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Phase I pharmacokinetic and sequence finding study of the combination of docetaxel and methotrexate in patients with solid tumours

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Abstract

This phase I study was performed to assess the feasibility and possible enhanced antitumour activity of the sequential administration of methotrexate (MTX) and docetaxel (D) in patients with solid tumours. Pharmacokinetic analysis was performed to investigate the pharmacokinetic interaction of the two agents. A total of 22 patients were enrolled, a total of six dose levels were investigated. MTX (days 1 + 15) 30, 40 and 50 mg/m^2 + D (day 2 or day 1) 75 and 85 mg/m^2 with supportive care measures. Both haematological and non-haematological toxicities were significant, preventing dose escalation above MTX 40 mg/m^2 + D 75 mg/m^2. Four partial responses were documented, three in patients with breast cancer, one in a patient with urothelial cell cancer. Pharmacokinetic data did not give an explanation for the significant toxicity as they revealed no interaction of D and MTX kinetics. Methotrexate and 7-OH MTX kinetics seemed to be independent of the administration of D and the moment of D administration appeared not to influence MTX kinetics. The sequential administration of MTX and D results in significant toxicity without any evidence of a clinical benefit.

Keywords: Phase I; Pharmacokinetics; Methotrexate; Docetaxel; Sequential; Synergism

1. Introduction

Docetaxel (D), a microtubule polymerisation promotor, has proven to be effective as a single agent in the second-line treatment of patients with metastatic breast cancer previously treated with anthracycline-based chemotherapy 5-fluorouracil, epirubicin and cyclophosphamide (FEC) or 5-fluorouracil, doxorubicin and cyclophosphamide (FAC), with response rates up to 55% [1,2]. To date, D single agent given at 100 mg/m^2 every 3 weeks has become standard second-line chemotherapy for patients with metastatic breast cancer [3]. Methotrexate (MTX), a dihydrofolate reductase inhibitor, also has demonstrated single agent activity in first- as well as second-line treatment of metastatic breast cancer [4,5].

As anthracycline-based chemotherapy is considered to be more effective than classical cyclophosphamide, MTX and 5-fluorouracil (CMF) and the first is better tolerated with the availability of $5HT_3$ antagonists, the FEC or FAC combination regimens have become the most widely applied first-line treatment in metastatic breast cancer [6]. As a consequence, MTX is not frequently used in first-line, and with the established role of D as second-line therapy, MTX is no longer incorporated in second-line regimens either.

We conducted a phase I study of the combination of D and MTX as a second-line regimen in breast cancer patients. Since D and MTX can be considered effective agents in other solid tumours, patients with non-small cell lung cancer, head and neck cancer, urothelial cancer or gastric cancer were also eligible for this dose-finding study. The study included a pharmacokinetic analysis.

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2. Patients and methods

2.1. Eligibility

Breast cancer patients relapsing after or progressing during anthracycline-based combination chemotherapy were eligible for the study. Patients with other solid tumours for whom treatment with D and/or MTX was considered of therapeutic intent were also eligible for study entry. Additional eligibility criteria were: histologically-confirmed solid tumours; age $\geqslant 18$ years; World Health Organization (WHO) performance status 0-2; adequate haematopoietic (absolute neutrophil count $\geqslant 1.5\times10^9$/l and platelet count $\geqslant 100\times10^9$/l), hepatic (total serum bilirubin $<1\times$ upper normal limit, transaminases $<1.5\times$upper normal limit, alkaline phosphatase $<2.5\times$upper normal limit (except in the presence of bone metastases only and in the absence of any liver disorders) and renal function (serum creatinine <100 μmol/l or creatinine clearance $\geqslant 1$ ml/s); no extensive radiotherapy for at least 4 weeks prior to study entry; indicator lesions should not have been irradiated, previous chemotherapy had to be stopped for at least 4 weeks before study entry; hormonal treatment had to be stopped before study entry; life expectancy of at least 12 weeks; no childbearing potential or using adequate contraception; no previous chemotherapy for systemic disease comprising MTX or a taxoid; no clinically relevant contra-indications for the use of corticosteroids; no somatic or psychic illness that could interfere with the planned treatment or follow-up; no concomitant use of other investigational drugs or anticancer treatment. The clinical protocol was approved by the institutional ethics committee and all patients provided written informed consent.

