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3′-Deamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin (FCE 23762): A New Anthracycline Derivative With Enhanced Cytotoxicity and Reduced Cardiotoxicity

Romano Danesi, Cristiana Agen, Maria Grandi, Vincenzo Nardini, Generoso Bevilacqua and Mario Del Tacca

The purpose of this study was to examine the cytotoxicity and cardiotoxicity of the new doxorubicin derivative, 3′-deamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin (FCE 23762). The concentration of FCE 23762 that resulted in a 50% reduction in colony formation of DU 145, COLO 320DM, A549 and A2780 human cancer cell lines ranged from 1.1 and 3.2 nmol/l and was 3–9 times as low as doxorubicin. In the isolated perfused rat hearts, doxorubicin 10^{-5} mol/l induced a significant prolongation of SαT segment and Q–F_{max} interval, and reduction in dF/dt_{max} and coronary flow while only FCE 23762 10^{-5} mol/l induced a widening of QRS complex. Anaesthetised rats given a single intravenous (i.v.) dose of doxorubicin 10 mg/kg showed significant changes in both ECG (SαT segment and QRS complex enlargement) and haemodynamic parameters (increase in mean arterial blood pressure and reduction in systemic arterial dP/dt_{max}), while animals given FCE 23762 (0.1 and 0.3 mg/kg) had a significant increase in QRS complex duration after the highest dose. In the chronic cardiotoxicity study animals receiving FCE 23762 (0.03 mg/kg i.v. once a week for 3 weeks) did not show any significant alteration of ECG and minor changes of cardiac histological picture; by contrast doxorubicin (3 mg/kg i.v. once a week for 3 weeks) induced a severe cardiomyopathy, characterised by progressive widening of SαT segment, increase in T wave and histological damage consisting of vacuolations and loss of myofibrils. These results indicate that FCE 23762 is more active *in vitro* than doxorubicin and markedly less cardiotoxic *in vivo* at the doses used in the present study. *Eur J Cancer*, Vol. 29A, No. 11, pp. 1560–1565, 1993.

INTRODUCTION

CURRENT THERAPEUTIC use of the anthracycline antibiotic doxorubicin (DOX), one of the most effective anticancer agents, in repeated courses of treatment has been limited by either a severe dose-related cardiomyopathy [for review see 1,2] and development of drug-resistance [3]. As a consequence of these limitations several derivatives have been synthesised; the most promising of them are compounds in which a new morpholinyl ring incorporating the amino nitrogen is obtained by the reductive alkylation of the sugar amino group of DOX [4]. The morpholinyl anthracyclines constitute a family of highly potent analogues including 3′-deamino-3′-(4-morpholinyl)-DOX, 3′-deamino-3′-(3-cyano-4-morpholinyl)-DOX [4] and 3′-deamino-3′-morpholino-13-deoxo-10-hydroxycarminomycin (MX2) [5].

Compared to their parent drugs, the most relevant features of such compounds are the increased affinity for lipids, which is associated with a rapid diffusion through the cell membrane and improved cellular uptake, and the marked increase in cytotoxicity when these drugs are administered *in vivo*, suggesting that a biotransformation occurs releasing a highly cytotoxic metabolite [4–8]. The new derivative of DOX, 3′-deamino-3′-(2-methoxy-4-morpholinyl)-DOX (FCE 23762, Fig. 1) is not cross-resistant to DOX in multidrug resistant cells and appears more cytotoxic than the parent drug on human and murine cell lines [9]. FCE 23762 is about 80 times as potent as DOX *in vivo*, is highly lipophilic and presents equivalent antitumour activity when administered by intraperitoneal (i.p.), intravenous (i.v.) or oral routes [10].

The present study evaluates the *in vitro* cytotoxicity of FCE 23762 on four human tumour cell lines of different histogenesis

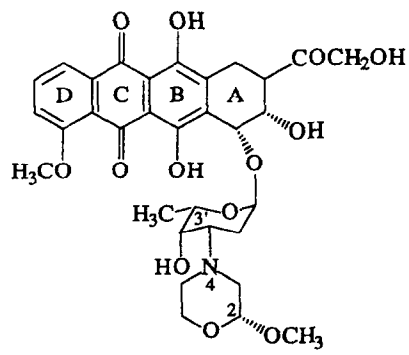

Fig. 1. Chemical structure of 3′-deamino-3′-(2-methoxy-4-morpholinyl)-DOX (FCE 23762).

