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Phase I–II Intraperitoneal Mitoxantrone in Advanced Pretreated Ovarian Cancer

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36 previously treated patients (25 with anthracyclines) with advanced epithelial ovarian cancer have been treated with intraperitoneal (i.p.) mitoxantrone (M) at increasing doses. The response was evaluated through repeated laparoscopy with multiple biopsies and serial measurement of Ovarian Cancer Antigen 125 (CA 125); 11/36 patients had a complete (6 patients) or partial (5 patients) response. Toxicity (both local and general) was observed starting from 25 mg/m² of M per cycle. The amount of drug reaching systemic circulation was monitored by measuring M plasma value after i.p. treatment. This study showed wide variations in serum levels obtained after i.p. doses ranging from 23 to 36 mg/m². The area under the curve (AUC) of mitoxantrone plasma samples, did not correlate with the i.p. administered dose. Conversely, a correlation seems to exist between the plasma AUC and the responder status. Patients who showed clinical responses to i.p. treatment with mitoxantrone had AUCs and plasma peak levels of the drug that were significantly higher than those in non-responders ($P = 0.03$, Fisher's exact test).
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INTRODUCTION

INTRAPERITONEAL (i.p.) treatment of ovarian cancer with drugs delivered in large fluid volumes represents a valid alternative to the more traditional intravenous (i.v.) route [1–4].

Mitoxantrone (M) is a new anthraquinone derivative of anthracene with established activity in breast cancer, acute leukaemia and malignant lymphoma [5, 6].

In ovarian cancer the clinical efficacy of this new drug given by conventional i.v. treatment has been only moderate in various experiences [5–10] (Table 1). In contrast, M proved to be the most effective cytotoxic agent against human ovarian cancer cells in the human clonogenic assay [11]. The concentrations tested *in vitro* (0.1–10 μg/ml) however, are not commonly attained after i.v. administration [12].

We started treating patients with intraperitoneal M to ascertain whether local high drug concentrations could improve the benefit/toxicity ratio.

PATIENTS AND METHODS

Patients' characteristics

36 patients entered this trial from June 1987 to October 1990. Patients' characteristics are shown in Table 2. All patients had recurrent or advanced ovarian cancer and all were previously treated with multiple chemotherapy regimens; 25 of them had received anthracyclines at the total mean dose of 258 mg/m² (range 128–500). A total of 116 M cycles were administered (Table 2). Patients entered into this trial if they met the following criteria: (a) life expectancy of at least 2 months and a WHO performance status less than 3; (b) laboratory evidence of normal renal and hepatic functions, with white blood cells (WBC) more than 4000 and platelet count more than 100 000 per μl; (c) no clinical evidence of cardiac disease (assessed by electrocardio-

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Table 1. Studies on mitoxantrone. Doses and efficacy in patients with advanced ovarian cancer

Reference	Dose of M	Schedule	No. of patients evaluable	Activity (CR)
7	14–16 mg/m² i.v.	Every 3 weeks	46	12 (2)
10	12–14/20 mg/m² i.v./i.p. mitox 75–100/180 mg/m² i.v./i.p. DDP	Every 3 weeks Every 3 weeks	32/4 32/4	23 (11) adjuv.
3	12/38 mg/m² i.p.	Every 4 weeks	17	5
16	10–40 mg/m² i.p.	Every 4 weeks	21*	5†
9	12–14 mg/m² infusion 14–36 mg/m² i.p.	Every 3 weeks Every 4 weeks	20 22	1 7 (4)
28	0.5–1.3 mg/m²/day i.v. infusion of 21 days	Every 6 weeks	25	phase I
15	30 mg/m² i.p.	Monthly	29	9 (5)

* 9 gastrointestinal, 6 ovarian, 2 unknown, 4 primary cancer and 1 peritoneal carcinomatosis.
† 2 ovary, 1 gastric, 1 breast, 1 mesothelioma.
adjuv. = adjuvant setting. DDP = cisplatin.

gram, blood pressure and pre-ejection time/left ventricular ejection time—PEP/LVET); (d) previous treatment with less than 400 mg/m² total doxorubicin dose.

