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Cardiac toxicity and antitumor activity of 4'-deoxy-4'-iodo-doxorubicinol

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Summary. The acute and chronic cardiotoxicity as well as the cytotoxicity of 4'-deoxy-4'-iodo-doxorubicinol (I-DXRol), the major metabolite of the doxorubicin (DXR) derivative 4'-deoxy-4'-iodo-DXR (I-DXR), were compared with those of I-DXR and DXR. In the acute study, anesthetized rats received i.v. DXR (10 mg/kg), I-DXR (4 mg/kg), or I-DXRol (4 mg/kg) and were monitored for ECG (SαT segment and T wave), systolic (SBP) and diastolic (DBP) blood pressure, the first derivative of the systemic arterial pressure (SA dP/dt_{max}), and heart rate. Treatments induced a significant widening of the SαT segment, but I-DXRol was significantly less toxic than I-DXR or DXR. As compared with control values, DXR induced a marked increase in SBP and DBP and a decrease in SA dP/dt_{max}, whereas I-DXR and I-DXRol induced modest changes in hemodynamic parameters. In the chronic study, 3 mg/kg DXR given to rats by i.v. bolus once a week for 3 weeks resulted in severe chronic cardiotoxicity that lasted 6 weeks and was characterized by SαT-segment widening, T-wave flattening, and severe cardiac histological damage. Doses of 1.2 mg/kg I-DXR and 1.2 and 2.4 mg/kg I-DXRol, given i.v. once a week for 3 weeks, and 3.6 mg/kg I-DXRol given as a single dose were associated with a significant T-wave voltage reduction; I-DXR and 2.4 mg/kg I-DXRol induced significant histological alterations of cardiac tissue as compared with control values, whereas modest alterations of heart tissue were observed after injections of 1.2 and 3.6 mg/kg I-DXRol in three doses and in a single dose, respectively. The cytotoxicity of the three anthracyclines against one glioblastoma cell line and two human small-cell lung cancer lines was similar. Results indicate that the acute cardiotoxicity of I-DXRol is lower than that of I-DXR and DXR, whereas the chronic heart damage is similar to that induced by I-DXR and significantly lower compared than that caused by DXR. Moreover, the cytotoxicity of the metabolite appears to be similar to that of I-DXR and DXR. The lack of additional cardiac toxicity due to I-DXRol further supports the lower overall cardiac toxicity of I-DXR, which retains a cytotoxic activity similar to that of the parent drug.

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Introduction

The pathogenesis of the acute and chronic cardiotoxicity of doxorubicin (DXR) has been the object of an exhaustive search (for review see [15]). Significant attention has been devoted to the relationship between metabolism and cardiotoxicity since doxorubicinol (DXRol), the C_{13}-dihydro metabolite of DXR, accumulates in cardiac tissue during repeated treatment with DXR [10] and is cardiotoxic to rats [7]. Moreover, DXRol has been shown to be a potent inhibitor of several cationic pumps involved in the regulation of cell calcium in the muscle cell [3]; the metabolite also inhibits the isometric contraction and increases the resting tension of isolated papillary muscle from rabbit heart [3]. The data obtained would suggest that DXRol importantly contributes to DXR cardiotoxocity, as previously postulated [10], and suggest the need for characterization of the cardiac toxicity of metabolites in evaluation of the pharmacological profile of a new anthracycline.