Correspondence to M. Del Tacca.
M. Del Tacca and C. Agen are at the Institute of Medical Pharmacology, University of Pisa, Via Roma 55, I-56126 Pisa; R. Danesi is at the School of University Studies and Doctoral Research S. Anna, Via Carducci 40, I-56127 Pisa; M. Grandi is at the Oncology Department, Farmitalia C. Erba Research Center, Via Giovanni XXIII 14, I-20014 Nerviano, Milano; and V. Nardini and G. Bevilacqua are at the Institute of Pathological Anatomy, University of Pisa, Via Roma 57, I-56126 Pisa, Italy.
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and its acute and chronic cardiotoxicity in the rat. The results demonstrate that FCE 23762 is more potent than DOX in inhibiting the colony formation of human cancer cell lines *in vitro* while the enhanced chemotherapeutic activity is not associated with any substantial increase in cardiotoxicity.

MATERIALS AND METHODS

Drugs, chemicals and supplements for cell culture

Doxorubicin and FCE 23762 were obtained from Farmitalia-Carlo Erba (Milano, Italy). The anthracyclines were dissolved in sterile isotonic saline just before use, and solutions were protected from the light until administration. RPMI 1640 and Ham's F12K media, fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, phosphate-buffered saline (PBS), and trypsin–EDTA solution (0.5 g/l trypsin–0.2 g/l EDTA·4Na) in Hank's balanced salt solution (HBSS) were from Sigma. Crystal violet was purchased from Carlo Erba (Milano, Italy) and urethane was from Fluka AG (Buchs, Switzerland); plastics for cell culture were from Costar (Cambridge, Massachusetts, U.S.A.).

In vitro *cytotoxicity*

The following human cancer cell lines were used: DU 145, originated from a metastatic brain lesion of an androgen-insensitive prostate cancer; COLO 320DM, obtained from a moderately undifferentiated adenocarcinoma of the sigmoid colon; A549, derived from a non-small cell lung cancer, and A2780, originated from an adenocarcinoma of the ovary. With the exception of the latter cell line, which was kindly provided by Dr E. Reed (National Cancer Institute, Bethesda, Maryland, U.S.A.), cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, Maryland, U.S.A.). DU 145, COLO 320DM and A2780 cell lines were grown in RPMI 1640 medium; A549 cell line was cultured in F12K medium. Media were added with 10% FBS, antibiotics (penicillin 100 IU/ml and streptomycin 100 μg/ml) and L-glutamine (2 mmol/l). Stock cultures were harvested with trypsin-EDTA solution in HBSS and routinely subcultivated in 175 cm^2 flasks incubated at 37°C in 5% CO_2 with 100% relative humidity. Cells in exponential phase of growth were used for the experiments described in this study.

The chemosensitivity of cancer cell lines was estimated by the colony formation assay. Cells were plated in triplicate in 6-well tissue culture plates in 2 ml of medium at 1.5×10^3 cells/well; 24 h after seeding they were treated with DOX or FCE 23762 for 48 h. Media containing drugs were replaced with 2 ml of drug-free medium and culture plates were incubated for an additional 4–7 days; the resulting colonies were fixed with 1 ml of methanol and stained with crystal violet. Colonies with more than 50 cells were scored as survivors and counted. The relative cloning efficiency was expressed as the percentage of the number of colonies formed in the presence of drugs vs. colonies formed in control wells. The drug concentration which inhibits 50% of the colony formation (IC_{50}) was determined from the cytotoxicity data using a mathematical transformation in which the log of the relative cloning efficiency was plotted vs. the log of the drug concentration; the resulting equation obtained with linear regression analysis was then solved to determine the log of the IC_{50}. The mean control cloning efficiency was 22% (DU 145), 8% (COLO 320DM), 19% (A549), and 12% (A2780).

Animals

Adult female Wistar rats with a mean body weight of 200 ± 8.5 g (S.D.) were used. They were fed standard laboratory chow and tap water *ad libitum* and were not used for at least 1 week after their delivery to the laboratory. The animals were maintained on a 12-h lighting cycle at an environmental temperature of 22–24°C, and approximately 50–60% relative humidity; their care and handling were in accordance with the provisions of the European Economic Community (EEC) Council Directive 86–609 recognised and adopted by the Italian Government. The distribution of animals in groups, and the treatment given to each group were randomised,