Efficacy evaluation and toxicity was assessed according to WHO criteria [13].

Tumour extent, or stage, was assessed according to the FIGO classification 1986 [14].

Treatment

Mitoxantrone was supplied by Lederle Italia (Catania, Italy) as a parenteral formulation in 10 ml ampoules at a concentration of 2 mg/ml; M was present as free base in acetic buffer.

The drug was dissolved in the entire volume of the infusate (2l) of normal saline previously warmed up to 37°C immediately before the intraperitoneal administration, and infused through an i.v. plastic catheter (B.D. i.v. catheter Becton Dickinson and Company, Rutherford, New York, U.S.A.) positioned under local anaesthesia through a 19G inner steel needle. Unlike other authors [15, 16], we changed the intraperitoneal catheter at every cycle for all cases. The administration time normally lasted 0.5–1 h.

After the infusion no attempt was made to tap the peritoneal space, apart from in 4 patients, so that the drug was allowed to dwell within it indefinitely. If abdominal pain arose during M infusion, we added i.p. bupivacaine hydrochloride (*N*-butyl-2-piperidincarbon)-2,6-dimetilanilide 0.25%, 10–20 ml and up to 40 ml if pain persisted.

The initial M dose started at 23 mg/m², as advised by Alberts *et al.* [12]. We reduced the initial dose to 14 mg/m² in 2 patients: in 1 for three cycles and in the other for only the first cycle, because these patients were considered to have low performance status (according to WHO). In another 3 patients, whose initial doses were 25, 32 and 25 mg/m², respectively, the doses were then reduced by 25% because these patients had grade 3 local toxicity (abdominal pain).

The initial dose was progressively increased by 1–3 mg/m² only in the absence of grade 3 toxicity. The cycle was repeated every 28 days. The treatment was stopped if no clinical response was evident after 2–4 cycles, because most patients were already heavily pretreated.

Quantitative analysis of M in plasma samples

The procedure described by Peng *et al.* and then by Van Belle *et al.* [17, 18] was followed with slight modifications.

Frozen plasma samples were quickly warmed up and vortexed for a few seconds until a clear solution was observed.

One millilitre of plasma was transferred into a centrifugation tube; 500 ng ametantrone was then added to each tube as an internal standard along with a borax buffer (1 ml, 0.1 mol/l, pH 9.4).

After vortexing for 1 min, 5 ml dichloromethane was added. The mixture was shaken for 5 min and then allowed to stand for 20 min. Finally, it was centrifuged (30 min, 3500 rpm) until the organic layer was clear. The dichloromethane phase was then transferred into glass vials and evaporated to dryness under nitrogen flow.

Two hundred millilitres of water solution containing 0.002 mol/l hexanesulphonic acid and 0.05% trifluoroacetic acid was added to the dry residue.

Ion paired reversed-phase high performance liquid chromatography (HPLC) was utilised for the quantitative analysis of M: a Perkin Elmer series 3B apparatus was used.

Separation was achieved at room temperature by means of a 30 × 0.4 cm Bondpak C18 Column (Waters Assoc.). A gradient elution was performed using between 72% and 62% of solution B, containing 99.5% water (2×10^{-3} mol/l hexanesulphonic acid) and 0.05% trifluoroacetic acid. The flow rate was 1.5 ml/min. Detection was performed at 600 nm using a Perkin Elmer LC/79 spectrophotometric detector.

Efficacy evaluation

In 5 patients evaluable, with 2–5 cm residues, the efficacy was evaluated clinically, cytologically and echotomographically. In only 1 patient was it not possible to perform laparoscopy, because of multiple adhesions. Another patient refused to undergo the procedure. These 2 cases were excluded from the evaluation of treatment efficacy.

19 out of 24 patients with less than 2 cm peritoneal deposits or with microscopic residues were evaluated with repeated laparoscopy (or laparotomy in 1 case) and multiple random biopsies. 5 patients were evaluated only by clinical and cytological examinations, because of evident disease progression. 1 patient with initial cytological evaluation attained complete response (CR) at the cytological control. Even though M induces adhesion formations [15], it was possible to perform laparoscopy with multiple biopsies, in all non-progressive cases except one.

