Cancer Chemother Pharmacol (1992) 29: 261–265

Cancer Chemotherapy and Pharmacology

Reduced cardiotoxicity and increased cytotoxicity in a novel anthracycline analogue, 4′-amino-3′-hydroxy-doxorubicin

Romano Danesi[1], Nunzia Bernardini[1], Cristiana Agen[1], Mario Costa[1], Lucia Zaccaro[2], Donatella Pieracci[2], Gino Malvaldi[2], and Mario Del Tacca[1]

[1] Institute of Medical Pharmacology, and [2] Institute of General Pathology, University of Pisa, Pisa, Italy

Received 20 April 1990/Accepted 18 September 1991

Summary. The acute and chronic cardiotoxicity and cytotoxicity of the novel doxorubicin (DXR) derivative 4′-amino-3′-hydroxy-DXR were compared with those of 4′-deoxy-DXR and DXR. In the acute cardiotoxicity study, the ECG and hemodynamic changes recorded in anesthetized rats that had been treated i.v. with 10 mg/kg 4′-amino-3′-hydroxy-DXR or 8.6 mg/kg 4′-deoxy-DXR were significantly less severe than those caused by 13 mg/kg DXR. In the chronic cardiotoxicity study, rats received 3 weekly i.v. injections of 3 mg/kg DXR, 3 mg/kg 4′-amino-3′-hydroxy-DXR, or 2 mg/kg 4′-deoxy-DXR during the first 14 days of the study and were observed for an additional 35-day period. DXR induced severe cardiomyopathy that was characterized by ECG changes in vivo (SαT-segment widening and T-wave flattening) and by impairment of the contractile responses (F_{max}, $\pm dF/dt_{max}$) to adrenaline of hearts isolated from treated animals. 4′-Deoxy-DXR caused a progressive enlargement of the SαT segment in vivo and a significant impairment of the $-dF/dt_{max}$ value in vitro, which were less severe than those produced by DXR. The least cardiotoxic drug was 4′-amino-3′-hydroxy-DXR, which induced minor ECG changes without causing significant alterations in the contractile responses of isolated hearts to adrenaline. On the basis of the drug concentration required to inhibit 50% of the colony formation (IC_{50}) of cell lines in vitro, 4′-amino-3′-hydroxy-DXR was less active than 4′-deoxy-DXR but at least twice as active as DXR against human cancer and murine transformed cell lines. These data indicate that 4′-amino-3′-hydroxy-DXR is significantly less cardiotoxic and more cytotoxic than DXR.

Introduction

The discovery made >20 years ago that antitumor therapy with DXR could produce irreversible and possibly life-threatening cardiac injury has led to limitations on the application of this drug in clinical practice despite its being one of the most effective single agents used for the treatment of cancer (for review see [12]).

New anthracycline analogues have been synthesized with the aim of reducing the cardiac toxicity and improving the antitumor activity of DXR [1]. Among the glycoside analogues, 4′-deoxy-DXR differs from DXR in the absence of an oxygen atom at position 4′ on the aminosugar. This drug, which is more lipophilic than DXR, exhibits increased cellular uptake and cytotoxicity both in vivo [6] and in vitro [15] as compared with the prodrug. The encouraging results that have been obtained by modifying the structure of the sugar moiety have led to the synthesis of a large number of anthracycline analogues; among these, 4′-amino-3′-hydroxy-DXR has been selected for preclinical toxicology studies on the basis of a promising spectrum of antitumor activity [1].

To investigate from a toxicological point of view the properties conferred by exchanging the positions of the amino and hydroxyl groups on the daunosamine, the aim of the present study was to compare both the acute and chronic cardiotoxicity of 4′-amino-3′-hydroxy-DXR, 4′-deoxy-DXR, and DXR in the rat and their cytotoxicity toward human cancer and murine transformed cell lines. Under the experimental conditions used in the present study, 4′-amino-3′-hydroxy-DXR was characterized by a significantly lower degree of cardiotoxicity as compared with DXR; this was not associated with reduced antiproliferative activity since the analogue was more cytotoxic than the prodrug.