Isolated heart study

Normal untreated rats were given heparin 500 IU/kg i.p. and then killed by cervical dislocation. Hearts (wet weight 0.95–1.10 g) were quickly removed and rinsed in cold NaCl 0.9%. The aorta was cannulated with a polyethylene catheter (inside diameter 1.3 mm) with the tip of the cannula positioned just above the semilunar valves. The cannula, which was tied in place, served to suspend hearts in the thermostatic chamber and to retrogradely perfuse them with the Krebs–Henseleit solution (composition in mmol/l: NaCl, 118; KCl, 4.7; EDTA·2Na, 1.0; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 25; glucose, 11) at 37°C, oxygenated with a mixture of 95% O_2 and 5% CO_2 in a non-recirculating system; perfusion pressure was kept at 60 mmHg. A suture was tied to the apex of hearts to connect them to an isometric force transducer (Basile model DY2, Basile, Comerio, Italy) and allowed to equilibrate for 30 min under 1 g of resting tension. Epicardial ECGs were recorded by means of two wick-type electrodes applied to the surface of the heart (right atrium and apex of the right ventricle), so that the perfusion solution flowing over the surface of the heart bridged the small gap between electrodes and myocardium, providing a pathway for conduction of electrical impulses between them. Hearts were perfused with either DOX 10^{-5} mol/l, or FCE 23762 10^{-7}–10^{-5} mol/l. ECG parameters, including SαT segment, QRS complex, PR interval (all expressed in ms), T wave amplitude (mV), and isometric contractile force tracings including maximum developed force (F_{max}, *g*), time interval between the onset of QRS complex of ECG and F_{max} (Q–F_{max}, ms), maximum rate of rise of force (dF/dt_{max}, *g*/s), and heart rate (beats/min) were recorded [11]. The coronary flow was evaluated by measuring the volume of solution spontaneously draining from hearts over 1 min.

Acute cardiotoxicity study

Rats were anaesthetised with a single dose of urethane (1 g/kg i.p.), tracheotomised and allowed to breathe spontaneously; after this dose of urethane animals did not require mechanical ventilation [12]. Blood pressure, ECG and heart rate were stable for at least 1 h and within the values published elsewhere [for review see ref. 13]. Arterial blood pressure was recorded continuously from a cannulated carotid artery via a pressure transducer (Statham model P23ID) connected to an APC channel of a Battaglia-Rangoni ESO 600 polygraph (Battaglia-Rangoni, Bologna, Italy) and the mean arterial blood pressure (mmHg) was calculated. Heart rate (beats/min) was derived from the pulse wave of the carotid pressure. The maximum rate of rise of systemic arterial (SA) blood pressure (SA dP/dt_{max}, mmHg/s), an indirect index of left ventricular contractility [14], was measured by differentiating the pressure signal by means of an analogue device (AO/DP/NS channel, Battaglia-Rangoni, Bologna, Italy). ECG (lead II) was recorded as previously reported [15] and the SαT segment, QRS complex duration (ms) and T wave amplitude (mV) were measured.

After the animals had been in stable ECG and haemodynamic conditions, DOX (10 mg/kg) and FCE 23762 (0.1 and 0.3 mg/kg) were administered by i.v. infusion through the femoral vein; the fluid volume, 1 ml/kg, was injected over 5 min. Controls were treated with 0.9% aqueous NaCl. ECG and haemodynamic parameters were recorded during a period of 60 min starting from the beginning of drug infusion.

Chronic cardiotoxicity study

Animals were given three i.v. bolus injections of DOX 3 mg/kg or FCE 23762 0.03 mg/kg once a week and were observed for a recovery period of 4 weeks. Body weight was recorded during the study; ECG (lead II) was monitored before dosing and once a week during the recovery period, as previously reported [15]. QRS complex and SαT segment duration (ms), and T wave voltage (mV) were measured.

At the end of the experiment (6th week), animals were killed by cervical dislocation. Hearts were immediately removed and washed in cold isotonic saline (0.9% NaCl) and fixed by retrogradely perfusing them through the aorta with 4% paraformaldehyde in 0.1 mol/l phosphate-buffered saline, pH 7.3 (see isolated heart study for details on perfusion procedure). Each heart was cut into 1 mm-thick transverse sections which were embedded in glycol methacrylate plastic resin. Sections (thickness: 1 μm) including epicardial and endocardial surfaces of left and right ventricles were obtained using an LKB Historange microtome (LKB, Bromma, Sweden) and stained with haematoxylin and eosin. The severity of cardiac lesions was evaluated by an investigator, who had no prior knowledge of the treatment given to animals.