In 18 patients the Ovarian Cancer Antigen (CA 125) plasma levels were determined [19] (immunoradiometric assay).

Toxicity evaluation

The evaluation of haematological toxicity, cardiotoxicity, abdominal pain and other gastro-intestinal side effects was performed according to WHO criteria [13].

Complete blood cell and platelet counts were obtained before starting treatment and at 15 and 25 day intervals, after each cycle. Serum multiple analysis (SMA 24) including renal and liver function tests and electrolytes were also obtained before each cycle. Drug dosage was to be decreased by 25% in presence

Table 2. Characteristics and clinical outcome in our series

N	Age	Stage	Residuum	Histol.	Grade	Cumulative dosage (mg/m²)	No. of cycles	Activity	Duration of response (months)	Mean plasma levels (ng/ml)	Peritoneal surface	Present condition
1	79	3C	< 2 cm	S.P.	2	78	4	PD	—	66.6	N.P.	Dead
2	60	3B	< 2 mm	S.P.	N.D.	72	2	PD	—	N.P.	N.P.	Dead
3	52	3A	Microsc.	Endom.	3	145	5	CR	8	N.P.	N	Alive
4	54	3C	< 2 cm	UD	3	202	7	PR	4	N.P.	Light blue	Dead
5	53	1C	Cytology	S.P.	2	116	4	CR	16+	N.P.	N.P.	Alive
6	45	3C	6 mm	S.P.	1	112	5	CR	24+	403	Blue	Disease free
7	64	4	< 1 cm	S.P.	3	90	3	MC	1	N.P.	N	Dead
8	63	3B	< 2 mm	S.P.	2	136	5	CR	8	N.P.	Some light blue spots	Dead
9	64	3C	2 mm	Endom.	2	50	2	PD	—	N.P.	N.P.	Dead
10	52	3C	0.5 cm	S.P.	2	102	3	MC	1*	61.2 65	N N	Alive
11	74	3B	< 2 cm	S.P.	3	97	3	PR	3	77.2	N	Alive
12	39	3B	< 2 cm	S.P.	1	91	3	PD	—	23.5 18.1	Some light blue spots	Alive
13	51	3C	0.5 cm	S.P.	2	81	3	CR	3	N.P.	Peritonites	Dead
14	50	3B	1 cm	Endom.	2	70	3	MC	2	43 N.P.	N Not possible for	Dead
15	67	3B	2 cm	S.P.	2	46	2	Not evaluable	—		adhesions	Alive
16	73	3C	< 2 cm	UD	3	53	3	PD	—	N.P.	N	Dead
17	51	3C	2 cm	S.P.	3	83	3	MC	5*	98.9 48.9	N N	Alive
18	59	3B	2 cm	S.P.	2	45	2	Not evaluable	—	8.4	N.P.	Alive
19	62	3B	< 2 cm	S.P.	2	47	2	NC	1	N.P.	N.P.	Dead
20	81	3C	> 5 cm	S.P.	1	42	3	NC	1	N.P.	N.P.	Dead
21	62	3C	> 5 cm	S.P.	2	107	4	MC	1	N.P.	N.P.	Dead
22	63	3B	< 2 cm	S.P.	2	86	3	PR	1*	35.9 30.7	N N	Alive
23	29	3A	> 2 cm	S.P.	3	90	3	NC	—	27.5	Normal	Alive
24	73	2B	< 2 cm	Muc.	3	84	3	NC	1	N.P.	N.P.	Dead
25	52	2B	Cytology	S.P.	3	33	1	CR	1†	84.1	Normal	Dead†
26	73	4	> 2 cm	UD	3	70	2	PD	—	N.P.	N	Dead
27	46	3A	< 2 cm	S.P.	2	87	3	NC	1*	117.2	N	Dead
28	49	3C	< 2 cm	S.P.	2	166	6	PR	4*	N.P.	Blue	Disease free
29	47	3B	< 1 cm	S.P.	2	153	6	PR	24+	164.9 112.9	Light blue Light blue	Disease free
30	55	3B	< 1 cm	Muc.	N.D.	87	3	PD	—	N.P.	N	Dead
31	54	3C	> 2 cm	S.P.	3	57	2	PD	—	N.P.	N.P.	Dead
32	51	3A	2 cm	End.	2	58	2	PD	—	23.5	N.P.	Dead
33	65	3C	> 5 cm	S.P.	2	50	2	PD	—	N.P.	N.P.	Dead
34	71	3B	> 5 cm	S.P.	3	75	3	PD	—	N.P.	N.P.	Dead
35	68	3C	< 2 cm	S.P.	3	75	3	PD	—	33.6	N	Dead
36	56	3B	< 2 cm	UD	3	77	3	MC	9	N.P.	N	Dead

* After treatment, patients underwent other therapy.

† Dead 24 h after second look for myocardial infarction.

N.D. = not defined; S.P. = serous papillary; Endom. = endometrioid; UD = undifferentiated; Muc. = mucinous; Microsc. = microscopic; N.P. = (laparoscopy or pharmacokinetic) not performed; CR = complete remission; PR = partial remission; MC = minimal change; NC = no change; PD = progression of disease; N = normal.

of grade 3 haematological toxicity and/or abdominal pain, or discontinued following an episode of grade 4 toxicity of any type.

Pharmacokinetic study

In selected patients with pre-treatment residuum ≤ 2 cm, M plasma samples were obtained during and after the i.p. infusion at the following time intervals: 0.5, 1, 2, 4, 8, 24, 48, up to 240 h.

Blood samples were put into glass tubes, immediately centrifuged and plasma was quickly separated and mixed with ascorbic acid and frozen to −70°C for M analysis [17, 18]; samples were all analysed at the Faculty of Chemistry laboratory, University of Padova. The limited number of data points did not permit a reliable compartmental analysis because of the small number of samples.

Thus, the following non-compartmental parameters were calculated: the area under the curve (AUC), the peak concentration (Pc), the time to Pc (Tp), the mean plasma concentration. The AUC was computed with the trapezoid rule from time 0 to the time of the last detectable plasma concentration, without extrapolating the curve to infinity.

Statistical considerations

Comparisons between means were performed using the t-test. Fisher's one sided exact test (two sided) was employed to

compare proportions. The log-rank test was used to test differences between survival curves.

RESULTS

Therapeutic results

The therapeutic effectiveness of intraperitoneal M was evaluable in 34 cases, in whom a total of 112 cycles were administered (Table 2).

Clinical responses were seen starting from 23 mg/m^2 dose level.

We observed 11/34 major responses (32%), namely 6 CR and 5 partial responses (PR) with median survival of 13 months.

The median duration of CR was 8 months (range 1–24+) and the median duration of PR was 4 months (range 1–24+). 2 patients with PR were switched to other treatments and another patient died from myocardial infarction 2 months after M administration. This last patient had a family history of myocardial infarction. The autopsy in this case excluded any connection to the chemotherapy.

Ovarian cancer antigen (CA 125) was also monitored as a non-invasive marker for therapeutic activity in 18 out of 34 patients. In 9 patients who did not respond to treatment, the basal CA 125 levels did not change significantly after M.

We noted that 3 patients with CR showed an initial rise in the CA 125 level with return towards normal values after 4–5 months.

The survival in responders was significantly higher (P = 0.0139, log-rank test) than in non-responders (Fig. 1).

We obtained four responses (2 CR, 2 PR) in 8 patients pretreated only with cisplatin; six responses (3 CR, 3 PR) in 24 patients who were pretreated with cisplatin–anthracycline combination; and no responses in 3 patients pretreated only with anthracycline.

Thus a major response rate was observed in the group of patients pretreated with cisplatin, while patients pretreated with doxorubicin showed a poorer clinical outcome. However the one tail Fisher test is not yet significant (P = 0.08). The total M dose and number of cycles received was not related to the clinical outcome.

The pretreatment size of residue seems to be a major factor of the therapeutic response. In fact, 11 of 24 patients (46%) with a residuum less than 2 cm, showed complete or partial remission, while none of those with residues greater than 2 cm responded to the drug. On the contrary, the histological grade was not significantly correlated to the response.

Table 3. Toxicity according to WHO score associated with intraperitoneal mitoxantrone: 117 cycles

	WHO grade				
Toxicity	0	1	2	3	4
Haematological	100	9	4	4	0
Abdominal pain	59	16	13	25	4
Gastrointestinal	81	6	13	15	2
Cardiotoxicity	116	1	0	0	0
Infections	114	2	1	0	0

It is worth noting that 5 of the 11 patients with partial or complete response showed at the laparoscopic examination a widespread or localised light blue colour on the peritoneal surface, (similar to that of the drug solution) while only one of the non-responders showed the same reaction (Table 2).

Toxicity

Moderate leukopenia was seen starting from the 25 mg/m^2 dose. No patient experienced thrombocytopenia. Increasing M dosage produced moderately severe (grades 1, 2, 3) haematological toxicity, which in only 1 case reached grade 4. Leukopenia was mild and fully reversible within 10–14 days.

We did not observe cardiotoxicity with routinely performed, ECG, PEP/LVET determination and clinical examination.

Severe abdominal pain (grade 3 and 4) was also observed for the first time at the dose level of 23 mg/m^2. Nausea and vomiting occurred (grade 3) starting from 23 mg/m^2 (Table 3). Impaired bowel function (transient paralytic ileus) was observed in 4 patients at 23, 26, 29, 36 mg/m^2, respectively. This side effect resolved within 10 and 30 days after non-steroidal analgesics (anti-spastic drugs increased paralysis of the ileus). 2 cases had peritoneal infection accompanied by leucocytosis, fever and abdominal tenderness. These patients were treated with oral trimethoprim–sulphamethoxazole (800 mg/day for 6 days). Fever and peritonitic signs completely disappeared on the third and fourth day of treatment, respectively.

Pharmacokinetic data

Plasma M concentrations were monitored during 20 cycles (15 patients) to evaluate the drug levels attained in plasma after intraperitoneal treatment (Fig. 2). The pharmacokinetic parameters are shown in Table 4.

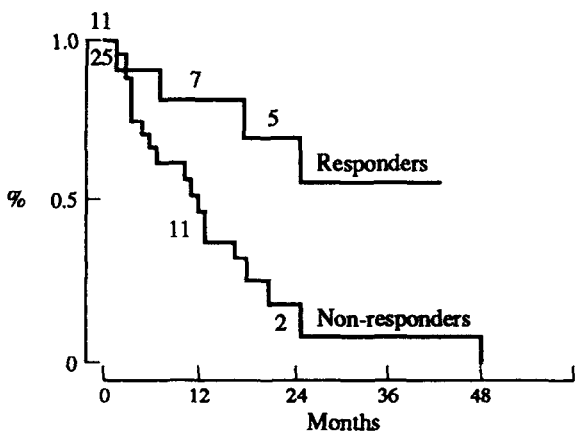

Fig. 1. Overall survival in 36 M-treated patients (P = 0.0139 log-rank test).

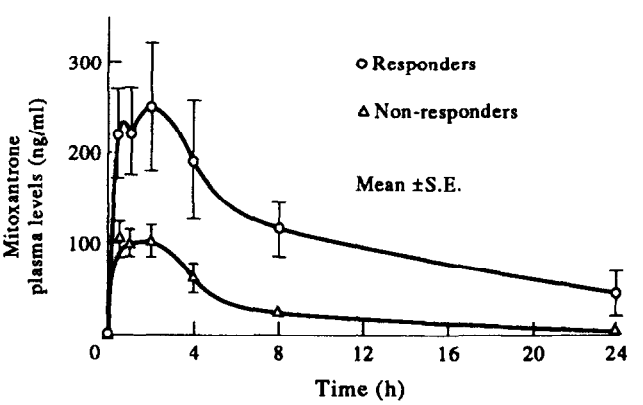

Fig. 2. Serum levels of M in treated patients.

Plasma AUCs were highly variable, ranging from 188 to 11870 ng*h/ml. On average, responders (CR+PR+MC) have a significantly higher AUC (3439 ± 3969 ng*h/ml vs. 639 ± 630 ng*h/ml) and peak plasma levels (278 ± 164 ng/ml vs. 105 ± 54) than non-responders (NC+PD) ($P < 0.05$, one tail t-test). Figure 2 shows the time course of mean plasma levels in responders and non-responders (MC = minimal change; NC = no change) and Fig. 5 shows the concentration–time curves in the individual patients.

No significant correlation was found between total i.p. administered dose and mean or peak plasma concentrations.

Studies of intraperitoneal levels were performed only in 3 patients because liquid extraction was unfeasible in the other 5 patients tested: of these, 2 patients had low serum levels and high intraperitoneal levels (> 10000 ng/ml) in the first 6 h after treatment and 1 of the 2 responded. In the other patients (CR) peritoneal fluid was obtained only after 20 min from administration with a M concentration of > 20000 ng/ml. After this first extraction it was no longer possible to obtain liquid peritoneal samples (Fig. 3). It is also interesting to notice the large number of chromatographic peaks (possibly drug metabolites) that appear in the intraperitoneal liquid after local injection (Fig. 4).

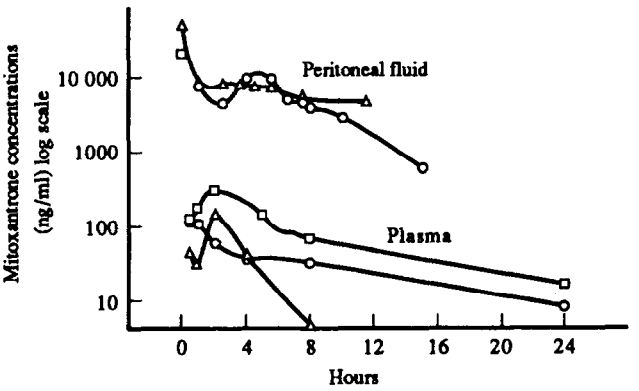

Fig. 3. Plasma and peritoneal levels of M. □ ○ = Responders; △ = non-responders.

DISCUSSION

The rationale for giving M intraperitoneally in patients with ovarian cancer lies in the ability to achieve high local drug concentrations without a parallel increase in systemic side-effects [20–22]. In our study the initial concentration of M in the infused solution was between 12 and 18 μg/ml, i.e. largely superior to the concentration range found to be active in the clonogenic *in vitro* assay (1–10 μg/ml). Our dosing approach resulted in a 32% achievement of CR or PR.

The most relevant side effect in more than half of the patients was severe abdominal pain due to the local irritating effect of M solution: this represents the truly limiting side-effect [23] in the majority of patients.

One may speculate that the occurrence of peritoneal adhesions secondary to the local irritating effect of the drug could have biased the laparoscopic evaluation of the efficacy.

It is possibly due to our administration approach (no permanent catheter was left after each infusion, no attempt to withdraw fluid was made, the drug was evenly diluted in a large volume) that diffuse peritoneal adhesions rarely occurred.

Other authors [24] found laparoscopy a reliable tool to predict the response to chemotherapy in ovarian carcinoma. Furthermore, in heavily pretreated patients we preferred laparoscopy, which is less invasive than laparotomy, for ethical reasons.

On the basis of the efficacy and toxicity results here presented

Table 4. Pharmacokinetic data

Patient no.	Cycle no.	Dose (mg/m²)	AUC (ng*h/ml)	Mean plasma conc. (ng/ml)	Pc (ng/ml)	Tp (h)	Therapeutic activity
1	II	15	532	67	140	1	PD
6	II	20	3224	403	590	2	CR
10	II	34	490	61	130	2	NC
10	III	34	260	65	100	1	NC
11	I	32	1852	77	430	0.5	PR
12	I	30	565	23	40	0.5	PD
12	II	30	435	18	40	0.5	PD
14	III	20	344	43	70	2	NC
17	II	28	2372	99	240	0.5	MC
17	III	28	391	49	140	2	MC
18	I	28	201	8	170	0.5	PD
22	I	28	287	36	70	2	PR
22	II	28	737	31	110	1	PR
25	I	33	2019	84	310	2	CR
23	II	30	660	27	75	0.5	NC
29	V	28	11870	165	330	2	PR
29	VI	28	8197	114	280	2	PR
27	II	29	937	117	190	2	NC
32	I	29	188	23	46	0.5	PD
35	I	29	2422	34	150	0.5	PD

Pc = peak concentration.
Tp = time of Pc.