Materials and methods

Experimental animals. Guidelines for the care and use of laboratory animals were followed as described by the "Guide for Care and Use of Laboratory Animals" (NIH publication 85–23, 1985 revision). Female Wistar rats weighing 190±8.5 g were obtained from Nossan (Milano, Italy). The rats were housed in plastic cages lined with wood-chip bedding in animal quarters under conditions of controlled temperature (22°–25°C), humidity (45%–55%) and light (12-h cycles) and were

Offprint requests to: M. Del Tacca, Institute of Medical Pharmacology, University of Pisa, 55, Via Roma, I-56 126 Pisa, Italy

given distilled drinking water and standard laboratory food ad libitum. Animals were randomized prior to their assignment to one of eight treatment groups and were subjected to a 10-day quarantine before the initiation of the experiments.

Drugs, chemicals, and supplements for cell culture. 4′-Amino-3′-hydroxy-DXR, 4′-deoxy-DXR, and DXR hydrochloride salts were obtained from Farmitalia-Carlo Erba (Milano, Italy). Solutions of drugs in 0.9% NaCl were freshly prepared immediately before their use and were protected from light. Urethane, adrenaline bitartrate, and the lactate dehydrogenase diagnostic kit were supplied by Sigma Chemical Co. (St. Louis, Mo., USA). All other chemicals were of analytical grade. RPMI 1640 medium, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS, pH 7.4), L-glutamine, antibiotics (penicillin and streptomycin), and 0.025% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) in Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution (HBSS) were obtained from Gibco (Paisley, Scotland, UK). Plastic equipment for cell culture was supplied by Costar (Cambridge, Mass., USA).

Acute cardiotoxicity study. Animals were divided into four groups of eight rats each. The experimental procedure has been described in detail elsewhere [8]. Briefly, a polyethylene catheter filled with heparinized saline (0.9% NaCl) was placed in the common carotid artery of rats in which anesthesia had been induced using 1 g/kg i. p. urethane, a dose that is suitable for cardiovascular studies [18]. The heart rate (beats/min), mean arterial blood pressure (MABP, mmHg), and systemic arterial (SA) dP/dt_{max} value (mmHg/s), which is an indirect index of left ventricular contractility [4], were measured. Needle electrodes were inserted under the skin of the animals' legs to record lead-II ECG, and SαT segments (ms) and T waves (μV) were measured and taken as cardiotoxicity endpoints [7]. After the establishment of stable hemodynamic conditions, the rats received a bolus injection of either 10 mg/kg 4′-amino-3′-hydroxy-DXR, 8.6 mg/kg 4′-deoxy-DXR, 13 mg/kg DXR, or vehicle (0.9% NaCl) and parameters were measured for 60 min post-dosing. The doses injected were chosen on the basis of equivalent antitumor activity against a murine solid tumor model [14].

Chronic cardiotoxicity study. Animals were divided into 4 groups of 12 rats each, were given 3 mg/kg 4′-amino-3′-hydroxy-DXR, 2 mg/kg 4′-deoxy-DXR, 3 mg/kg DXR, or vehicle (0.9% NaCl) on days 0, 7, and 14 of the study and were observed for a recovery period of 35 days. DXR and 4′-deoxy-DXR doses corresponded to the optimal therapeutic doses used in multiple-dose antitumor activity studies in animals [3]. The dose of 4′-amino-3′-hydroxy-DXR used in this study was equal to that of DXR since the two anthracyclines exhibit equal antitumor activity against a panel of murine solid tumors [14]. Starting from the first administration of drug (day 0), body weights and ECGs (lead II) were recorded weekly immediately prior to each injection with the animals under light ether anesthesia and on the same day of the week during the recovery period; SαT-segment duration (ms) and T-wave amplitude (μV) were also measured. At the end of the study, rats were injected i. p. with 500 IU heparin and killed by cervical fracture, and their hearts were immediately removed for the isolated heart study. Because of the reliability of the SαT-segment value in monitoring the development of anthracycline cardiotoxicity and its correspondence to the DXR-induced morphological alterations in the myocardium [7], histopathology of the cardiac tissue was not performed.

