PERGAMON

European Journal of Cancer 37 (2001) 431–437

European Journal of Cancer

www.ejconline.com

Tissue distribution, antitumour activity and *in vivo* apoptosis induction by MEN10755 in nude mice

O. Gonzalez-Paz [a,1], D. Polizzi [a], M. De Cesare [a], F. Zunino [a], M. Bigioni [b], C.A. Maggi [b], S. Manzini [b], G. Pratesi [a,*]

[a] *Istituto Nazionale Tumori, Via Venezian 1, 20133 Milan, Italy*
[b] *Menarini Ricerche S.p.A., 00040 Pomezia, Rome, Italy*

Received 7 July 2000; received in revised form 4 October 2000; accepted 5 October 2000

Abstract

MEN10755 is a disaccharide analogue of doxorubicin (DXR) endowed with a broader spectrum of activity compared with DXR in a panel of human tumour xenografts. In an attempt to investigate the pharmacological basis of the improvement of therapeutic efficacy of the analogue, a comparative pharmacokinetic (tissue and tumour distribution) and pharmacodynamic (antitumoral activity and ability to induce apoptosis) study of MEN10755 and DXR was performed in athymic nude mice bearing a human ovarian carcinoma xenograft (A2780). Drug level was quantified by high performance liquid chromatography (HPLC) with fluorimetric detection after a single intravenous (i.v.) injection of 7 mg/kg of MEN10755 or DXR. The results indicated a reduced accumulation of MEN10755 compared with DXR in all tissues investigated (tumour, heart, kidney and liver). The reduction was more marked in normal than tumour tissues. Moreover, in spite of the reduced drug uptake by tumour tissues, the new disaccharide anthracycline given in its optimal regimen showed an enhanced antitumour efficacy, compared with DXR. The drug effects on tumour growth paralleled a marked activation of apoptosis. In conclusion, the pattern of tissue distribution and the pharmacokinetic behaviour were consistent with a better tolerability of the novel analogue which allowed a higher cumulative dose to be delivered. The superior therapeutic efficacy of the analogue over DXR, in spite of a reduced tumour accumulation, supports an increased tumour selectivity.

Keywords: Anthracyclines; Biodistribution; Topoisomerase inhibitors; Apoptosis; MEN10755

1. Introduction

Doxorubicin (DXR) still remains one of the most effective antitumour agents in clinical use today [1]. The efficacy of DXR has stimulated several structure–activity relationship studies aimed at identifying critical modifications that might improve the therapeutic profile. A series of disaccharide anthracyclines has recently been synthesised and investigated [2,3], and one of them, MEN10755, was selected for clinical development on the basis of its promising preclinical profile [4,5]. The novel analogue exhibits improved efficacy related to the induction of apoptosis [4] and an enlarged spectrum of activity in human tumour xenografts [5]. Cellular pharmacology studies have indicated that MEN10755, compared with DXR, despite a lower uptake and accumulation in tumour cells, is endowed with an increased ability to induce DNA breaks and eventually apoptosis [4,6].

In an attempt to investigate the pharmacological basis of the improvement in the therapeutic efficacy of the analogue, a comparative pharmacokinetic and tissue distribution study of MEN10755 and DXR was carried out. The aims of the present study were: (a) to compare the *in vivo* distribution and accumulation of the two anthracyclines in tumour tissues and in selected organs (heart, liver and kidneys) of athymic nude mice bearing the human ovarian carcinoma xenograft A2780; (b) to investigate the ability of the two anthracyclines to inhibit tumour growth and to promote *in vivo* apoptosis. The results indicated that, in spite of a reduced drug accumulation in tumour tissue, the new analogue has an increased ability, compared with DXR, to induce apoptosis and growth inhibition of the A2780 tumour. The reduction of tissue accumulation of MEN 10755, compared with DXR, was more marked in normal, than in tumour tissues. The pattern of distribution of the two drugs is consistent with an improved tolerability of

* Corresponding author. Tel:. +39-2-239-0626; fax: +39-2-239-0692.

E-mail address: pratesi@istitutotumori.mi.it (G. Pratesi).

[1] Present address: Department of Pharmacology, IRBM, 00040 Pomezia, Roma, Italy.

0959-8049/01/$ - see front matter
PII: S0959-8049(00)00414-7

MEN 10755 as documented by the appreciable increase of tolerated doses of the analogue [4,5].

2. Materials and Methods

2.1. Animals and drugs

Female athymic Swiss nude mice (Charles River, Calco, Italy), 10–12 weeks of age, were used throughout the study. Mice were maintained in laminar flow rooms, according to the United Kingdom Coordinating Committee on Cancer Research guidelines [7]. Experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Istituto Nazionale Tumori.