Statistics

Presented data are means ± S.E. of n number of observations. Data relative to cytotoxicity (IC_{50}), isolated heart study (ECG, contractility parameters and coronary flow) and to acute and chronic cardiotoxicity studies (haemodynamics, ECG parameters and body weight) were analysed by a one-way analysis of variance, followed by the Student-Newman-Keuls test [16] to evaluate the effect of treatments. A P value less than 0.05 was considered significant.

RESULTS

In vitro *cytotoxicity*

Over a complete dose-response range *in vitro* both FCE 23762 and DOX dose-dependently inhibited the ability of individual cells to form colonies, COLO 320DM being the most sensitive cell line (Fig. 2). The concentrations of FCE 23762 required for half-maximal inhibition of colony formation (IC_{50}) of the cell line tested were 3–9 times as low as those of DOX; this difference was statistically significant (Fig. 2).

Isolated heart study

Control hearts or hearts perfused with the Krebs–Henseleit solution containing FCE 23762 10^{-6} or 10^{-7} mol/l did not show any significant change in ECG, contractility parameters and coronary flow (Fig. 3). Perfusion with DOX 10^{-5} mol/l and FCE 23762 10^{-5} mol/l were associated with significant alterations of heart function consisting of a persistent widening of SαT segment ($P < 0.05$ at 15–60 min, compared to controls, FCE 23762 10^{-6} and 10^{-7} mol/l; Fig. 3), a progressive increment in PR interval ($P < 0.05$ at 30–60 min, compared with controls, FCE 23762 10^{-6} and 10^{-7} mol/l; data not shown), and a decrease in dF/dt_{max} ($P < 0.05$ at 15–60 min, compared to controls, FCE

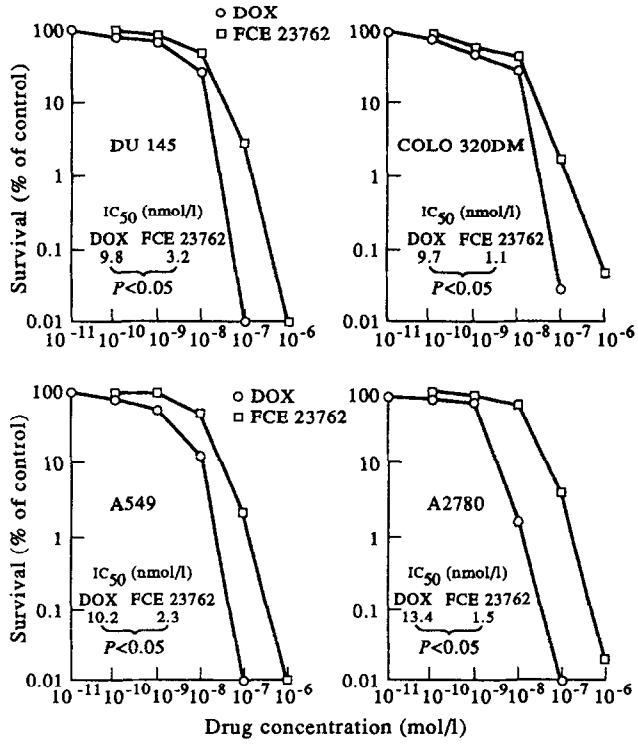

Fig. 2. Effect of increasing concentrations of DOX or FCE 23762 on colony forming ability of DU 145, COLO 320DM, A549 and A2780 human cancer cell lines. Cells were exposed to drugs for 48 h and colonies were counted after culture in drug-free medium for 4–7 days. The mean value of IC_{50} and the statistical significance are shown in the graphs. Growth inhibition is expressed as percentage of untreated control. Data points are the mean of nine experiments; the S.E. never exceeded 10%.