Isolated heart study. The isolated heart study was carried out as previously described [8]. Briefly, hearts were rinsed in cold 0.9% NaCl and the aorta was cannulated. Hearts were rapidly transferred to a thermostatic chamber and were retrogradely perfused in a flow-through system with Locke-phosphate buffer consisting of 157 mM NaCl, 5.63 mM KCl, 2.09 mM $CaCl_2$, 1.75 mM $NaHCO_3$ and 5.55 mM glucose at 37° C under oxygenation with 100% O_2 (pH adjusted to 7.4); the perfusion pressure was kept at 60 mmHg. Hearts were stimulated with increasing doses of adrenaline (0.1, 1 and 10 μg) that were added cumulatively (0.1 ml) to the perfusion medium just above the aortic cannula, and isometric contractile-force tracings were recorded as previously described [10]; the parameters measured included the maximal developed force (F_{max}), the maximal rate of increase in force (+ dF/dt_{max}), and relaxation (– dF/dt_{max}).

Cell culture and cytotoxicity assay. The human cell lines HOS (osteosarcoma) and DU 145 (prostate cancer) were purchased from the American Type Culture Collection (ATCC, Rockville, Md., USA). The human cell lines SNB-19 (glioblastoma) and A2780 (ovarian cancer) were kindly provided by Dr. RV La Rocca and Dr. E Reed, respectively (National Cancer Institute, Bethesda, Md., USA). The V79/AP4 cell line, a transformed Chinese hamster fibroblast cell line [17], was also used for cytotoxicity studies. The growth medium for HOS, DU 145, SNB-19, and A2780 was RPMI 1640 supplemented with 10% FBS; V79/AP4 cells were grown in DMEM containing 5% FBS. Media were supplemented with antibiotics (penicillin, 100 IU/ml; streptomycin, 100 μg/ml) and L-glutamine (2 mM). Cells were harvested with 0.025% trypsin and 0.02% EDTA in Ca^{2+}/Mg^{2+}-free HBSS from exponential-phase maintenance cultures that had been cultivated in 175-cm^2 flasks by incubation at 37° C in an atmosphere containing 5% CO_2 at 100% relative humidity.

Chemosensitivity studies were carried out as clonogenic survival assays [16]. HOS, DU 145, SNB-19, and A2780 cells (1,000/well) were plated in serum-supplemented medium in six-well culture plates; the next day, drugs were added to triplicate wells for 48 h. The drugs were removed and the cells were washed once in an equal volume of medium containing serum and then incubated for 7–10 days, depending on the growth rate. The medium was removed and the cells were fixed in methanol and stained with crystal violet for 10 min. The dye was removed, the plates were washed and dried, and colonies of >50 cells were scored as survivors. The cloning efficiency under these conditions was ≥30%. The relative colony-forming efficiency was expressed as a percentage of the number of colonies formed in the presence of drugs relative to the number of colonies formed in control wells containing no drugs. The IC_{50} values were determined from the cytotoxicity data using a mathematical transformation in which the log of the fraction of dead cells divided by the fraction of surviving cells was plotted against the log of the drug concentration; the resulting equation was then solved to determine the IC_{50}.

The effect of a short-term exposure to anthracyclines in serum-free cell culture on V79/AP4 viability was evaluated using the colony-formation assay as well as the release of the cytosolic enzyme lactate dehydrogenase (LDH). For the colony-formation assay, cells in exponential growth were plated at a density of 1×10^5 cells/cm^2 in FBS-supplemented DMEM in 25-cm^2 flasks. After 24 h, the cells were washed; the medium was replaced with FBS-free DMEM containing 4′-amino-3′-hydroxy-DXR, 4′-deoxy-DXR, and DXR; and the cells were incubated for 2 h. Treatments were terminated by rinsing the plates with PBS; the cells were then trypsinized and 100 of them were plated in 60-mm cell-culture dishes containing 5 ml of FBS-supplemented DMEM and then incubated. After 8 days, colonies of >100 cells were counted as described above and the IC_{50} value was calculated. Cloning efficiency was 70% ± 12% for untreated cells.