DXR was supplied by Pharmacia-UpJohn (Milan, Italy). MEN10755 (7-(0-4′-0-alpha-L-daunosaminyl-2′-deoxy - alpha - L - fucosyl) - 4 - demethoxyadriamycinone, MW 680) was synthesised at the Chemistry Department of Menarini Ricerche as previously described [4]. Drugs were dissolved in water and delivered intravenously (i.v.) in a volume of 10 ml/kg body weight.

2.2. Antitumour activity studies

The human A2780 ovarian tumour line, derived from the injection of 5×10^6 cells from *in vitro* cultures, was maintained by subcutaneous (s.c.) passages of tumour fragments in nude mice [8]. For the evaluation of antitumour activity, each control or drug-treated group included five mice bearing bilateral s.c. tumours. Tumour weight (TW) was calculated by measuring tumour diameters with a Vernier caliper and using the formula: TW (mg) = tumour volume (mm^3) = $d^2\times D/2$, where d and D are the shortest and the longest diameter, respectively. Drug efficacy was assessed as the percentage of TW inhibition (%) in treated versus control mice, and as $\log_{10}$ cell kill (LCK) achieved by the drug treatment, according to the formula: $T-C/\mathrm{DT}\times3.32$, where T is the mean time (days) required for the treated tumours and C for the control tumours to reach 1 g of weight, and DT is the mean doubling time of control tumours (1.9±0.4 days). Mice body weight loss percent (BWL%) induced by drug treatment was calculated as: $100-(100\times BW_x/BW_1)$, where BW_x is the mean BW at the day of maximal (TWI)% and BW_1 is the mean BW on the 1st day of treatment. No mouse died due to toxicity.

Drugs were delivered i.v. according to two treatment schedules, i.e. every 7th day, 3 times (q7d×3) or every 3rd/4th day, 5 times (q3/4d×5) starting when the tumour weight was approximately 50 mg. Two consecutive drug injections of 5 mg/kg (1 h interval) were delivered for MEN10755 when the weekly schedule was investigated, since i.v. doses higher than 7 mg/kg may cause immediate death in mice [5]. Doses corresponding to the maximum tolerated ones were compared [5].

2.3. Biodistribution studies

Mice bearing the A2780 tumours in both flanks were treated i.v. 10 days after tumour transplant with DXR or MEN10755 at the same dose (7 mg/kg). The dose of 7 mg/kg was chosen since this dose level is the maximum tolerated dose as a single injection of MEN10755. In the schedule used for antitumour activity experiments, an increase of daily dose is possible using two injections. This modality of administration is not suitable for a quantitative comparison of biodistribution. The dose of 7 mg/kg is considered optimal also for DXR in a standard treatment schedule (q7d×3) [5]. Thus, this dose level, which is in the range of therapeutic doses for both agents, is expected to provide a correct view of the difference of the pharmacokinetic behaviour between the drugs. At 0.5, 1, 2, 4, 6, 10, 24, 48 and 72 h after treatment, three mice per time point were sacrificed and their tissues (heart, liver, kidneys and tumour) removed. At the time points 0.5 and 2, 6 and 24 h plasma was also collected by bleeding under light anaesthesia. Samples were maintained at −20°C until analysis.

2.3.1. Analytical assay

DXR and MEN10755 were quantified by high-performance liquid chromatography (HPLC) with fluorimetric detection according to a previously described technique [9], with minor modifications. After homogenisation in water, tissue samples, with daunorubicin added as internal standard, were deproteinised with silver nitrate (33%), extracted with 8 ml of propanol, and centrifuged at $1200g\times10$ min. The organic phase was evaporated to dryness under vacuum and then resuspended in a mobile phase. Extracts were transferred into autosampler vials and 100 μl of sample were injected on to the LC column. A Waters HPLC system was used with fluorescence detection at an excitation wavelength of 475 nm and an emission wavelength of 580 nm. Separation was achieved with an isocratic solvent system of water–acetonitrile–phosphoric acid (0.1 M) (42:32:26) using a 30 cm μBondapack C18 column (Waters, London, UK). Recovery of DXR and MEN10755 extracted after adding a known amount of drug to plasma or tissue homogenate was in the range of 85–90% and the sensitivity was 10 ng/ml for plasma and 20 ng/g for tissue. A non-compartmental analysis of kinetic parameters for tissue distribution was performed with an appropriate computer program (PK Solutions 2.0, Summit Research Services, Ashland, OH).