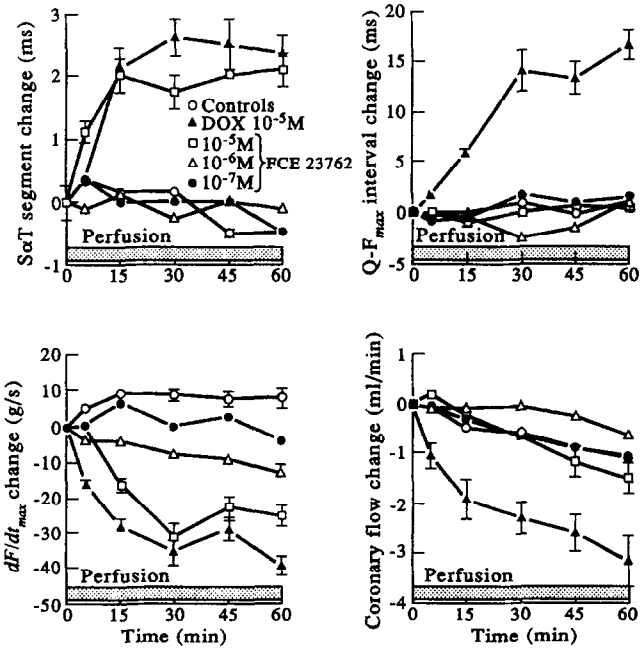

Fig. 3. Effects of the anthracyclines on SαT segment, Q–F_{max} interval, dF/dt_{max} and coronary flow of isolated hearts perfused with DOX 10^{-5} mol/l, FCE 23762 10^{-5}, 10^{-6} and 10^{-7} mol/l for 60 min (stippled column). Changes are expressed as differences from baseline values (time 0). Data points are the mean of five hearts, with vertical bars representing the S.E. Mean ± S.E. baseline values were: SαT segment: 14.8 ± 2.1 ms; Q–F_{max} interval: 48.8 ± 5.7 ms; dF/dt_{max}: 70.8 ± 9.1 g/s; coronary flow: 5.4 ± 3.9 ml/min.

23762 10^{-6} and 10^{-7} mol/l; Fig. 3). In hearts perfused with DOX 10^{-5} mol/l, Q-F_{max} interval was markedly increased ($P < 0.05$ at 5–60 min, compared to controls, FCE 23762 10^{-5}, 10^{-6} and 10^{-7} mol/l; Fig. 3), while heart rate ($P < 0.05$ at 5–60 min, compared to controls, FCE 23762 10^{-5}, 10^{-6} and 10^{-7} mol/l; data not shown) and coronary flow ($P < 0.05$ at 5–60 min, compared to controls, FCE 23762 10^{-5}, 10^{-6} and 10^{-7} mol/l; Fig. 3) were markedly reduced.

Acute cardiotoxicity study

No significant changes were observed in both ECG and haemodynamic parameters of control rats treated with vehicle (0.9% NaCl) or animals receiving FCE 23762 0.1 mg/kg. DOX 10 mg/kg induced an early and persistent increase in SαT segment duration ($P < 0.05$ at 5–60 min, compared to controls, FCE 23762 0.1 and 0.3 mg/kg; Fig. 4). QRS complex duration increased immediately after the beginning of DOX infusion: at 5–60 min it was significantly different ($P < 0.05$) from controls and FCE 23762 0.1 mg/kg (Fig. 4). Adminstration of FCE 23762 0.3 mg/kg was associated with a significant increase in QRS complex duration ($P < 0.05$ at 15–60 min compared with controls and FCE 23762 0.1 mg/kg; Fig. 4). Other haemodynamic changes in animals given DOX included an increase in mean arterial blood pressure ($P < 0.05$ at 15–45 min) and impairment of SA dP/dt_{max} ($P < 0.05$ at 15–60 min, compared with controls and FCE 23762 0.1 and 0.3 mg/kg; Fig. 4); these parameters were not significantly affected in animals given FCE 23762 0.1 and 0.3 mg/kg (Fig. 4).

Chronic cardiotoxicity study

Rats injected with DOX showed inactivity, anorexia and diarrhoea. The body weight changes of animals from each experimental group are shown in Fig. 5. DOX-treated animals showed a significant reduction in body weight increase compared with controls and rats given FCE 23762 0.03 mg/kg ($P < 0.05$ at 1–6 weeks). During the study the ECGs of controls and FCE 23762-treated rats remained unchanged. On the contrary, DOX-treated animals presented a progressive widening of SαT segment ($P < 0.05$ at 2–6 weeks, compared to controls and FCE 23762 0.03 mg/kg; Fig. 5) and changes of T wave amplitude ($P < 0.05$ at 1–6 weeks, compared to controls and FCE 23762 0.03 mg/kg; Fig. 5). The histological analysis of hearts from animals treated with FCE 23762 0.03 mg/kg revealed the presence of minimal cardiac alterations, consisting of rarefaction of nuclear chromatin (Fig. 6). The examination of myocardial tissue from DOX-treated animals revealed the presence of myofibrillar loss and cellular vacuolation in the majority of heart sections observed (Fig. 6). These lesions were more pronounced in the samples from the left ventricles.