For measurement of the LDH activity in the culture medium, 1.8×10^5 cells were plated in 24-well plates containing FBS-supplemented DMEM. After 24 h, the cells were washed with PBS and treated as described for the colony-formation assay. After 2 h, the LDH activitiy released by anthracycline-treated cells was measured in the culture medium as described elsewhere [9] and then compared with the activity released by vehicle-treated cells. The drug concentration that induced a 50% increase in the release of LDH in the supernatant relative to control values was calculated.

Statistical analysis. The results of statistical analysis of the data were recorded as mean values (± SE) for n observations. Longitudinal measurements of ECG, hemodynamics, body weight, and cardiac contractility parameters were evaluated using one-factor analysis of variance (ANOVA, two-tailed) followed by the Student-Newman-Keuls test [19] to analyze the effect of anthracycline treatments. The level of significance chosen was 95% ($P < 0.05$).

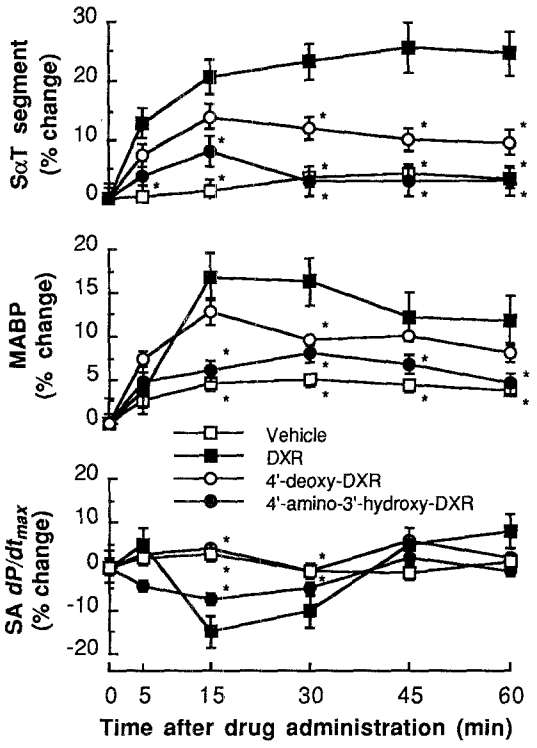

Fig. 1. Hemodynamic and ECG responses to i. v. bolus injections of 4′-amino-3′-hydroxy-DXR (10 mg/kg), 4′-deoxy-DXR (8.6 mg/kg), DXR (13 mg/kg), and vehicle (0.9% NaCl) in anesthetized female Wistar rats. All data are expressed as mean percentages of pretreatment values ± SEM (n = 8). Where error bars are not visible, the SEM was smaller than the symbol size. * P <0.05 vs DXR-treated animals at the same time point