2.2. Pretreatment and follow-up

Before the start of treatment a medical history was taken and physical examination, laboratory studies, electrocardiogram and imaging studies for tumour measurement were performed. Laboratory studies included a complete blood cell count analysis and measurement of white blood cell differential, sodium, potassium, creatinine, creatinine clearance if indicated, serum calcium, total protein, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH) and urinanalysis.

History, physical examination and toxicity scoring according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) were performed every week. Blood counts were performed weekly. The other laboratory tests were repeated on days 1 and 15. Tumour measurements were performed at 6 week intervals until documentation of progressive disease (PD). In patients with evaluable and measurable disease, standard WHO response criteria were used.

2.3. Drug administration

MTX was administered as an intravenous (i.v.) push on days 1 and 15. Docetaxel was administered as a 1-h infusion on day 2. The sequential administration of MTX and D was based on preclinical data showing synergistic antitumour activity when D was administered at least 12 h after MTX [7,8]. Following dose-limiting toxicity (DLT) observed in the first 3 patients at the first dose level and a recent report on a more favourable dose–toxicity profile by the simultaneous administration of both agents on day 1 [9], the protocol was amended to test this regimen in the second cohort before proceeding with our original study. In this cohort, less toxicity was observed. Since, apparently, the 24-h interval augmented the clinical interaction and in order to obtain the recommended dose and to investigate potential evidence for enhanced antitumour efficacy with that schedule, we continued our study with the initial MTX on day 1 and D on day 2 schedule, complemented with maximum supportive measures to reduce myelosuppression and mucositis. These measures consisted of leucovorin 15 mg orally (p.o.) 4 times daily, starting 24 h after each MTX administration for a total of 2 days, plus lenograstim 263 μg subcutaneously (s.c.) once daily, days 4–10. Treatment was repeated every 3 weeks. All patients received premedication with dexamethasone, p.o. 8 mg twice daily (b.i.d.), starting 1 day before each infusion of D, and given for 3 days.

In each cohort, 3 patients were treated until DLT during the first cycle was observed in 1 patient. If two or more DLTs were observed, that dose was considered too high. In the case of one DLT, the accrual of 3 additional patients was required. If DLT was seen in no more than 1 patient on that dose level, the dose was to be escalated. From then onwards 6 patients were to be entered at each dose level and dose escalation was to continue if a DLT was seen in no more than 1 patient. The dose level at which 2 or more patients experienced DLT was to be considered the maximum tolerated dose (MTD). DLT was defined as grade 4 neutropenia lasting $\geqslant 5$ days, or neutropenia with fever causing hospitalisation for administration of i.v. antibiotics, or grade 4 thrombo-cytopenia, or grade 2 mucositis lasting $\geqslant 5$ days, or any grade 3 or 4 non-haematological toxicity (except grade 3 nausea). The following dose levels and escalating steps were planned: MTX 30 mg/m^2–D 75 mg/m^2; MTX 30 mg/m^2–D 85 mg/m^2; MTX 30 mg/m^2–D 100 mg/m^2; MTX 40 mg/m^2–D 100 mg/m^2; and MTX 50 mg/m^2–D 100 mg/m^2. If a patient required dose reductions after experiencing DLT, the next cycles in this patient were evaluated for toxicity at the lower dose level.