2.4. Apoptosis induction

In experiments designed for determination of the apoptotic index, mice bearing A2780 tumours in both

flanks were treated at day 7 either once with the same dose (7 mg/kg) of each drug to allow a comparison with the *in vivo* distribution, or according to the therapeutic schedules of DXR (q7d×3) or MEN10755 (q3/4d×5). On days 1, 3, 6 and 8 after drug treatment (corresponding to days 22, 24, 27 and 29 from tumour inoculum in the groups receiving multiple treatments), mice were sacrificed, and their tumours were excised, immediately fixed in 4% buffered formaldehyde, and then embedded in paraffin (the two tumours together) as previously described [4]. Tissue sections were then treated according to the method described by Gavrieli and colleagues [10]. For the TUNEL (i.e. terminal deoxynucleotide transferase-mediated digoxigenin-uridine triphosphate nick-end labelling) reaction, the *in situ* cell death detection POD kit (Boehringer Mannheim, Ingelheim, Germany) was used. The apoptotic index was determined by using a light microscope at ×400 magnification and by recording the number of apoptotic nuclei per field (10 fields/tumour). A percentage was calculated as: apoptotic index = 100×mean number of apoptotic nuclei/mean number of cells. The mitotic index was calculated according to the same procedure.

The one-way ANOVA followed by the Newman–Keuls test was used for statistical comparison between apoptotic and mitotic index values.

3. Results

3.1. Tissue distribution

Fig. 1 shows the time course of levels of DXR and MEN10755 in the tumour and selected organs (heart, kidney and liver). Calculated C max, area under the concentration curve (AUC) as well as T max are reported in Table 1. The tissue distribution of MEN10755 was markedly different from that of DXR. In particular, lower drug levels were achieved in the tumour and normal tissues at all times. With both anthracyclines, the greatest AUC was measured in the tumour. The T max in the tumour was achieved earlier (0.5 h) with the disaccharide anthracycline than with DXR (2 h). In the tumour, in spite of a relatively small difference observed between the C max values of the two drugs (DXR/MEN10755, ratio 1.4), a 4.1-fold higher AUC value was