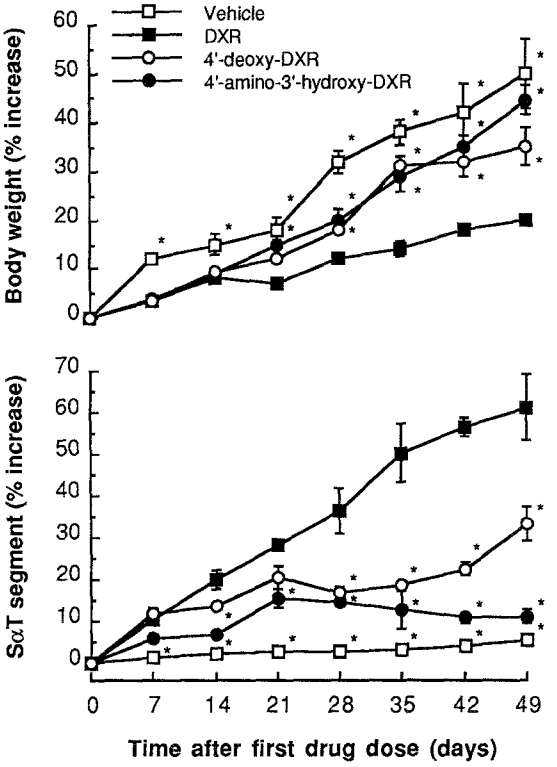

Fig. 2. Effect of i. v. bolus injections (days 0, 7 and 14) of 4′-amino-3′-hydroxy-DXR (3 mg/kg), 4′-deoxy-DXR (2 mg/kg), and DXR (3 mg/kg) on the changes in body weight and the SαT-segment duration in female Wistar rats. All data are expressed as mean percentages of pretreatment values ± SEM (vehicle and 4′-amino-3′-hydroxy-DXR, n = 12; 4′-deoxy-DXR, n = 10; DXR, n = 9). Where error bars are not visible, the SEM was smaller than the symbol size. * P <0.05 vs DXR-treated animals at the same time point

Results

Acute cardiotoxicity study

The increase in SαT-segment duration induced by 4′-amino-3′-hydroxy-DXR and 4′-deoxy-DXR was significantly less pronounced than that produced by DXR (P <0.05 at 15–60 and 30–60 min; Fig. 1). The MABP increase following DXR dosing was more severe than that induced by either 4′-amino-3′-hydroxy-DXR (P <0.05 at 15–60 min) or 4′-deoxy-DXR (P <0.05 at 30 min; Fig. 1). Similarly, the SA dP/dt_{max} value was impaired by treatment with DXR as compared with 4′-amino-3′-hydroxy-DXR and 4′-deoxy-DXR (P <0.05 at 15–30 min; Fig. 1). Although no arrhythmia was recorded following DXR administration, a significant increase in heart rate was induced only by DXR at 15 min after dosing (data not shown).

Chronic cardiotoxicity study

Sporadic episodes of arrhythmia were recorded during treatment with DXR and 4′-deoxy-DXR; however, for ECG measurements, tracings that exhibited arrhythmia were not used. 4′-Amino-3′-hydroxy-DXR and 4′-deoxy-DXR were less toxic than DXR with regard to the body growth rate of treated animals (P <0.05 at 21–49 and 28–49 days; Fig. 2). DXR induced a marked increase in SαT-segment duration (Fig. 2), whereas 4′-amino-3′-hydroxy-DXR and 4′-deoxy-DXR were significantly less toxic (P <0.05 at 14–49 and 28–49 days, respectively; Fig. 2) than DXR. Based on SαT-segment changes, 4′-amino-3′-hydroxy-DXR was the least cardiotoxic drug, whereas 4′-deoxy-DXR induced a progressive, moderate degree of cardiotoxicity. Treatment with DXR was associated with a flattening of the T wave (data not shown); this effect was not observed in rats that had been given 4′-amino-3′-hydroxy-DXR or 4′-deoxy-DXR. No mortality was associated with 4′-amino-3′-hydroxy-DXR treatment, but two animals in the 4′-deoxy-DXR group and three rats in the DXR group died.

Isolated heart study

The basal values for both the wet weight and the contractility of the heart did not differ significantly among the experimental groups (Table 1). Adrenaline stimulation of control hearts produced a dose-dependent increase in F_{max} and $+dF/dt_{max}$ values and a decrease in $-dF/dt_{max}$ (Table 1). The contractile responses of hearts isolated from rats that had been treated with DXR and 4′-deoxy-DXR were significantly impaired as compared with control values (Table 1); in particular, DXR significantly impaired the F_{max} and $-dF/dt_{max}$ values, whereas 4′-deoxy-DXR

Table 1. Effects of incremental doses of adrenaline on isometrically contracting hearts obtained from rats treated with the vehicle 0.9% NaCl, DXR, 4′-deoxy-DXR, or 4′-amino-3′-hydroxy-DXR[a,b]