2.4. Sampling schedule and drug analysis

Blood specimens were obtained in all patients during the first and second courses of treatment. Blood volumes of 5 ml were drawn directly into Vacutainer glass tubes containing lyophilised lithium heparin (Becton Dickinson, Meylan, France) from a peripheral venous access device. Samples for MTX analysis were collected immediately before treatment and at 0.08 (end of infusion), 0.5, 2, 4, 6, 24 and 48 h after dosing. All blood samples were centrifuged immediately for 5 min at 3000*g* to yield plasma, which was frozen in polypropylene vials at −80 °C until the time of analysis by reversed-phase high-performance liquid chromatography (HPLC) as described in Ref. [10]. In brief, samples were purified by protein precipitation with acetone followed by solvent extraction with a mixture of *n*-butanol and diethyl ether. The water phase was subsequently further processed with methanol and dried under N_2. The compounds of interest, including the unchanged parent drug and its 7-hydroxyl metabolite (7-OH-MTX), were separated on an analytical column (150×4.6 mm, I.D.) packed with (5 μm P.S.) RP Inertsil ODS-80A material (delivered by Alltech, Breda, The Netherlands) and were eluted in a solvent system containing 5% (v/v) tetrahydrofuran in water (pH 2.0). The column effluent was monitored by ultraviolet (UV) absorption measurements using a Spectra Physics Model UV-2000 detector (San Jose, CA, USA) set at 313 nm. Detection and integration of chromatographic peaks was performed by the Fisons ChromCard data analysis system connected to an ICW workstation (Milan, Italy). Calibration curves were fitted by weighted ($1/x^2$) linear-regression analysis by using the peak area of MTX versus the concentrations of the nominal standard (x). The detector response was linear in a concentration range of 10–10 000 ng/ml, with a lower limit of quantitation of MTX and 7-OH-MTX of 10 ng/ml using 1-ml sample aliquots. Values for accuracy and within-run and between-run precision were 95.5–111 and 3.69–11.0%, respectively.

Evaluation of D pharmacokinetics was performed using plasma samples obtained before drug infusion and at 0.5, 1, 2, 4, 6 and 24 h after the start of the drug infusion. Samples were analysed by a HPLC method described in detail elsewhere in Ref. [11]. This method is based on the extraction of D from the plasma matrix with a mixture of acetonitrile and *n*-butyl chloride (1:4, v/v), using paclitaxel as an internal standard. Chromatographic separations were performed on the same analytical column (see above) using a mobile phase composed of water–methanol–tetrahydrofuran (37.5:60.0:2.5, v/v/v) containing 0.1% (v/v) ammonium hydroxide, with the pH adjusted to 6.0 (formic acid). During analysis, the column was maintained at 60 °C using a Spark Model SpH99 column oven (Meppel, The Netherlands), and the eluent was monitored at a wavelength of 230 nm. The lower limit of quantitation was 10 ng/ml, with the accuracy and precision ranging from 95.5 to 106% and 0.33 to 3.34%, respectively.

2.5. Pharmacokinetic data analysis

Individual plasma concentration–time data were analysed using the Siphar v4.0 software package (SIMED, Créteil, France), by determination of slopes and intercepts of the plotted curves with multi-exponential functions. The program determined initial parameter estimates and these were improved using an iterative numerical algorithm based on Powell's method [12]. Model discrimination was assessed by a variety of considerations, including visual inspection of the predicted curves, dispersion of residuals, minimisation of the sum of weighted squares residuals, and the Akaike information criterion [13]. In all cases, concentration–time profiles of both MTX and D were best fitted to a bi-exponential equation after zero-order input with weighting according to y_{obs}^{-1}. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic parameters using standard equations [14].

2.6. Statistical considerations for pharmacokinetics

Parameters of all compounds are reported as mean values ± standard deviation. The difference in pharmacokinetic parameters between the MTX administration days and between patient cohorts was evaluated statistically using a two-sided parametric matched-pairs Student's *t*-test (after testing for normality) *plus* the 95% confidence intervals and the Kruskal–Wallis statistic, respectively. The effect of D interval time on the pharmacokinetics of MTX was analysed by the Mann–Whitney test. Probability values (two-sided) of less than 0.05 were considered statistically significant. All calculations were done on the Number Cruncher Statistical Systems v5. software package [29].