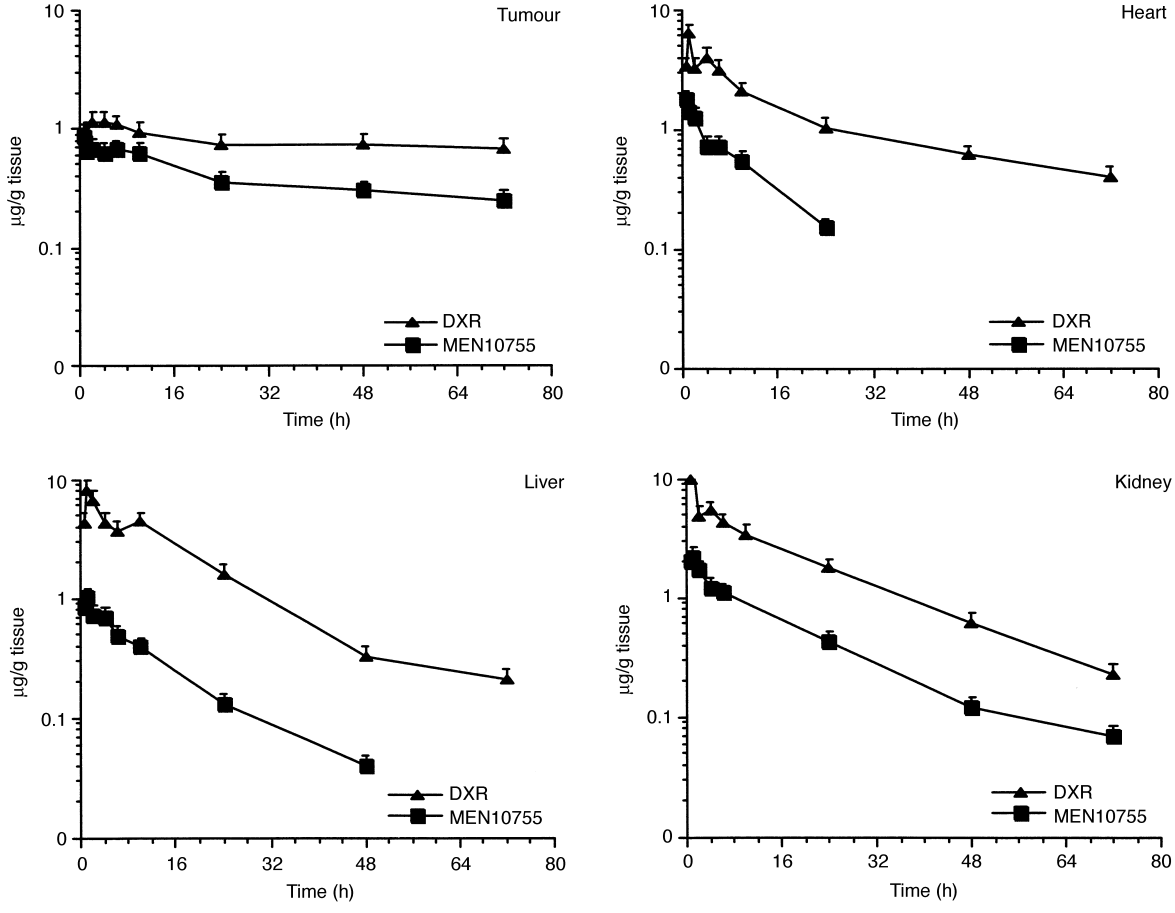

Fig. 1. Tissue distribution (tumour, heart, kidney and liver) of doxorubicin (DXR) (▲) and MEN10755 (■) in nude mice bearing the A2780 human tumour xenograft. Drugs (7 mg/kg) were delivered i.v., 10 days after tumour implantation. Three samples/point were evaluated. Mean values ± standard deviations (S.D.) are reported.

Table 1
Tissue and tumour levels of DXR and MEN10755 in mice bearing the A2780 human ovarian tumours

Tissue	Drug	T max[a] (h)	C max		AUC[d]	
			μg/g[b]	Ratio[c]	μg/g×h	Ratio[c]
Heart	DXR	1	6.3±0.8	3.5	109.1	7.3
	MEN10755	0.5	1.8±0.5		14.9	
Liver	DXR	1	8.1±1.2	8.1	106.2	9.5
	MEN10755	0.5	1±0.04		11.2	
Kidney	DXR	1	10.2±0.3	4.6	123.2	4
	MEN10755	1	2.2±0.1		30.8	
Tumour	DXR	2	1.1±0.5	1.4	249	4.1
x	MEN10755	0.5	0.8±0.3		60.4	

[a] Time required to reach the peak value of concentration (C max).
[b] Drug content/g tissue, mean values±standard deviation (S.D.).
[c] Ratio between the values of the two drugs.
[d] Extrapolated to infinity, area under the concentration curve.

Table 2
Plasma level (μg/ml) of MEN10755 and DXR (both i.v., 7 mg/kg) in nude mice bearing the A2780 human ovarian tumours

Time after administration (h)	μg/ml[a]	
	MEN10755	DXR
0.5	1.83±0.06	0.45±0.02
2	0.33±0.01	0.36±0.01
6	0.24±0.01	0.38±0.05
24	0.12±0.01	0.27±0.01

i.v., intravenous.
[a] Mean value± standard deviation (S.D.).

achieved for DXR, likely due to a slower elimination rate. With both drugs, at times longer than 24 h, the tumour drug concentration approached a plateau. At 72 h after treatment, MEN10755 and DXR were both easily detectable in the tumour tissues. In all examined organs, the tissue levels (as determined by C max and AUC values) of DXR were always markedly higher than those of MEN10755, and the ratios between the two drugs were generally higher than that observed in the tumour (Table 1), mainly due to a faster clearance of the disaccharide anthracycline from the tissues. At 72 h in the heart and liver tissues, the levels of MEN10755 (but not DXR) were below the detection limit, and the drug was barely detectable in the kidney.

Plasma drug levels, measured at selected times (Table 2), indicated an initially greater peak for MEN10755 than for DXR, followed by a faster plasma clearance of the disaccharide anthracycline. Indeed, MEN10755 was barely detectable at 24 h.

3.2. Antitumour activity studies

Against the A2780 ovarian carcinoma, the comparison of the antitumour efficacy of the two anthracyclines was performed delivering DXR or MEN10755 according to the two schedules (weekly and twice weekly) (Table 3). With both schedules, the analogue was better tolerated than the parent drug, the total tolerated cumulative dose being 30 mg/kg for MEN10755 versus 21 or 22.5 mg/kg for DXR. With the weekly schedule, the efficacy between the two drugs was comparable. However, the optimal activity of MEN10755 was achieved when the same total dose of the drug was delivered according to a more fractionated treatment schedule (q3/4d×5), leading to a LCK greater than 6. On the contrary, according to such a schedule DXR was less effective. The greater activity of the analogue compared with DXR is reflected also in the higher percentage of long-term survivors in the A2780 tumour-bearing mice [5].