	Basal values	Adrenaline (μg)		
		0.1	1	10
Vehicle:				
F_{max}(g)	1.8±0.2	3.4± 0.5	3.9± 0.5	4.2± 0.5
$+dF/dt_{max}$ (g/s)	74.5±5.8	142.7± 9.9	163.9±15.1	189 ±15.5
$-dF/dt_{max}$ (g/s)	–35 ±4.3	–102.8± 8.9	–133.9±12.5	–165.6±18.1
DXR:				
F_{max} (g)	1.5±0.4	2.7± 0.4	2.6± 0.3*	2.8± 0.2*
$+dF/dt_{max}$ (g/s)	69.9±7.4	129.8± 9.7	135.3±14.3	148.8±10.2*
$-dF/dt_{max}$ (g/s)	–40.2±6.6	–69.2± 7.4*	–80.5± 8.9*	–105.6± 8.7*
4′-deoxy-DXR:				
F_{max} (g)	1.6±0.3	3 ± 0.5	3.3± 0.5	3.8± 0.3
$+dF/dt_{max}$ (g/s)	71.7±9.5	139.8±10.2	149.5±10	170.1±18.9
$-dF/dt_{max}$ (g/s)	–38.4±4.5	–70.9± 6.7*	–78.7± 8.9*	–110.3± 9.2*
4′-amino-3′-hydroxy-DXR:				
F_{max} (g)	1.9±0.4	3.1± 0.4	3.6± 0.3	4 ±0.4
$+dF/dt_{max}$ (g/s)	75.6±8.8	144.9±10.7	159.3±12	179.8±15
$-dF/dt_{max}$ (g/s)	–39.6±4.9	–89.9±9.9	–120.4±11.4	–155.7±13.9

[a] Heart wet weight did not significantly differ among the experimental groups (vehicle, 0.8±0.05 g; DXR, 0.75±0.04 g; 4′-deoxy-DXR, 0.79±0.05 g; 4′-amino-3′-hydroxy-DXR, 0.79±0.09 g)

[b] Data represent mean values ± SEM (vehicle and 4′-amino-3′-hydroxy-DXR, $n = 12$; 4′-deoxy-DXR, $n = 10$; DXR, $n = 9$)

* $P < 0.05$ vs vehicle-injected animals

Table 2. In vitro cytotoxic activity of DXR, 4′-deoxy-DXR, and 4′-amino-3′-hydroxy-DXR in various cell lines

	IC_{50} (nM)[a]				
	HOS	A2780	DU 145	SNB-19	V79/AP4
DXR	15.8	13.5	9.77	4.37	254.8
4′-deoxy-DXR	0.56	2.51	0.79	1.29	4.01
4′-amino-3′-hydroxy-DXR	7.41	1.38	1.91	2.09	0.34

[a] Data represent the mean values for triplicate experiments, and the SEM never exceeded 10%. For cell-culture conditions, see Materials and methods

treatment resulted in a significant impairment of $-dF/dt_{max}$. No significant change was associated with 4′-amino-3′-hydroxy-DXR treatment (Table 1).

Cytotoxicity study

4′-Amino-3′-hydroxy-DXR, 4′-deoxy-DXR, and DXR produced a concentration-dependent inhibition of the colony-forming ability of the HOS, A2780, DU 145, and SNB-19 cell lines; based on IC_{50} values, 4′-amino-3′-hydroxy-DXR was at least twice as active as DXR (Table 2). The cytotoxic activity of anthracyclines in the V79/AP4 cell line following a 2-h incubation is shown in Table 2. This cell line was highly sensitive to the cytotoxic activity of 4′-amino-3′-hydroxy-DXR. The 50% increase in LDH activity released in the culture medium by cells that had been exposed to anthracyclines as compared with vehicle-treated cells was obtained at a concentration of 4.3 nM 4′-amino-3′-hydroxy-DXR; for 4′-deoxy-DXR and DXR, this concentration was >172 nM and 17.2 μM, respectively.

Discussion

The results of the present study indicate that the new DXR derivative 4′-amino-3′-hydroxy-DXR is characterized by significantly lower acute and chronic cardiotoxicity in the rat and increased cytotoxicity against human cancer and murine transformed cell lines as compared with DXR.