3. Results

A total of 22 eligible patients entered this study, receiving a total of 79 cycles. Patient characteristics are shown in Table 1. All patients were evaluable for toxicity. 18 of the 22 patients were also evaluable for tumour response. Toxicity data are shown in Tables 2 and 3. Myelosuppression, mucositis and fatigue were the principal DLTs observed with this regimen. Neutropenia grade 4 prevented the administration of MTX day 15 in 33% of the cycles at the first two dose levels.

Table 1
Patient characteristics

Patients treated			22	
Number of cycles			79	
Age median (range) years			49 (30–64)	
WHO performance score				
	0		5	
	1		14	
	2		3	
Sex				
	Male		9	
	Female		13	
Prior chemotherapy				
		No	Yes	Total
Primary tumour				
Breast cancer		–	9	9
Non-small cell lung cancer		–	3	3
Stomach cancer		2	–	2
Urothelial cell cancer		3	–	3
Head/neck cancer		3	–	3
Sarcoma		1	–	1
Adenocarcinoma of the unknown primary		1	–	1

WHO, World Health Organization.

The following dose levels were explored:

1. MTX (days 1 + 15) 30 mg/m^2–D (day 2) 75 mg/m^2 3 patients
2. MTX (days 1 + 15) 30 mg/m^2–D (day 1) 75 mg/m^2 4 patients
3. MTX (days 1 + 15) 30 mg/m^2–D (day 2) 75 mg/m^2 + supportive care measures 3 (4) patients
4. MTX (days 1 + 15) 30 mg/m^2–D (day 2) 85 mg/m^2 + supportive care measures 3 patients
5. MTX (days 1 + 15) 40 mg/m^2–D (day 2) 75 mg/m^2 + supportive care measures 7 (9) patients and
6. MTX (days 1 + 15) 50 mg/m^2–D (day 2) 75 mg/m^2 + supportive care measures 2 patients.

(Numbers of patients in parentheses represent the total number of patients evaluated for toxicity at a dose level, including patients who were treated at this dose level after a dose reduction.)

At the first dose level (MTX30/D75 day 2), all 3 patients obtained a DLT in the first course. 2 patients had significant mucositis (grades 3 and 2 lasting more than 5 days). In the third patient, MTX day 15 was postponed because of neutropenia grade 4 lasting more than 5 days. According to the amended protocol, at the second dose level (MTX30/D75 day 1) patients received MTX and D both on day 1 followed by MTX on day 15. One out of the 4 patients treated with this schedule experienced a grade 4 neutropenia preventing the administration of MTX at day 15. There were no DLT events observed. Hence, the toxicity at this dose level compared favourably with that obtained with the sequential administration in dose level 1. At the next dose levels, again using the 24-h interval between MTX and D, the supportive care measures (s.c.m.) were introduced.

At dose level 3 (MTX30/D75 day 2 + s.c.m.), febrile neutropenia was observed in 1 patient, which was considered a DLT. No other significant toxicities were seen at this dose level. Since there were no additional cases of neutropenic fever amongst the 6 patients on dose levels 1 and 3, escalation to dose level 4 was performed. All patients received MTX on day 15 in all courses. At dose level 4 (MTX30/D85 day 2 + s.c.m.), 1 out of 3 patients experienced mucositis grade 2 concomitant with fatigue grade 3 which were considered dose-limiting and 1 patient obtained mucositis grade 3 in the second course that was also considered dose-limiting since this patient already experienced significant mucositis grade 2 in the first course.