Table 3
Antitumour activity of DXR and MEN10755 against A2780 human ovarian tumour xenografts

Drug	Dose (mg/kg)		Schedule[a]	BWL[b] (%)	maxTWI%[c] (day)	LCK[d]
	Single	Total				
DXR	7	21	q7d×3	4	94 (27)	3.4
	4.5	22.5	q3/4d×5	1	82 (27)	2.6
MEN10755	5×2	30	q7d×3	0	93 (26)	2.1
	6	30	q3/4d×5	2	99 (33)	>6

[a] Drugs were delivered i.v., every 7th day 3 times (q7d×3) or every 3rd–4th day 5 times (q3/4d×5).
[b] Mice body weight loss% at the day of max TWI% (see Materials and Methods).
[c] Tumour weight inhibition (TWI)% in treated over control mice. In parentheses, day of evaluation.
[d] Log_{10} cell kill (LCK) (see Materials and Methods).

3.3. Apoptosis induction

The ability of the drug to induce *in vivo* apoptosis in the A2780 tumours was assessed after a single i.v. injection of the same dose of the two anthracyclines (7 mg/kg). Tumour-bearing mice were treated when the tumour weight averaged 150 mg (day 7 from tumour implantation). Apoptotic and mitotic indices determined at the various times after treatment are reported in Fig. 2. In the untreated tumours (controls), the apoptotic index remained constant during the observation period (Fig. 2a). Both drugs strongly stimulated the induction of apoptosis in the tumours (P at least <0.05 versus controls at any time). MEN10755 was more efficacious than DXR in eliciting an early apoptotic response (day 1, $P<0.05$ versus DXR) under conditions where a reduction of tumour weight was not detectable. As regards the mitotic index, it progressively increased in controls up to day 6 (corresponding to day 13 from tumour implantation). In treated tumours, mitotic index values were in general significantly lower than in controls (Fig. 2b). The finding is consistent with the accumulation of proliferating cells in the G2/M phase following anthracycline treatment.

In order to assess the relevance of apoptotic induction for tumour response to the two anthracyclines, the apoptotic index in the A2780 tumours was also measured after a therapeutic cycle at the optimal schedule for each drug (i.e. 7 mg/kg, q7d×3 for DXR and 6 mg/kg, q3/4d×5 for MEN10755). The first treatment was delivered at day 7 from tumour implantation and the last one at day 21. The time courses of apoptosis and mitosis after the end of treatment are reported in Fig. 3. Compared with control tumours, MEN10755 was a more effective apoptotic inducer than DXR at any time, in keeping with the enhanced antitumour efficacy against the tumour (Table 3).

4. Discussion

The present study provides further evidence that the improved preclinical profile of MEN10755 over DXR reflects an increased tumour selectivity of the disaccharide analogue. This interpretation is supported by the observation that, in spite of a reduced tumour