In both acute and chronic studies, 4′-amino-3′-hydroxy-DXR induced the lowest degree of cardiac alterations in vivo as compared with the changes produced by DXR or 4′-deoxy-DXR. The isolated heart experiments did not demonstrate any alteration in the contractile response to adrenaline by hearts from animals that had been treated with 4′-amino-3′-hydroxy-DXR, in contrast to the findings in rats that had received 4′-deoxy-DXR or DXR. The acute and chronic cardiotoxicity studies indicated that 4′-deoxy-DXR induced significant cardiac damage as demonstrated by the ECG analysis. The present results are in agreement with a previously published study on the acute cardiotoxicity of anthracycline derivatives [6], which reported that both DXR and 4′-deoxy-DXR induced cardiac ultrastructural alterations characterized by mitochondrial swelling and disruption of cristae, myocyte vacuolization, and the appearance of dense inclusion bodies and that no significant difference was observed in the severity and frequency of ultrastructural lesions induced by the two drugs at 24 h after a single dose in the rat. In the chronic study, 4′-deoxy-DXR was less cardiotoxic than DXR; however, its cardio-

toxicity was more severe than that caused by 4′-amino-3′-hydroxy-DXR, as the former analogue induced a significant widening of the SαT-segment duration and impaired the adrenaline-induced relaxation ($-dF/dt_{max}$) of isolated heart preparations, whereas the latter did not. The present data suggest that the rat model is useful for the study of anthracycline cardiotoxicity, since other experimental models have failed to reveal any cardiotoxic effect for 4′-deoxy-DXR [11].

The severity of cardiac toxicity induced by a doxorubicin analogue may be dependent on both the pharmacokinetic profile of the drug and its ability to stimulate the metabolism of reactive oxygen species [13]. Futile cycles of reduction and oxidation of the anthracycline quinone occurring in proximity to the Ca^{2+} pump of the sarcotubular system or to complex I of the mitochondrial electron-transport chain can generate a reactive oxygen flux of sufficient magnitude to produce site-specific tissue injury [12]. The anthracycline-induced mitochondrial damage can be evaluated in vitro by measurement of the nucleotide levels (adenosine triphosphate and guanosine triphosphate) and the respiration rate in cardiac tissue from animals that have been treated with anthracyclines. In agreement with the present results, recent in vitro experiments have demonstrated the reduced toxicity of 4′-amino-3′-hydroxy-DXR at the level of mitochondrial biochemical functions as evaluated by the cellular respiration rate and nucleotide levels in rat cardiac tissue; under the same experimental conditions, 4′-deoxy-DXR and DXR induced a marked impairment of the respiration rate and of nucleotide biosynthesis [5].

The results of the present cytotoxicity studies on human cancer and murine transformed cell lines demonstrated that exchanging the positions of the amino and the hydroxyl groups on the sugar moiety of DXR, which leads to 4′-amino-3′-hydroxy-DXR, is associated with a significant increase in the antiproliferative activity of the compound. Our data are in agreement with those of Belvedere et al. [2], who observed a marked increase in the cytotoxicity of 4′-amino-3′-hydroxy-DXR as compared with DXR in the LoVo colon-cancer cell line.

In conclusion, a major goal in the search for new anthracycline analogues is the development of a derivative that exhibits lower cardiotoxicity and equivalent or higher antitumor activity than does DXR. The present results demonstrate that 4′-amino-3′-hydroxy-DXR is a possible candidate for use in humans; therefore, further biochemical and pharmacokinetic studies should be carried out to obtain a better understanding of the promising pharmacological profile shown by 4′-amino-3′-hydroxy-DXR in the present investigation.

Acknowledgements. The generous contribution of the Italian Association for Cancer Research (AIRC, Milano, Italy) is gratefully acknowledged. This study was performed with the excellent technical assistance and collaboration of Mr. B. Stacchini.