New DXR derivatives have been synthesized to reduce the cardiac toxicity and improve the antitumor activity of DXR [1]. Among the glycoside analogues, 4'-deoxy-4'-iodo-DXR (I-DXR) differs from DXR in the presence of an iodine atom at position 4' on the daunosamine sugar group; it is more lipophilic than DXR, is cytotoxic against tumor cell lines sensitive and resistant to the parent drug [26], and has been reported not to induce cardiotoxicity in mice [2]. Pharmacokinetic studies in mice have demonstrated that I-DXR is largely converted to its major metabolite, I-DXRol [11]. Since no evidence is present in the literature about the pharmacological properties of I-DXRol, the present study investigated the acute and chronic cardiac toxicity and the in vitro cytotoxicity of the metabolite in the rat and compared them with those of

DXR and I-DXR. On the treatment schedule used in the present study, the results demonstrated a lower acute cardiotoxicity for the metabolite as compared with the parent drug, although the chronic cardiotoxicity was similar; however, both compounds were significantly less cardiotoxic than DXR. In addition, the in vitro cytotoxicity of the drugs was similar.

Materials and methods

Experimental animals. Adult female Wistar rats with a mean body weight of 190 ± 8.5 g were used. They were fed standard rat diet and tap water ad libitum and were not used for at least 1 week after their delivery to the laboratory. The animals were housed in temperature-controlled rooms on a 12-h lighting cycle at 22°–24° C and approximately 50%–60% relative humidity. The distribution of animals in groups and the treatment allotted to each group were randomized.

Drugs and chemicals. DXR, I-DXR and I-DXRol hydrochloride salts were obtained from Farmitalia-Carlo Erba (Milano, Italy). Aqueous solutions of drugs were freshly prepared immediately before use and protected from light. Urethane and hematoxylin and eosin were from Sigma Chemical Co. (St. Louis, Mo., USA). All other chemicals were of analytical grade.

Acute cardiotoxicity study. Animals were divided in four groups of eight rats each and were treated as reported in Table 1. For comparison of the cardiotoxicity of the anthracyclines, the doses of I-DXR and I-DXRol vs DXR were chosen on the basis of equivalent toxicity and corresponded to the LD_{50} for each drug in the female rat. Rats were anesthetized i. p. with 1 g/kg urethane and were allowed to breathe spontaneously through a tracheal cannula; at this dose, urethane anesthesia has been shown to be suitable for cardiovascular investigations [18, 28]. Heart rate (beats/min), systolic (SBP) and diastolic (DBP) blood pressure (mm Hg), and the first derivative of the systemic arterial pressure (SA dP/dt_{max}; mm Hg/s), taken as an indirect index of left ventricular contractility [4], were recorded as previously reported [8]. A lead-II ECG was recorded and the SαT-segment duration (ms) and T-wave amplitude (μv) were measured as cardiotoxicity endpoints [6]. After a stabilization period of 20 min, the ECG and the hemodynamic parameters were registered before and 60 min after treatment.

Chronic cardiotoxicity study. In preliminary experiments animals were treated with equimolar doses of the drugs (3 mg/kg DXR, 3.6 mg/kg I-DXR, and 3.6 mg/kg I-DXRol; once a week for 3 weeks). This protocol schedule was discontinued due to the high mortality of animals treated with I-DXR and I-DXRol; their ECGs, however, showed only modest abnormalities, and deaths were most likely due to extracardiac causes (data not shown). As in the acute study, for comparison of the cardiotoxicity of the various anthracyclines, the doses of DXR, I-DXR, and I-DXRol were chosen on the basis of the LD_{50} for each drug in the female rat. Animals were divided into six groups of six rats each and were treated as reported in Table 1; for each drug, the cumulative dose given corresponded to 90% of the LD_{50}. In the case of I-DXRol, this dose was delivered on two schedules: three weekly injections of 1.2 mg/kg or a single dose of 3.6 mg/kg. The cardiotoxicity of an additional treatment was also studied using three weekly doses of 2.4 mg/kg; in this case the total dose given was 180% of the LD_{50} for the metabolite. Body weight and an ECG (lead II) were recorded weekly, and the heart rate, SαT-segment duration, and T-wave amplitude were measured.