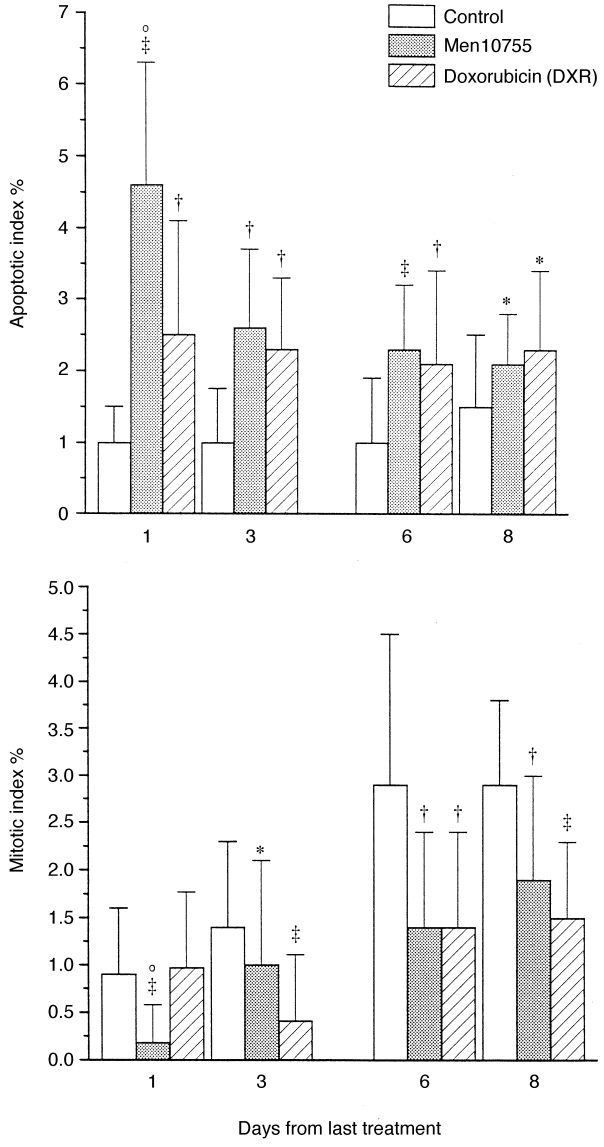

Fig. 2. Time course of (a) apoptotic and (b) mitotic indices in A2780 tumours after single drug treatment. DXR and MEN10755 (7 mg/kg) were delivered intravenously (i.v.) at day 7 from tumour implantation. Mean values ± standard deviations (S.D.) are reported. $P<0.05$ (*), <0.01 (†), <0.001 (‡) versus control tumours. $P<0.05$ (o) versus DXR-treated tumours, by one-way ANOVA followed by Newman–Keuls test.

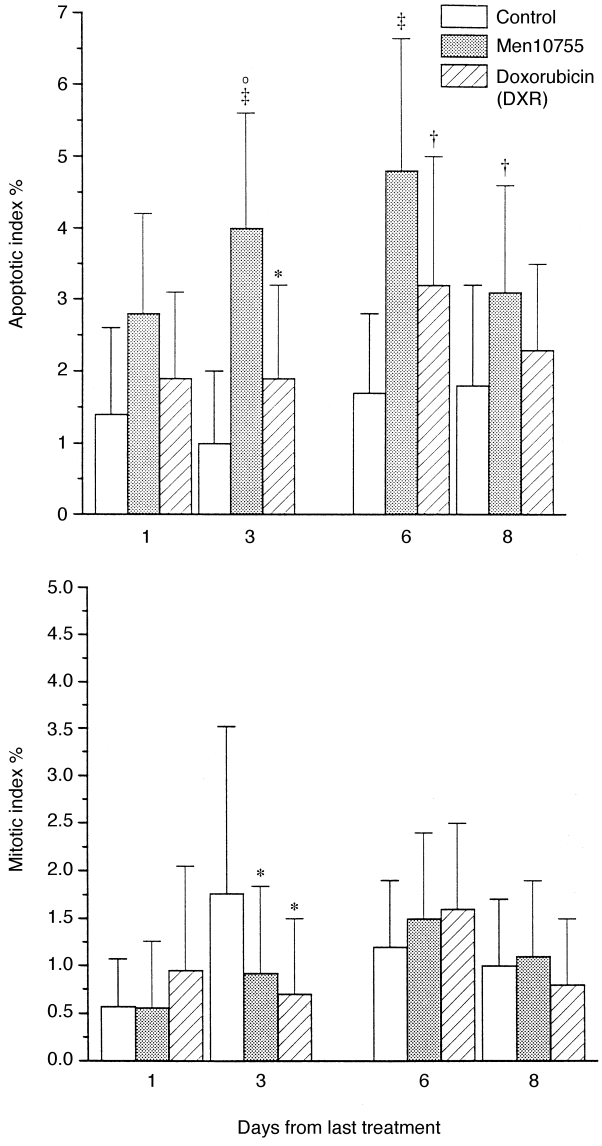

Fig. 3. Time course of (a) apoptotic and (b) mitotic indices in A2780 tumours after repeated drug treatments. DXR (7 mg/kg, q7d×3) and MEN10755 (6 mg/kg, q3/4d×5) were delivered intravenously (i.v.) from day 7 to day 21 from tumour implantation. Mean values ± standard deviation (S.D.) are reported. $P<0.05$ (*), <0.01 (†), <0.001 (‡) versus control tumours. $P<0.05$ (o) versus DXR-treated tumours, by one-way ANOVA followed by Newman–Keuls test.

accumulation, the novel drug given in its optimal regimen was more effective than DXR in inhibiting tumour growth and stimulating apoptosis of the A2780 ovarian carcinoma. The *in vivo* effects are reminiscent of the cellular behaviour, since the analogue exhibits comparable cytotoxic potency in spite of reduced intracellular accumulation compared with DXR [4]. The results at the cellular level support a greater intrinsic ability of MEN10755 over DXR to produce lethal DNA damage, eventually leading to apoptosis of the tumour cells. It is likely that the superior *in vivo* efficacy of the anthracycline disaccharide over DXR reflects the contribution of improved tolerability, allowing a more intensive treatment schedule and a higher cumulative dose, and additional (topoisomerase II-independent) cellular effects, as suggested by a prompt apoptotic response.