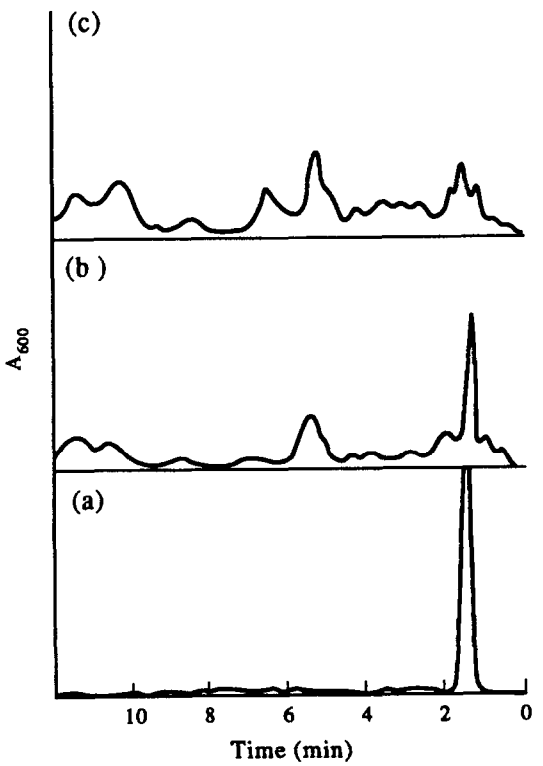

Fig. 4. HPLC elution profile of mitoxantrone (absorbance at 600 nm) at different times (t) after peritoneal injection. (a) t = 0; (b) t = 1 h; (c) t = 3 h.

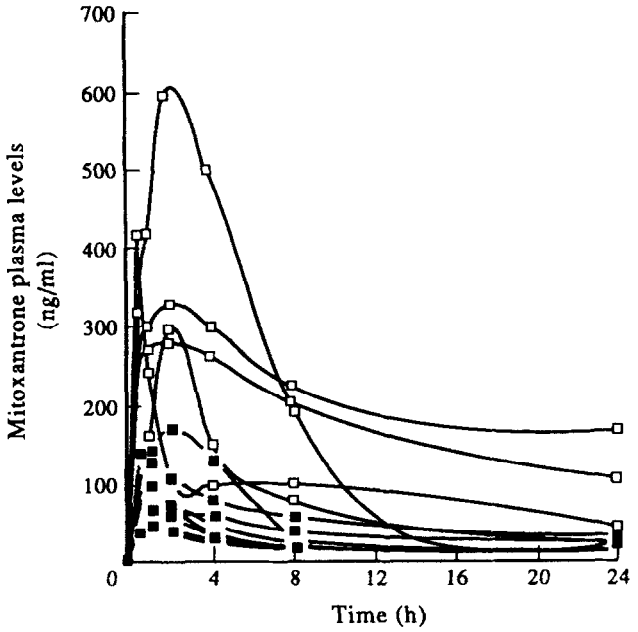

Fig. 5. Plasma levels in responders ■ and in non-responders □.

and in agreement with what has been already stated by other authors [16], it appears that a 28 mg/m^2 dose is generally tolerated and possibly effective.

Plasma AUC in 20 cycles (of 16 patients) who underwent the pharmakokinetic study was highly variable. This could be due to different elimination rates or variable amounts of drug reaching the circulation from the peritoneal cavity. When comparing our pharmacokinetic results with those found by other authors who used the same route of administration [3, 16], it appears that there is a general agreement as far as peak plasma concentration is concerned (40–590 ng/ml vs. 1–488 ng/ml).

However, the AUC were substantially higher in our patients (188–11870 ng*h/ml) and closer to the AUC found by others after i.v. administration [25, 26]. A possible reason for this discrepancy may be a slower elimination rate or an interference by long lasting plasma metabolites with drug level assay.