At dose level 5 (MTX40/D75 day 2 + s.c.m.), febrile neutropenia was observed in 1 out of 6 patients treated. The first 2 patients who were treated at dose level 6 (MTX50/D75 day 2 + s.c.m.) required a dose reduction after obtaining DLT. One patient had grade 3 fatigue, the second patient had grade 3 myalgia. This dose level

Table 2
Myelotoxicity (worst toxicity per cycle) at each dose level per cycle[a]

MTX (mg/m^2)	Tax. (mg/m^2)	Pts (*n*)	Cycles (*n*)	WBC grade (CTC scale)				ANC grade (CTC scale)				PLTS grade (CTC scale)			
				1	2	3	4	1	2	3	4	1	2	3	4
30	75 d2	3	12	0	0	6	4	1	1	1	7	1[b]	1	0	0
30	75 d1	4	21	9	2	6	0	3	5	4	4	1[b]	4	0	0
30	75 d2 Supportive care	3 (4)	15 (16)	1	2	10	1	0	2	3	7	1[b]	1	0	0
30	85 d2 Supportive care	3	9	0	1	4	3	0	1	1	6	3	0	0	0
40	75 d2 Supportive care	7 (9)	10 (19)	0	1	7	2	0	0	2	6	2[b]	0	0	0
50	75 d2 Supportive care	2	2	0	0	0	1	0	0	0	1	0	0	0	0

pts, patients; WBC, white blood cells; ANC, absolute neutrophil count; CTC, Common Toxicity Criteria; PLTS, platelets; MTX, methotrexate; Tax, taxane; d, day.

[a] Numbers in parentheses represent total of patients/cycles evaluated for toxicity at a certain dose level, including patients treated/evaluable cycles at that dose level after a dose reduction.

[b] Denotes dose-limiting toxicity events.

Table 3
Main toxicities (worst per cycle) at each dose level, expressed in the number of cycles that they occurred[a]

MTX (mg/m^2)	Tax. (mg/m^2)	Pts. (*n*)	Cycles (*n*)	Mucositis grade (CTC scale)				Fatigue grade (CTC scale)				Myalgia grade (CTC scale)			
				1	2	3	4	1	2	3	4	1	2	3	4
30	75 d2	3	12	6	1[b]	1[b]	0	2	5	1	0	1	2	0	0
30	75 d1	4	21	9	2	0	0	10	0	1	0	1	1	0	0
30	75 d2 Supportive care	3 (4)	15 (16)	7	5	0	0	8	5	0	0	0	0	0	0
30	85 d2 Supportive care	3	9	0	2 1[b]	1[b]	0	0	5	1[b]	0	1	1	0	0
40	75 d2 Supportive care	7 (9)	10 (19)	5	3	0	0	4	7	0	0	2	5	0	0
50	75 d2 Supportive care	2	2	0	0	0	0	0	1	1[b]	0	0	1	1[b]	0

pts, patients; CTC, Common Toxicity Criteria; MTX, methotrexate; Tax, taxane; d, day.

[a] Numbers in parentheses represent total of patients/cycles evaluated for toxicity at a certain dose level, including patients treated/evaluable cycles at that dose level after a dose reduction.

[b] Denotes dose-imiting toxicity events.

was considered the MTD for the escalation of MTX. In the subsequent courses at reduced dose (level 5), no serious toxicities were reported. Dose level 5 (MTX40/D75 day 2 + s.c.m.) was determined to bethe recommended dose level. At dose levels with supportive care measures, neutropenia grade 4 was a common side-effect (50% of cycles), but this was generally brief and did not interfere with the planned treatment schedule as observed in dose level 1.

Overall, nausea and vomiting were mild (grade 1). Mucositis, fatigue and myalgia were significant non-haematological side-effects.

Of the 10 (out of the total of 22) patients who obtained DLT, 7 had previously been treated with chemotherapy.