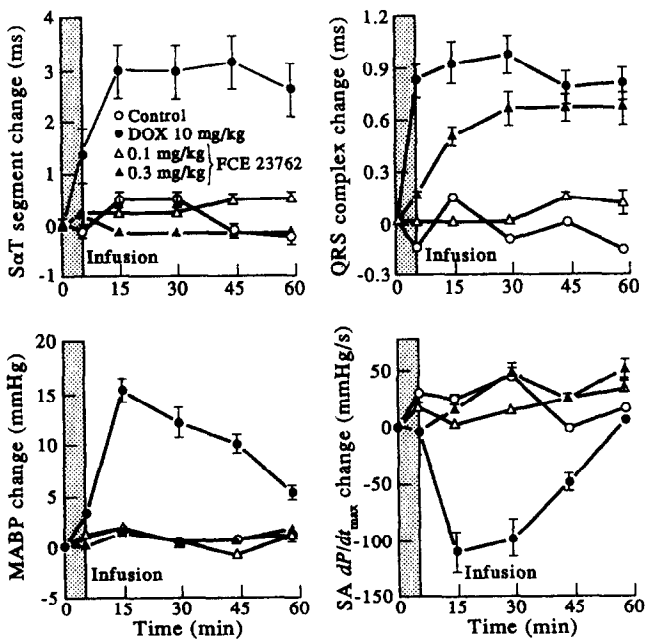

Fig. 4. Effects of the anthracyclines on SαT segment, QRS complex, mean arterial blood pressure (MABP) and systemic arterial (SA) dP/dt_{max} of anesthetised rats treated with DOX 10 mg/kg, FCE 23762 0.1 or 0.3 mg/kg as i.v. infusion lasting 5 min (stippled column). Changes are expressed as differences from baseline values (time 0). Data points are the mean of six animals, with vertical bars representing the S.E. Mean ± S.E. baseline values were: SαT segment: 18.1 ± 2.8 ms; QRS complex: 13.9 ± 1.8; MABP: 72.1 ± 9.4 mmHg; SA dP/dt_{max}: 2735.8 ± 190.5 mmHg/s.

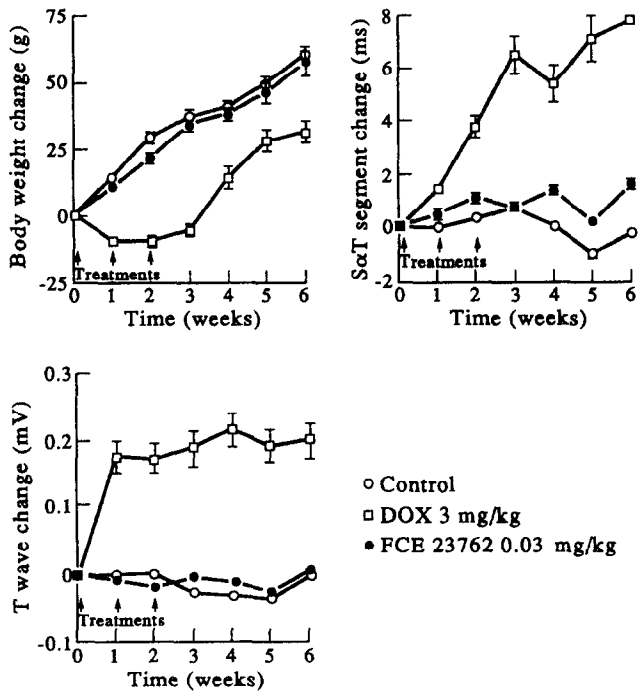

Fig. 5. Effects of the anthracyclines on body growth rate, SαT segment and T wave of rats given 3 weekly doses (arrows) of DOX 3 mg/kg or FCE 23762 0.03 mg/kg. Changes are expressed as differences from baseline values (time 0). Data points are the mean of six animals, with vertical bars representing the S.E. Mean ± S.E. baseline values were: body weight: 215.9 ± 18.5 g; SαT segment: 18.5 ± 3.0 ms; T wave: 0.2 ± 0.018 mV.

DISCUSSION

Several anthracycline derivatives modified on sugar moiety have been synthesised to enhance the antitumour activity, reduce the toxicity and circumvent the development of multidrug-resistance associated with DOX treatment. Morpholinyl anthracyclines, including 3'-deamino-3'-(4-morpholinyl)-DOX and 3'-deamino-3'-(3-cyano-4-morpholinyl)-DOX [4], 3'-deamino-3'-morpholino-13-deoxo-10-hydroxy-carminomycin (MX2) [5] and FCE 23762 [9, 10], appear to be the most promising drugs. Previous data have demonstrated that their antitumour activity against cancer cell lines is increased with respect to DOX: the analysis of the 50% lethal concentration of 3'-deamino-3'-(3-cyano-4-morpholinyl)-DOX demonstrated that it was 1400 times