References

1. Arcamone F (1985) Properties of antitumor anthracyclines and new developments in their application: Cain Memorial Award lecture. Cancer Res 45: 5995–5999
2. Belvedere G, Suarato A, Geroni C, Giuliani FC, D'Incalci M (1989) Comparison of intracellular drug retention, DNA damage and cytotoxicity of derivatives of doxorubicin and daunorubicin in a human colon adenocarcinoma cell line (LoVo). Biochem Pharmacol 38: 3713–3721
3. Cagnasso M (ed) (1982) IMI-58-4′-deoxydoxorubicin. Basic information. Farmitalia Carlo Erba, Milano
4. Chan SHH, Ong BT (1987) A simple experimental index for the evaluation of inotropic responses. J Pharmacol methods 18: 23–29
5. Cini-Neri G, Neri B, Bandinelli M, Del Tacca M, Danesi R, Riccardi R (1991) Anthracycline cardiotoxicity: in vivo and in vitro effects on biochemical parameters and heart ultrastructure in rats. Oncology 48: 327–333
6. Cummings J, Willmott N, More I, Kerr DJ, Morrison JG, Kaye SB (1987) Comparative cardiotoxicity and antitumor activity of doxorubicin (Adriamycin) and 4′-deoxydoxorubicin and the relationship to in vivo disposition and metabolism in the target tissues. Biochem Pharmacol 36: 1521–1526
7. Danesi R, Del Tacca M, Soldani G (1986) Measurement of the SαT segment as the most reliable electrocardiogram parameter for the assessment of Adriamycin-induced cardiotoxicity in the rat. J Pharmacol Methods 16: 251–260
8. Danesi R, Bernardini N, Marchetti A, Bernardini MC, Del Tacca M (1990) Protective effects of fructose-1,6-diphosphate on acute and chronic doxorubicin cardiotoxicity in rats. Cancer Chemother Pharmacol 25: 326–332
9. Decker T, Lohmann-Matthes ML (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. J Immunol Methods 15: 61–69
10. Del Tacca M, Danesi R, Solaini G, Bernardini M, Bertelli A (1987) Effects of 4′-*O*-tetrahydropyranyl-doxorubicin on isolated perfused rat heart and cardiac mitochondrial cytochrome c oxidase activity. Anticancer Res 7: 803–806
11. Dickinson AC, De Jordy JO, Boutin MG, Teres D (1984) Absence of generation of oxygen-containing free radicals with 4′-deoxydoxorubicin, a non-cardiotoxic anthracycline drug. Biochem Biophys Res Commun 125: 584–591
12. Doroshow JH (1991) Doxorubicin-induced cardiac toxicity. N Engl J Med 324: 843–845
13. Gervasi PG, Agrillo MR, Lippi A, Bernardini N, Danesi R, Del Tacca M (1990) Superoxide anion production by doxorubicin analogs in heart sarcosomes and by mitochondrial NADH dehydrogenase. Res Commun Chem Pathol Pharmacol 67: 101–115
14. Grandi M, Giuliani FC, Verhoef V, Filippi J (1988) Screening of anthracycline analogs. In: Lown JW (ed) Bioactive molecules, vol 6. Anthracycline- and anthracenedione-based anticancer agents. Elsevier, Amsterdam, pp 571–597
15. Kerr DJ, Kerr AM, Freshney RI, Kaye SB (1986) Comparative intracellular uptake of Adriamycin and 4′-deoxydoxorubicin by non-small-cell lung tumor cells in culture and its relationship to cell survival. Biochem Pharmacol 35: 2817–2823
16. Leyland-Jones BR, Townsend AJ, Tu C-PD, Cowan KH, Goldsmith M (1991) Antineoplastic drug sensitivity of human MCF-7 breast cancer cells stably transfected with a human α class glutathione S-transferase gene. Cancer Res 51: 587–594
17. Simi S, Colella CM, Mariani T, Piras A, Rainaldi G (1988) Quantitative analysis of chromosomal evolution in a colcemid-treated Chinese hamster population. Teratogenesis Carcinog Mutagen 8: 45–54
18. Wildt DJ de, Jong Y de, Hillen FC, Steerenberg PA, Hoesel QGCM van (1985) Cardiovascular effects of doxorubicin-induced toxicity in the intact Lou/M Wsl rat and in isolated heart preparations. J Pharmacol Exp Ther 235: 234–240
19. Zar JH (1984) Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey