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Doxorubicin Compared with Related Compounds in a Nude Mouse Model for Human Ovarian Cancer

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Eight human ovarian cancer lines grown in nude mice were used to compare the activity of doxorubicin, epirubicin, mitoxantrone and menogaril. The tumour lines were different in histological subtype, tumour doubling time and sensitivity to doxorubicin. The compounds were administered intravenously at the maximum tolerated dose twice with one week in between when tumours measured 50-150 mm³. Growth inhibition greater than 50% was obtained for doxorubicin in 8/8, for epirubicin in 4/8, for mitoxantrone in 5/8 and for menogaril in 2/8 tumour lines. In MRI-H-207, doxorubicin was the only drug able to induce complete remission. Compared with doxorubicin, mitoxantrone and menogaril were given in proportionally higher doses than those administered to patients, but did not result in superior antitumour activity.
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INTRODUCTION

DOXORUBICIN is one of the most powerful chemotherapeutic drugs available with activity in leukaemias, lymphomas, breast cancer, ovarian cancer, soft tissue sarcomas and small cell lung cancer. There are three mechanisms of action ascribed to doxorubicin: DNA intercalation, membrane binding and free-radical formation. Maximum cell kill occurs during the S phase of the cell cycle. Toxicity consists of nausea and vomiting, myelosuppression and alopecia. A side-effect of major concern is the induction of congestive heart failure; damage to the myocardial tissue is proportional to the total dose. Analogues or related compounds with similar antitumour activity but hopefully diminished cardiotoxicity have been investigated [1]. We have compared epirubicin [2], mitoxantrone [3] and menogaril [4, 5] with doxorubicin in a nude mouse model for human ovarian cancer.

MATERIALS AND METHODS

Animals and tumour lines

Female NMRI/Cpb nude (nu/nu) mice (Harlan Cpb) were maintained in cages with paper filter covers. Cages, covers, bedding, food and water were changed and sterilized weekly. Animal handling was done under sterile conditions in a laminar down-flow hood.

Human ovarian cancer lines used in the experiments are listed in Table 1. They were selected for moderate to high sensitivity to doxorubicin. Ov.He, Ov.Me, Ov.Ri(C) and Ov.Pe were established in our laboratory, while MRI-H-207, A2780, and FMa were provided by other investigators and have been described before [6]. FCo was given by Dr W. Kleine, Albert-Ludwigs University, Freiburg. Tumour lines were maintained by serial subcutaneous transplantation of tumour fragments 2–3 mm in diameter in both flanks of 8- to 10-week-old animals.

Treatment and evaluation

Doxorubicin (Farmitalia Carlo Erba) and epirubicin (I.C.N. Pharmaceuticals) were dissolved in water at 2 mg/ml. Mitoxantrone (Lederle) was provided as 2 mg/ml solution and menogaril (Upjohn) was dissolved in water at 5 mg/ml. Drugs were injected intravenously twice with one week in between at the maximum tolerated dose (MTD) indicated in Table 2. At the MTD the mice lost approximately 10% of their initial weight within 1 week after the first injection.

Subcutaneous tumours were measured at least weekly in three dimensions with a slide caliper by the same observer. The volume in mm³ was calculated from length × width × thickness × 0.5.

In each experiment, mice bearing tumours of 50-150 mm³ were randomized into groups of 5–8 animals for treatment or control. Because of the variation in size at the start of treatment, volumes were calculated in relation to the initial tumour volume. The relative volume was expressed as V_T/V_0, where V_T is the volume at any given day and V_0 the volume at the start. The ratio of the mean relative volumes of treated tumours (T) over

Table 1. Human ovarian cancer lines, histology and growth rate

Line	Histology	T_D
MRI-H-207	Undifferentiated	3.5
A2780	Undifferentiated	3.5
Ov.He	Moderately differentiated, mucinous	9
Ov.Me	Carcinosarcoma	6
Ov.Ri(C)	Moderately differentiated, serous	11
FMa	Poorly differentiated, mucinous	5
Ov.Pe	Moderately differentiated, mucinous	8
FCo	Clear cell carcinoma	6.5

T_D = tumour doubling time (days).