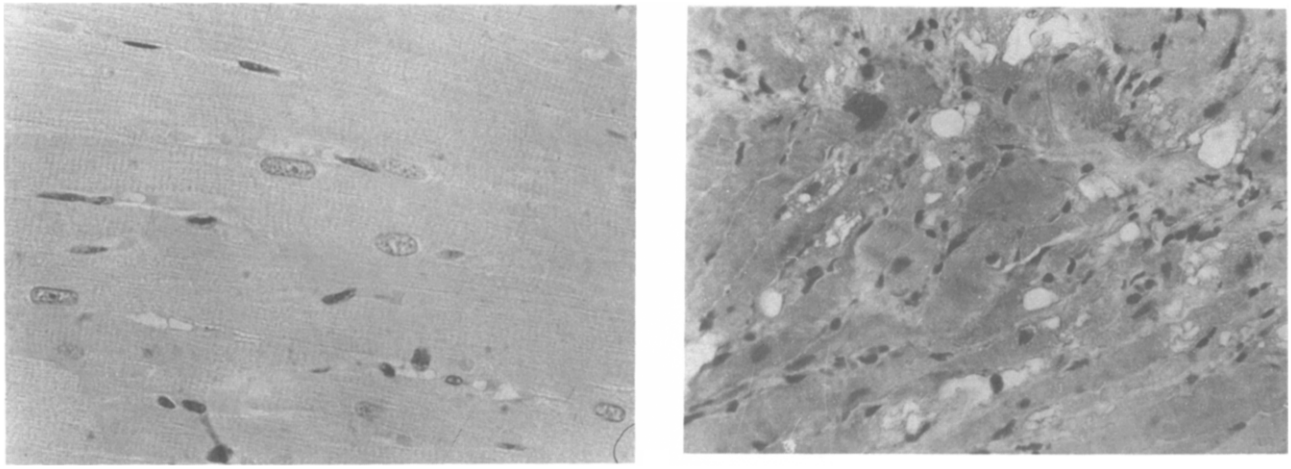

Fig. 6. Haematoxylin and eosin stained sections of myocardium from the left ventricle of a rat 4 weeks after treatment with three weekly doses of DOX 3 mg/kg (left) or FCE 23762 0.03 mg/kg (right). DOX induced severe morphological alterations, consisting of vacuolations and loss of myofibrils affecting nearly every myocyte, while FCE 23762 treatment was associated with modest nuclear changes consisting of chromatin rarefaction in isolated myocytes. Magnification: ×450 (left), ×400 (right).

as potent as DOX with respect to cytocidal activity against KBM-3 human leukaemia cells *in vitro* [7] and the concentration required to reduce cell viability of HT-29 human colon carcinoma *in vitro* by 90% was 150 times as low as DOX [17]. FCE 23762 has been reported to be between 3- and 15-fold more cytotoxic than DOX *in vitro* on different cell lines, including LoVo colon adenocarcinoma [9] and ES-2 ovarian cancer [18]. This drug was demonstrated to be 80–120 times as potent as the parent compound *in vivo* against tumours transplanted into mice [10]. Furthermore, morpholinyl analogues are noncross-resistant to DOX on multidrug resistant cells [19]; compounds tested included 3′-deamino-3′-(3-cyano-4-morpholinyl)-DOX on the Dx5 subline of the human sarcoma cells MES-SA [20], 3′-deamino-3′-(4-morpholinyl)-DOX on murine leukaemia P388/DOX [6], the MX2 derivative against K562/DOX and 2780^{AD} [5] and FCE 23762 on human colon adenocarcinoma LoVo/DOX [10].

The results of the chemosensitivity testing performed on human cancer cell lines in the present study indicate that the IC_{50} values of FCE 23762 were 3–9 times as low as those of DOX. The enhanced cytotoxic activity of FCE 23762 as well as of other morpholinyl anthracyclines may be the result of the combination of several factors. These drugs reach elevated intracellular concentrations because they are more lipophilic than the parent compounds and, consequently, they easily cross cell membranes [5, 6]. FCE 23762 achieved much higher intracellular levels than DOX in P388, LoVo and MCF-7 cells and in their resistant sublines [9]. The basic morpholinyl nitrogen present in 3′-deamino-3′-(4-morpholinyl)-DOX may become protonated under the slight acidic conditions of intracellular pH and subsequently it binds with high affinity to intracellular sites [6] allowing a prolonged exposure of target molecules to the drug. The above-mentioned mechanism, however, is not valid for 3′-deamino-3′-(3-cyano-4-morpholinyl)-DOX since it retains its neutrality over a broad range of pH [20].

Morpholinyl anthracyclines inhibited RNA synthesis to a greater extent than DNA synthesis, without significant differences with DOX [21]; at variance with DOX, however, they were found to be preferential inhibitors of rDNA transcription and might have a mechanism of action similar to actinomycin D on rRNA synthesis [22]. In addition to this, morpholinyl anthracyclines, but not DOX, stimulated topoisomerase I-mediated DNA cleavage of specific DNA sites [23]. In the study by Peters *et al.* [24] and Westendorf *et al.* [25], 3′-deamino-3′-(3-cyano-4-morpholinyl)-DOX but not other cyano-lacking morpholinyl anthracyclines produced DNA–DNA cross-links probably via CN displacement. Recent studies on FCE 23762 demonstrated that the methoxy-morpholino derivative of DOX produced single-strand breakage of DNA and its cytotoxicity was potentiated in the presence of human liver microsomes and NADPH [18]. In these conditions, DNA interstrand cross-links are produced since the drug is activated to a covalently reacting molecule, probably the *O*-demethyl metabolite [18]. Metabolic potentiation of cytotoxicity of FCE 23762 [18] or other morpholinyl derivatives of DOX [8] is markedly reduced by inhibition of the CYP IIIA isoform(s) of cytochrome P-450, suggesting that this enzyme system plays a major role in the activation of morpholinyl anthracyclines to a more active species [8]. Interestingly, no potentiation by microsomes and NADPH occurred with DOX or 3′-deamino-3′-(3-cyano-4-morpholinyl)-DOX [8].

The results of the present work demonstrated that no substantial cardiotoxicity occurred with FCE 23762 at the doses employed throughout the study; in particular, when comparing equimolar doses of FCE 23762 and DOX in the isolated heart study, the methoxymorpholinyl derivative was found to be less toxic than DOX. On the contrary, DOX produced alterations in all model systems used, the most characteristic of them were the significant widening of SαT segment of the ECG, indicating abnormalities of the repolarisation of cardiac tissue [15] and histologically detectable cellular vacuolation and myofibril loss. Several lines of evidence demonstrate that free radical formation via redox cycling of the quinone moiety of the anthracycline ring may be causative in the development of chronic cardiotoxicity [1, 2]. Substitution of the quinone of DOX and daunorubicin (DNR) by an imino group results in a selective reduction of cardiotoxicity of DOX and DNR without significant changes in anticancer activity [4]. The cardiotoxicity of one of the most extensively studied morpholinyl anthracyclines, 3′-deamino-3′-(3-cyano-4-morpholinyl)-DOX, demonstrated that at doses equivalent to DOX in terms of therapeutic activity, the derivative did not produce heart damage, indicating a clear separation of cardiotoxicity and antitumour activity in this compound [20].

On an equimolar basis, however, the cardiotoxicity of 3'-deamino-3'-(3-cyano-4-morpholinyl)-DOX and DOX were similar [20]; this result should be expected since the quinone group of the anthracycline ring is unchanged in 3'-deamino-3'-(3-cyano-4-morpholinyl)-DOX. Thus, the improvement in the therapeutic index of such derivatives is dependent on the marked increase in antitumour activity. Since the quinone is unsubstituted in FCE 23762, one might speculate that this anthracycline analogue might be cardiotoxic at very high doses, even though cardiac tissue distribution and metabolism should be considered for a complete toxicological profile of FCE 23762. The new morpholinyl derivative FCE 23762 should not produce cardiotoxicity at therapeutically effective doses, due to its unusual increase in therapeutic activity compared to the parent drug. Other signs of toxicity, such as neurological and dermatological, as shown for 3'-deamino-3'-(3-cyano-4-morpholinyl)-DOX [26], could not be demonstrated in the present study. It should be noted that, at variance with 3'-deamino-3'-(3-cyano-4-morpholinyl)-DOX, FCE 23762 is inactive in intracranially implanted murine leukaemias, suggesting that it does not cross the blood–brain barrier [10] and should not produce neurotoxicity.

In conclusion, the marked differences between chemotherapeutic activity and toxicity of FCE 23762 and DOX suggest that the methoxymorpholinyl substituent may represent an important improvement in the design of a new class of anthracycline analogues characterised by an increased growth inhibitory activity and reduced cardiac toxicity compared to DOX.

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