3.1. MTX and D pharmacokinetics

The possible effect of the drug schedule and interval time between drug administration on the pharmacokinetics of MTX was investigated in 10 patients (4 given D immediately following MTX and another 6 given D 24 h later). Unpaired analysis indicated that changing the treatment interval to 24 h had no significant influence on any of the studied parameters (P = N.S., Table 4). Plasma concentration–time profiles of MTX and its metabolite 7-OH-MTX in patients treated with MTX alone or in combination with D are displayed in Fig. 1. The time course of MTX concentrations in all patients was best described with a tri-exponential function. Concentrations of 7-OH-MTX increased slowly after MTX administration and peaked consistently at 4 h after dosing. The metabolite data best fitted a two-compartmental model with a lag-phase of approximately 10 min preceding the appearance of 7-OH-MTX in plasma. A summary of paired MTX pharmacokinetic parameters in the absence and presence of D obtained from 15 patients is given in Table 5. The total plasma clearance as well as the terminal disposition half-life of MTX was not significantly altered by D administration in any patient (P = N.S., Table 5). Similarly, the formation and subsequent disposition of the MTX metabolite was not substantially altered by D cotreatment ($P > 0.13$), although it is possible that minor alterations were obscured by the substantial interindividual variability in the generated data. Over the various dose levels examined, D pharmacokinetics was not dependent on the MTX dose level, with an overall mean D clearance of 17.3 ± 5.32 l/h/m^2 (Table 6).

3.2. Responses

18 of the 22 patients were evaluable for response. 3 patients with metastatic breast cancer treated at dose levels 1, 2 and 3 obtained a partial response, with a response duration of 13, 9 and 6 months, respectively.

Table 4
Effect of docetaxel (D) interval time on the pharmacokinetics of MTX[a]

Parameter	Interval time (h)		*P* value[b]
	0 ($n = 4$)	24 ($n = 6$)	
MTX			
C_{max} (ng/ml)	4803 ± 1828	9012 ± 7339	0.286
AUC (μg h/ml)	6.63 ± 1.96	8.72 ± 5.38	0.670
CL (l/h/m^2)	4.80 ± 1.29	4.41 ± 2.18	0.670
$T_{1/2}$ (h)	2.84 ± 1.09	1.88 ± 1.03	0.201
7-OH-MTX			
C_{max} (ng/ml)	110 ± 30.0	160 ± 42.7	0.088
T_{max} (h)	4.62 ± 0.95	4.44 ± 0.78	0.831
AUC (ng h/ml)	2.06 ± 0.69	2.97 ± 1.34	0.166
$T_{1/2}$ (h)	7.06 ± 0.31	8.56 ± 2.90	0.831
REC	0.33 ± 0.12	0.41 ± 0.26	0.999

MTX, methotrexate; C_{max}, peak plasma concentration; AUC, area under the plasma concentration–time curve; CL, total plasma clearance; $T_{1/2}$, apparent half-life of the terminal disposition phase; T_{max}, time to peak plasma concentration; REC, relative extent of conversion of MTX into 7-OH-MTX (i.e. $AUC_{7\text{-OH-MTX}}/AUC_{MTX}$).

[a] Data were obtained from patients treated with MTX at a dose level of 30 mg/m^2 with docetaxel (75 mg/m^2) given immediately following MTX or 24 h later.

[b] Mann–Whitney test.

One additional patient with urothelial cancer treated at dose level 4 obtained a partial response that lasted 7 months. No complete responses were documented. 9 patients had stable disease for a median duration of 3 months (range 6 weeks–6 months).

4. Discussion

In the present study, the feasibility and recommended dose of the sequential use of D and MTX was investigated. *In vitro* data had shown that the administration of edatrexate, a MTX analogue, 24 h prior to D resulted in greater cytotoxicity than the simultaneous administration or administration of D prior to edatrexate [7]. This interaction was also reported *in vitro* with the combination of MTX and paclitaxel [8]. A phase I investigation of the sequential use of MTX given on day 1 followed by paclitaxel on day 2 showed the combination to be feasible, but pronounced myelotoxicity indicated enhanced toxicity [15].

In the current study, MTX was given as an i.v. push on days 1 and 15. D was administered as a 1-h infusion on day 2. Treatment was repeated every 3 weeks. In the first cohort of sequential administration of the two agents, all 3 patients experienced DLT, consisting of mucositis and/or neutropenia grade 4. In a recent report by Guillot and colleagues [9], modest toxicity was reported when MTX and D were both administered on day 1. In order to investigate this possible clinically significant schedule-dependent difference in toxicity, by amending the protocol the next 4 patients received both agents on day 1 without changing the drug doses. Indeed, we found significantly less toxicity, suggesting a sequence-dependent pharmacodynamic interaction. Seeking suggestive evidence of an enhanced cytotoxic interaction, we continued to study the original drug sequence, adding optimal drug support, consisting of lenograstim and leucovorin prophylaxis. Despite the administration of these supportive care measures, toxicity remained significant, prohibiting dose escalation of MTXC above 40 mg/m^2 and D above 75 mg/m^2. The significant toxicity observed with this combination was not restricted to patients who had previously been treated with chemotherapy, as three out of the total of 10 DLTs were obtained in chemonaive patients. Moreover, the findings of no more than four partial responses in tumours potentially sensitive to D and/or MTX was disappointing and does not suggest enhanced antitumour effects.

We evaluated potential drug–drug interactions and the possible role of schedule of drug administration of MTX and D. D undergoes significant hepatic metabolism, mainly by the cytochrome P-450 3A4 isozyme [16,17], and its use has been associated with kinetic interactions, for example with doxorubicin [18], epirubicin [19,20], and topotecan [21]. In addition, a number of reports have shown that the D-formulation vehicle, polysorbate 80 (Tween 80), profoundly alters methotrexate pharmacokinetics by changing renal and biliary excretion profiles in mice [22]. The observed pharmacokinetic parameters of MTX and its aldehyde oxidase-mediated metabolite 7-OH-MTX demonstrated linear and dose-independent behaviour over the dose range studied, similar to single-agent data [23]. We also found that parameters of MTXC and 7-OH-MTX pharmacokinetics were independent of the concomitant administration of D and comparable data were obtained in the schedule with a 24-h interval between the admin-

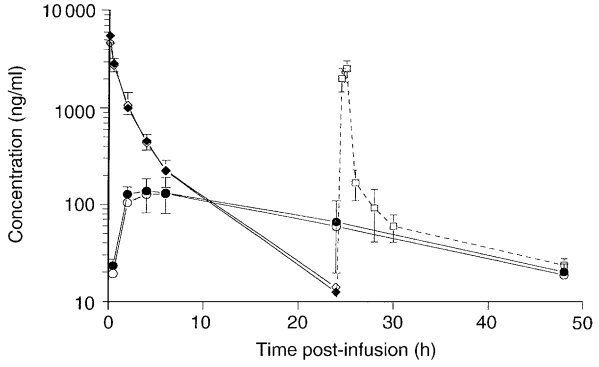

Fig. 1. Plasma concentration–time profiles of methotrexate (MTX) (diamonds) and 7-OH-MTX (circles) in patients treated with MTX alone at 40 mg/m^2 as a 5-min intravenous (i.v.) infusion (open symbols) or in combination with docetaxel (D) (closed symbols) given on day 2 at 75 mg/m^2 as a 1-h i.v. infusion. The plasma concentration–time profile of D in indicated by squares and the dotted line (infusion from 24 to 25 h after MTX dosing). Data are presented as mean values of 6 pharmacokinetically evaluable patients (symbol)±standard deviation (S.D.) (error bar).

Table 5
Summary of paired MTX pharmacokinetics in the absence and presence of D[a]

Parameter	MTX	MTX + D	95% C.L.	P value[b]
MTX				
CL (l/h/m^2)	4.68±1.40	4.35±1.40	−0.78, 0.12	0.135
$T_{1/2}$ (h)	2.48±1.05	2.60±1.28	−0.45, 0.24	0.675
7-OH-MTX				
T_{max} (h)	5.16±1.04	4.61±1.20	−1.34, 0.24	0.155
CL_{app} (ng h/ml)	16.9±22.7	17.2±16.2	−6.78, 7.45	0.921
$T_{1/2}$ (h)	8.63±3.90	9.23±3.40	−0.49, 1.68	0.260
REC	0.49±0.29	0.38±0.21	−0.26, 0.04	0.134

MTX, methotrexate; D, docetaxel; CL, total plasma clearance; $T_{1/2}$, apparent half-life of the terminal disposition phase; T_{max}, time to peak plasma concentration; CL_{app}, apparent total plasma clearance; REC, relative extent of conversion of MTX into 7-OH-MTX (i.e. $AUC_{7\text{-OH-MTX}}/AUC_{MTX}$); 95% C.L., 95% confidence limits for the mean difference.

[a] Data were obtained from 15 patients treated on the first day with MTX at a dose level of 30, 40 or 50 mg/m^2 and D given on day 2 at a dose level of 75 or 85 mg/m^2.

[b] Two-sided paired Student's *t*-test.

Table 6
Effect of MTX dose on the pharmacokinetics of D

D dose (mg/m^2)	75	75	75	85	
MTX dose (mg/m^2)	30	40	50	30	Overall mean
n	6	7	2	4	19
C_{max} (ng/ml)	2413±672	2691±726	2694±478	2898±223	–
AUC (ng h/ml)	3.92±1.34	4.85±0.99	4.97±1.04	6.06±1.62	–
CL (l/h/m^2)	21.1±7.33	15.9±2.66	15.4±3.23	14.9±4.34	17.3±5.32*
MRT (h)	4.51±3.23	10.4±6.33	5.87±3.33	5.56±4.73	7.04±5.26
$V_{d,ss}$ (l/m^2)	87.8±61.2	156±65.4	85.2±32.4	75.1±62.5	110±67.1
$T_{1/2}$ (h)	9.63±6.87	16.0±5.48	13.3±1.74	9.72±1.88	12.4±5.77

D, docetaxel; MTX, methotrexate; C_{max}, peak plasma concentration; AUC, area under the plasma concentration–time curve; CL, total plasma clearance; MRT, mean residence time; $V_{d,ss}$, volume of distribution at steady-state; $T_{1/2}$, apparent half-life of the terminal disposition phase.
*$P = 0.256$, Kruskal–Wallis test.

istration of both agents, indicating no apparent pharmacokinetic interaction. The lack of polysorbate 80 effect on MTX pharmacokinetics may be due to the fact that this surfactant is very extensively metabolised in humans within the systemic circulation into oleic acid and polyoxyethylene sorbitol [24]. In fact, the polysorbate 80 peak plasma levels observed in cancer patients receiving D (100 mg/m^2 over 1 h) were only 0.16±0.05 μl/ml (mean±S.D.), suggesting that it could not have interfered in MTX disposition in our patients [24]. The pharmacokinetic behaviour of D was also independent of the MTX dose, and similar to the single-agent data [25]. Thus, overall, our plasma pharmacokinetic data do not provide an explanation for the degree of toxicity observed with the combination of MTX and D. It is important to realise, however, that sequence and schedule-dependent differences in toxicity and pharmacokinetics can be obscured by large inter- and intra-individual variability in systemic exposure. Indeed, high (5- to 7-fold) variability has been reported in D and MTX area under the plasma concentration–time curve (AUC) [25,26]. However, because patients received MTX both in the presence and absence of D, grossly abnormal plasma clearance values required to explain the toxicity of the combination should have been noted in the present study. It is more likely that alternative mechanisms, undetected by the current analytical methods, have contributed to the enhanced toxicity associated with the combination of MTX and D. One of these mechanisms might be an effect of D on the intracellular polyglutamation of MTX and 7-OH-MTX by the enzyme folylpolyglutamate synthetase (FPGS). This protein is an important pathway for the selective intracellular retention of naturally occurring folates, and is also an important determinant of MTX-induced cytotoxicity [27,28]. Clearly, the pharmacological effects of the combination of antifolates and antimicrotubule anticancer agents seem to be rather complex and will need further (pre)clinical investigation to be fully understood. Hence, our pharmacokinetic data do not give an explanation for the amount of toxicity as they revealed no interaction of D and MTX kinetics. MTX and 7-OH-MTX kinetics seemed to be independent of the administration of D and the moment of D administration appeared not to influence MTX kinetics.

We conclude that the sequential administration of MTX and D results in significant toxicity without evidence of an enhanced activity.

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