In our experimental conditions, using the same dose for the two anthracyclines, lower levels of MEN10755 than DXR were found in all examined tissues. However, tumour was the tissue in which the smallest difference was achieved between the two drugs (C max ratio between DXR/MEN10755 = 1.4). The difference in distribution between tumour and normal tissues (heart and liver) was higher for MEN10755 (four–five-fold) than for DXR (two-fold). Furthermore, in tumour, MEN10755 reached the peak earlier (0.5 h) than DXR (2 h) (Table 1). Such behaviour could contribute to the enhanced antitumour effect of the disaccharide analogue against the A2780 tumours and the prompt apoptotic response. It is noteworthy that for both drugs the content in the tumours tended to level off and maintain a plateau over time, possibly indicating the persistent binding by tumour tissue. A similar relationship between biodistribution and the efficacy of the two drugs was observed in the human mammary carcinoma model MX-1 (data not shown). Interestingly, the MX-1 tumour is resistant to DXR but is particularly responsive to MEN10755 [4].

The ability to stimulate an early apoptotic response has been reported for MEN10755 in two human tumour xenografts (the mammary carcinoma MX-1 and the lung carcinoma POVD) resistant to DXR and has been related to the marked therapeutic efficacy of the analogue [4]. The A2780 tumour investigated in the present study carries the wild-type *TP53* gene and is very responsive to both anthracyclines. Thus, in such experimental models, no substantial differences can be expected in terms of tumour response. However, *in vitro* studies with A2780 cells indicated the ability of MEN10755 to elicit remarkable apoptosis despite a low intracellular content. Our *in vivo* study further supports a relationship between the therapeutic superiority of MEN10755 and an increased ability to induce apoptosis. The differences between MEN10755 and DXR, which were quantitative in the responsive A2780 model, were more marked and qualitative in other DXR-resistant tumour models. For example, in contrast to DXR, MEN10755 induced a marked Bcl-2 phosphorylation in the breast carcinoma model MX-1 [5,11].

Cumulative cardiac toxicity represents a major limiting factor in the clinical use of DXR [12,13]. The present study indicated that in tumour-bearing mice the cardiac accumulation of MEN10755 was markedly lower than that of DXR, as determined by C max and AUC values (approximately four-fold and seven-fold lower, respectively), whereas the rate of clearance by myocardial tissue was faster for MEN10755. However, our results indicate that, in spite of reduced cellular uptake, MEN10755 exhibited a strong antitumour efficacy in all tumour models investigated. Therefore, the reduction of the drug level in the heart is not sufficient to expect a toxicity advantage. Nevertheless, a recent study showed that MEN10755 produces fewer ECG alterations in the rat than an equimyelotoxic regimen of DXR. Furthermore, a long-term progression in the severity of functional and histological cardiotoxic effects has been observed in DXR- but not in MEN10755-treated rats [14].

In view of the limited sampling times (0.5, 2, 6 and 24 h), a comprehensive analysis of plasma kinetic data was not feasible in the tumour-bearing mice. However, the plasma concentrations achieved at the selected times correlated with the ones observed in normal mice [15] or rats [16] indicating higher levels of MEN10755 than of DXR at early times (0.5 h) and the opposite at 24 h. In both species, compartmental pharmacokinetic analysis indicates that MEN10755 possesses a higher C max and AUC, a shorter half-life, a greater clearance and a lower volume of tissue distribution compared with DXR [15,16]. Since in rat only a minimal conversion of MEN10755 to metabolites was found [16], no attempt was performed to detect metabolites in this study. In mice, the pharmacokinetic behaviour and the tissue distribution of the disaccharide analogue account, at least in part, for its pharmacological profile in antitumour efficacy studies. Indeed, the lower tissue level and the shorter plasma half-life achieved by MEN10755 compared with DXR suggested the use of a more fractionated treatment schedule (twice weekly versus weekly) as the most favourable to exploit the therapeutic potential. A twice-weekly schedule is less favourable for DXR: a tolerated dose (4.5 mg/kg) was less effective and a higher dose (5.8 mg/kg) was partially toxic [5].

In conclusion, the results of this study suggest that MEN10755 has a peculiar pharmacokinetic behaviour which accounts for the distinct features compared with DXR in terms of efficacy and toxicity. Regarding efficacy, there is a preferential penetration and accumulation in tumour tissues compared with normal tissues. In addition, there is a superior ability of MEN10755 compared with DXR for commitment of tumour cells to undergo apoptosis and for inhibition and delay of

tumour growth. As regards toxicity, the drug disposition indicated a lower tissue content of MEN10755 than DXR in organs targeted for toxicity, such as the heart, and a faster clearance from plasma. Such findings are in line with a better tolerability and with evidence of a lower cardiotoxicity in animals. On the whole, the preclinical studies substantiated the hypothesis that the superior antitumour efficacy of MEN10755 over DXR reflects an increased tumour selectivity. Two phase I clinical studies of MEN10755 have been performed [17,18] and the compound is currently in phase II studies.