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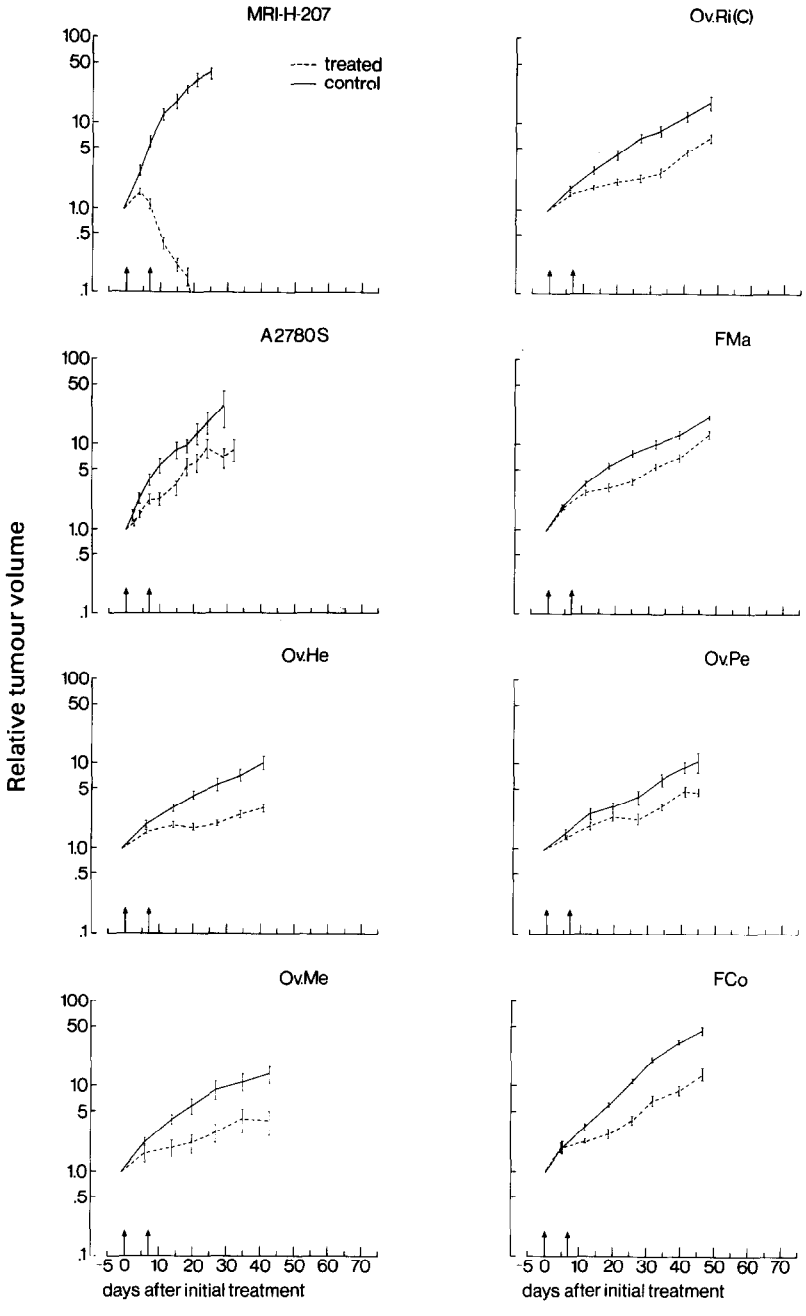

Fig. 1. Growth curves of human tumour lines derived from ovarian cancer. Tumour-bearing mice were divided into groups for control (—) or treatment with doxorubicin 8 mg/kg intravenously (----) on days 0 and 7. Mean (S.E.).

that of control tumours (C) multiplied by 100 ($T/C\%$) was calculated at each evaluation. Growth inhibition was expressed as $100\% - T/C\%$, and the highest value within 5 weeks after the final injection was considered the optimal growth inhibition. Deaths within 2 weeks after the last day of treatment were considered as toxic deaths and these animals were excluded from the study. Complete remission was defined as the total disappearance of the tumours without regrowth within the following month.

RESULTS

Figure 1 shows growth rates of the eight human ovarian cancer lines and their sensitivity to doxorubicin. In MRI-H-207 the drug induced cures of all tumours, while a growth inhibition of 53–69% was obtained in the other lines. Growth inhibition induced by the four compounds is listed in Table 3 and visualised in Fig. 2. Epirubicin and mitoxantrone inhibited growth by more than 50% in four and five tumour lines respectively, while menogaril inhibited growth in only two. Comparison of the activity of the four compounds showed superior efficacy for doxorubicin. Significantly less growth inhibition was observed in MRI-H-207 and Ov.Me with the other compounds ($P < 0.05$, t test). In MRI-H-207 doxorubicin was the only drug able to induce complete remission. Menogaril was remarkably ineffective in view of its presumed cross-resistance with doxorubicin.

Table 2. Maximum tolerated doses and schedules of doxorubicin, epirubicin, mitoxantrone, and menogaril in nude mice and in patients

Drug	Mouse		Patient	
	mg/kg*	Ratio	mg/m²†	Ratio
Doxorubicin	8	1	75	1
Epirubicin	10	1.25	90	1.2
Mitoxantrone	4	0.5	14	0.19
Menogaril	50	6.25	200	2.67

* Twice with one week in between.
† Every 21 days except menogaril, every 28 days.

For comparison, ratios of recommended doses of drugs given on a 3 to 4 weekly basis in good-risk patients [2, 3, 7–9] and those of MTDs in mice were calculated (Table 3). Epirubicin-doxorubicin ratios were similar in mice and in patients, whereas for mitoxantrone and menogaril these ratios were 2.5-fold higher in mice. Nonetheless, proportionally higher doses of mitoxantrone and menogaril did not result in superior antitumour activity in the human ovarian cancer lines compared with that of doxorubicin.

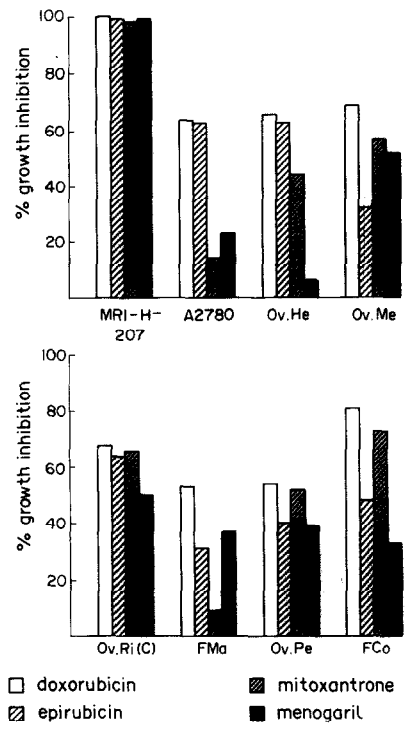

Fig. 2. Growth inhibition obtained with doxorubicin, epirubicin, mitoxantrone, or menogaril administered intravenously at MTD twice with one week in between.

DISCUSSION

Analogues and related compounds of doxorubicin have been developed for a lower frequency of cardiac toxicity with retention of antitumour activity. Comparative activity studies in our human ovarian cancer lines in nude mice have shown best growth inhibitory effects for doxorubicin, followed by epirubicin and mitoxantrone. Menogaril was least effective. MTDs administered were proportionally similar to those given to patients for doxorubicin and epirubicin, but 2.5-fold higher for mitoxantrone and menogaril. The results derived from the nude mouse model suggest that of the four compounds, doxorubicin remains the most active drug for incorporation in combination chemotherapy of ovarian cancer clinically.

Screening of new anticancer compounds by murine tumour models may result in a high number of (false positive) drugs without activity against solid tumour types in patients [10]. The incorporation of a series of human tumour lines of a specific tumour type grown in nude mice as a secondary screen may have a higher predictive potential for new cytostatic drugs [11]. This screen is human-based and disease-oriented and may therefore relate better to the clinical situation. For analogues of conventional cytostatic drugs, such as carboplatin, we have already shown the predictive capacity of the nude mouse model for human ovarian cancer [12].

Superior antitumour effects for any of the three related compounds over doxorubicin investigated have not yet been observed in clinical trials. Comparison of treatment results of epirubicin with those of doxorubicin at equitoxic doses has indicated similar efficacy in breast cancer patients [13]. At equimolar doses, response rates were slightly in favour of

Table 3. Growth inhibition (%) by doxorubicin, epirubicin, mitoxantrone and menogaril in human ovarian cancer lines

Tumour line	Doxorubicin	Epirubicin	Mitoxantrone	Menogaril	Day of maximum growth inhibition
MRI-H-207	CR	99*	98*	99*	25
A2780	64	63	14*	23*	15
Ov.He	66	63	44*	6*	34
Ov.Me	69	32*	57*	52*	27
Ov.Ri(C)	68	64	66	50	33
FMa	53	31*	9*	37	25
Ov.Pe	54	40	52	39	34
FCo	67	48*	63	33	32

*Less active than doxorubicin ($P < 0.05$).
CR = complete remission (growth inhibition 100%).

doxorubicin but not significantly so, as demonstrated in soft tissue sarcoma [14] and breast cancer [15]. Epirubicin has also been shown to be active in ovarian cancer first-line treatment [3]. Comparative randomized trials with mitoxantrone and doxorubicin in breast cancer patients, either studying single agents or the drugs incorporated in combination regimens, have demonstrated for mitoxantrone an efficacy slightly less than but not statistically different from doxorubicin [16–18]. Mitoxantrone has shown some efficacy in phase II trials in ovarian cancer patients [18–21]. Thus far, menogaril has shown activity in breast cancer, but was inactive in colorectal cancer, non-small cell lung cancer and ovarian cancer [4, 5, 22–25].

Although epirubicin, mitoxantrone and menogaril are structurally related to doxorubicin and are also excreted through the biliary system, the compounds show slight dissimilarities in their respective mechanism of action, metabolism and side-effects. In general, the impact of such differences cannot be interpreted from the nude mouse model to the clinical situation. Regardless of whether a compound is less effective at a dose equitoxic to doxorubicin, differences in side-effects may be of advantage if drugs have to be used for specific purposes. For instance, cardiotoxicity was less frequent with long-term administration of epirubicin and mitoxantrone compared with doxorubicin [2, 3]. For mitoxantrone, intraperitoneal and intrathecal administration can be done without the local toxicity to be expected with the parent compound [25]. Mitoxantrone is also known to induce less alopecia, nausea and vomiting [3]. These favourable features may be a reason to prescribe such drugs in selected patients.

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