	0.247	0.117
HAR . . .	0.924	0.678	0.405	0.190
EYR . . .	1.130	0.879	0.942	0.719
ROM . . .	1.640	1.197	0.533	0.426
$\bar{x}$	1.022	0.809	0.430	0.283
t paired	3.556		7.42	
t ($\nu = 7$; $2\alpha = 0.05$)		2.365		
Significance	Yes		Yes	

[a] AUC (MTX and 7-OH-MTX) are expressed as 10^{-8} *M*/mg MTX injected per hour

plasma levels were 0.98×10^{-5} *M* (SD = 0.08) at the 24th hour and 1.01×10^{-5} *M* (SD = 0.07) at the end of the infusion. In contrast, in the presence of VDS the predetermined plasma MTX level (10^{-5} *M*) was never achieved (0.87×10^{-5} *M* (SD = 0.11) at the 24th hour and 0.89×10^{-5} *M* (SD = 0.11) at the 36th hour). Figure 2 b shows largely a decline in the 7-OH-MTX levels during MTX + VDS infusion: at the 36th hour, 7-OH-MTX levels were 0.38×10^{-5} *M* (SD = 0.06) with MTX alone and 0.21×10^{-5} *M* (SD = 0.06) for MTX + VDS infusions.

Statistical comparisons (*t* paired) of the areas under the curves for MTX and for 7-OH-MTX during infusions with and without VDS showed a significant decrease when VDS was administered simultaneoulsy.

Discussion

Because of the relatively poor response of head and neck carcinoma and disseminated breast cancer treated by monochemotherapy, a need has been felt for protocols associating more than one drug. Several treatments have been developed in the expectation of synergism between the drugs, especially with MTX, one of the most important agents in the treatment of these carcinomas [5, 11, 13].

On the basis of several in vitro studies [10, 25] and a schedule-dependent synergism between VDS and MTX against L1210 leukemia cells [6, 25], a therapeutic protocol associating these two drugs has been developed for the treatment of patients with head and neck carcinoma or disseminated breast cancer.

After a MTX IV push (50 mg/m^2) which allowed determination of the MTX infusion dose according to Monjanel's protocol [20], the patients received 36-h MTX

infusions with or without VDS (3 mg/m^2). In a previous study, we described a decrease in MTX plasma levels during MTX + VDS infusions. This modification was not due to an increase in the renal MTX elimination [19]. We therefore attempted to study the variation in MTX metabolism in the presence of VDS. Plasma concentrations of 7-OH-MTX were monitored further, with the aid of the sensitive and specific radioimmunoassay we developed for this circulating metabolite of MTX.

To observe any interference of VDS on both MTX pharmacokinetics and metabolism, a protocol including two IV boluses (with and without VDS) or at least two infusions, one of them without VDS, was followed. The different routes of MTX and VDS administration (IV bolus and infusion) enabled us to show that VDS influences not only the MTX pharmacokinetic behavior but also the 7-OH-MTX formation.

When MTX and VDS were administered by IV push, VDS did not modify the MTX pharmacokinetic parameters. With regard to 7-OH-MTX, the areas under the curve were largely reduced (29%). T max values were identical and C max decreased, so we can postulate that this reduction was not due to a decreasing rate but to inhibition of or competition in the hydroxylation process. This observation fits in with the results obtained on the isolated perfused rat liver by Strum [24], who showed that the vinca alkoloids inhibit the MTX hepatic uptake. Consequently, the hydroxylation which is catalyzed by an hepatic aldehyde oxidase [17] could be reduced. During the infusion protocols, the same difference was noted in the 7-OH-MTX formation (AUC were decreased of 31.7%), but in this case, the MTX areas under the curve were also reduced (18.4%).

The unmodified MTX pharmacokinetics after an IV push may be explained by the rapid plasma disappearance of the VDS, which could prevent the maintenance of high VDS plasma concentrations for a sufficiently long time.

The observation that VDS decreases the MTX hydroxylation both after an IV push and after a 36-h infusion could have important implications for protocols associating these two drugs. As 7-OH-MTX has been demonstrated to be less soluble than MTX at acid pH, it contributes to the renal toxicity of an HD-MTX regimen [16]. Moreover, this metabolite is an ineffective inhibitor of DHFR, and at high concentrations it could compete with MTX for the membrane transport carrier, then decreasing the MTX cytotoxic activity and consequently the efficiency of the treatment. During the rescue, since the leucovorin is transported by the same carrier as MTX and its metabolite, the influence of the 7-OH-MTX is also very important: 7-OH-MTX plasma concentrations are largely higher than those of MTX. For example, at the 72th hour, the plasma concentration ratio of 7-OH-MTX/MTX is 650 in MTX alone infusions and only 283 when VDS is added. So, the entrance of leucovorin which facilitates the efflux of MTX out of the cells and replenishes the reduced folate pools depleted by the inhibition of DHFR, is reduced by the presence of large amounts of 7-OH-MTX. At the moment we can state that no toxicity has been observed for patients treated by this protocol associating MTX and VDS.

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