In an attempt to identify factors which might have a bearing on prognosis, we examined the possible role played by histopathological features of residues and by previous drug treatments. As also pointed out by Markman *et al.* [15], we found that pretreatment size of residua is a major prognostic factor, in that only patients with a residua measuring less than 2 cm (independently of histological grade) showed a CR or PR; this finding is in agreement with previous experimental data [11]. The penetration of chemotherapeutic agents in the tumour mass is confined to few cell layers, or, at best, to a few millimetres of cancer tissue [21, 27]. On the other hand, the clinical outcome does not seem dependent on whether cisplatin or doxorubicin were previously given. The rise in CA 125 levels in responders may simply reflect mitoxantrone-induced peritoneal irritation, or reparative mesothelial proliferation.

An intriguing correlation has been found between the persistence of a light blue colour on the peritoneal surface and the degree of clinical response. This finding was not correlated to massive peritoneal adhesions and therefore attributable to a limited drug distribution. Since the M solution is blue, it could be suggested that responders had more sustained and persistant tissue/drug concentration than non-responders: this might be due to higher tissue binding or to a decreased rate of drug inactivation allowing for local persistance.

Interestingly, a relation was found between response and drug AUC/peak concentration, but not with the total administered dose or the number of cycles. Although the intravenous administration of M, was generally found to result in a poor clinical response [8, 26], the intraperitoneal administration associated with high systemic bioavailability and high plasma levels seems to achieve a better therapeutic result. This observation suggests a synergistic therapeutic activity of the drug delivered to the malignant cells through the vascular bed after local absorption [16], therefore revealing a favourable prognostic meaning to high M plasma levels.

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Immunoradiometric Assay of Pro-cathepsin D in Breast Cancer Cytosol: Relative Prognostic Value Versus Total Cathepsin D

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In breast cancer cell lines, the maturation of pro-cathepsin D into enzymatically active cathepsin D is altered, leading to its increased secretion. In order to specifically assay pro-cathepsin D (52 kD form) in breast cancer cytosol, we monitored a solid phase sandwich radioimmunoassay using D9H8 and D7E3 monoclonal antibodies raised against human pro-cathepsin D from MCF7 cells. Pro-cathepsin D was assayed in 108 primary breast cancer cytosols in which total cathepsin D was previously found to be correlated with metastasis. Pro-cathepsin D concentrations were found to be correlated with total cathepsin D and with lymph node invasion, and was slightly higher in premenopausal patients. By contrast, Cox multiparametric analysis showed that pro-cathepsin D status had no prognostic value for survival, or metastasis free survival contrary to total cathepsin D status. This first study shows the technical validity of the pro-cathepsin D assay but indicates that it has less value as a prognostic marker than total cathepsin D. This study also shows that the proportion of pro-cathepsin D recovered *in vivo* (1–6%) is much less than that produced in cell lines and suggests that the secreted pro-enzyme might be activated in the tumour extracellularly or following its reinternalisation.
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INTRODUCTION

CATHEPSIN D (cath D), a lysosomal protease is produced in excess by most breast cancer cell lines [1, 2]. Moreover, in many breast cancer cell lines, maturation of the pro-enzyme and its routing to lysosomes are known to be altered, resulting in its increased secretion. Several monoclonal antibodies have been raised against human pro-cath D secreted by MCF7 cells [3]. Two of them (D7E3 and M1G8) have been used in a solid phase immunoradiometric assay (IMRA) to measure total cath D concentration in breast cancer cytosol, including the pro form (52 kD), the intermediate form (48 kD) and one of the mature chain (34 kD) [4]. Studies using this assay [5] or ELISA [6] have shown that a high cath D level is associated with higher risk of relapse and metastasis even in node-negative breast cancer patients. One frequently proposed mechanism by which proteases might facilitate metastasis is by degradation of the extracellular matrix after secretion [7]. Moreover, pro-cath D has been shown to be mitogenic [8] and to interact with the mannose-6-phosphate/insulin-like growth factor II (Man 6P/IGFII) receptor [9]. Thus the pro-cath D assay might prove to be more