Acknowledgements

This work was partially supported by the Associazione Italiana Ricerca sul Cancro, Milan and by the Ministero della Sanita', Roma, Italy and by IMI grant no. 63216. We wish to thank Ms L. Zanesi for editorial assistance, Ms M. Tortoreto for technical support and Dr A.E.G. Crea and V. D'Aranno for helpful discussion and comments on kinetic data.

References

1. Arcamone F. *Doxorubicin, Anticancer Antibiotics*. New York, Academic Press, 1981.
2. Animati F, Berettoni M, Cipollone A, *et al*. New anthracycline disaccharides synthesis of L-daunosaminyl-α(1→4)-2-deoxy-L-rhamnosyl and of L-daunosaminyl-α(14)-2-deoxy-L-fucosyl daunorubicin analogues. *J Chem Soc, Perkin Trans 1* 1996, 1327–1329.
3. Arcamone F, Animati F, Bigioni M, *et al*. Configurational requirements of the sugar moiety for the pharmacological activity of anthracycline disaccharides. *Biochem Pharmacol* 1999, **57**, 1133–1139.
4. Arcamone F, Animati F, Berettoni M, *et al*. Doxorubicin disaccharide analogue: apoptosis-related improvement of efficacy *in vivo*. *J Natl Cancer Inst* 1997, **89**, 1217–1223.
5. Pratesi G, De Cesare M, Caserini C, *et al*. Improved efficacy and enlarged spectrum of activity of a novel anthracycline disaccharide analogue of doxorubicin against human tumor xenografts. *Clin Cancer Res* 1998, **4**, 2833–2839.
6. Bellarosa D, Ciucci A, Nardelli F, *et al*. Cellular and molecular basis of cytotoxic activity of anthracyclines: a comparative study of MEN10755 and doxorubicin. Biochemical mechanisms of induction of apoptosis. *J Pharmacol Exp Therap* 2000, in press.
7. UKCCCR guidelines for the welfare of animals in experimental neoplasia (second edition). *Br J Cancer* 1998, **77**, 1–10.
8. Pratesi G, Manzotti C, Tortoreto M, Prosperi E, Zunino F. Effects of 5-FU and cisDDP combination on human colorectal tumor xenografts. *Tumori* 1989, **95**, 60–65.
9. Broggini M, Italia C, Colombo T, Marmonti L, Donelli MG. Activity and distribution of i.v. and oral 4-demethoxydaunorubicin in murine experimental tumors. *Cancer Treat Rep* 1984, **68**, 739–747.
10. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 1992, **119**, 493–501.
11. Pratesi G, Polizzi D, Perego P, Dal Bo L, Zunino F. Bcl-2 phosphorylation in a human breast carcinoma xenograft: a common event in response to effective DNA-damaging drugs. *Biochem Pharmacol* 2000, **60**, 77–82.
12. Weiss RB. The anthracyclines: will we ever find a better doxorubicin? *Semin Oncol* 1992, **19**, 670–686.
13. Dorr RT. Cytoprotective agents for anthracyclines. *Semin Oncol* 1996, **23**, 23–34.
14. Cirillo R, Sacco G, Venturella S, Brightwell J, Giachetti A, Manzini S. Comparison of doxorubicin- and MEN10755-induced long-term progressive cardiotoxicity in the rat. *J Cardiovasc Pharmacol* 2000, **35**, 100–108.
15. D'Aranno V, Petrini A, Urso R, Giachetti A, Crea AEG. Pharmacokinetics of doxorubicin and MEN10755, a new anthracycline, in mice after intravenous administration. In *XXIX Congresso Nazionale della Societa' Italiana di Farmacologia*, 1999.
16. D'Aranno V, Curcuru' G, Urso R, Crea AEG. Pharmacokinetics of 14C-MEN10755, a new anthracycline, in the rat after intravenous administration. In *Proceedings of the 7th European Congress ECBP and 5th Congress EUFEPS*, Jerusalem, 1999.
17. Roelvink M, Aamdal S, Dombernowsky P, *et al*. Phase I study of MEN10755 in patients with a solid tumor as a short i.v. infusion given once every 3 weeks. *Eur J Cancer* 1999, **35**(Suppl. 4), 289.
18. Vermorken JB, Bos A, Schrijvers D, *et al*. Phase I study of MEN10755 in solid tumors. *Proceedings of ASCO* 2000, **19**, 202.