Table 1. Treatment schedule

Acute study	(mg/kg)[a]	Chronic study	(mg/kg)[b]
Control	–	Control	–
DXR	10	DXR	3
I-DXR	4	I-DXR	1.2
I-DXRol	4	I-DXRol	1.2
			2.4
			3.6 (sd)

[a] Drugs given by i. v. infusion lasting 3 min; the compound was dissolved in 2.5 ml/kg saline (0.9% NaCl). Controls received 2.5 ml/kg 0.9% NaCl

[b] Drugs given by i. v. bolus; the compound was dissolved in 2 ml/kg 0.9% NaCl and injected once a week for 3 weeks; sd, single dose. Controls received 2 ml/kg 0.9% NaCl

At the end of the experiment (6th week), the animals were killed by cervical dislocation. The hearts were promptly removed and washed in cold isotonic saline (0.9% NaCl) and were then retrogradely perfused in a Langendorff apparatus with phosphate-buffered saline (composition in grams per liter: KCl, 0.20; KH_2PO_4; 0.20; NaCl, 8; $Na_2HPO_4 \cdot 7H_2O$, 2.16) at pH 7.4 for 1 min and fixed by perfusion with 4% paraformaldehyde in the same buffer for 5 min to provide a homogeneous fixation throughout the thickness of the cardiac wall. Each heart was cut in ten transverse sections (1 mm thick), including both ventricles, starting 3 mm below the atrioventricular valves; these slices were left in the same fixative for 1 h. Blocks including both epicardial and endocardial surfaces were embedded in glycol-methacrylate resin; 1-μm-thick sections were obtained using an LKB Historange microtome (LKB, Bromma, Sweden) with glass knives and were stained with hematoxylin and eosin. The severity of DXR-induced cardiac lesions was assessed by light microscopic examination [9], and changes observed in 20 fields/slide were graded on a scale of 0–3 on the basis of the number of cardiocytes showing myofibrillar loss and cytoplasmic vacuolation: 0, normal myocardium; 1, single scattered cells with early changes (minute vacuoles), surrounded by normal myocardium; 2, small groups of cells with more advanced alterations, surrounded by normal tissue; 3, severe morphologic changes involving ≥ 50% of the myocytes.

Cell lines and growth inhibition studies. NCI-H69 [12] and NCI-H691, two human small-cell lung cancer cell lines, were obtained from American Type Culture Collection (Rockville, Md. USA) and were kindly provided by Dr. A. Gazdar (NCI-Navy Medical Oncology Branch, Bethesda, Md. USA). U-706T [16, 27], a human glioma cell line, was kindly supplied by Dr. B. Westermark (Uppsala, Sweden). These cell lines were routinely maintained as suspension cultures in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 m*M* L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml) (all from Biofluids, Inc., Rockville, Md. USA) and were incubated at 37° C in a humidified atmosphere containing 5% CO_2. Growth-curve studies were conducted by seeding $3.5–4.5 \times 10^4$ cells/ml in exponential growth phase in 6-well tissue-culture dishes (Costar 3506, Cambridge, Mass., USA). Drugs were dissolved in cell-culture-grade water and cells were exposed to DXR, I-DXR, and I-DXRol at respective concentrations of 0.1, 1, and 10 μ*M* for 144 h. Cell number was determined at the end of drug exposure by electronic particle counting (Model ZB_1 Coulter Counter; Coulter Electronics, Hialeah, Fla., USA), and results were expressed as the percentage of control growth [23].

Statistics. Statistical analysis was performed using NWA STATPAK software (Northwest Analytical, Inc., Portland, Ore., USA) and a Honeywell XP Computer. Data are presented as the mean (± SEM) of *n* observations. The data were analyzed by two-way analysis of variance (ANOVA) (ECG, hemodynamic parameters, and body weight) or one-way ANOVA (histopathology score), followed by the Tukey test with equal or unequal sample sizes. A P value of <0.05 was considered to be significant.

Results

Acute cardiotoxicity study

A significant increase in the duration of the SαT segment was observed after treatment with DXR and I-DXR vs

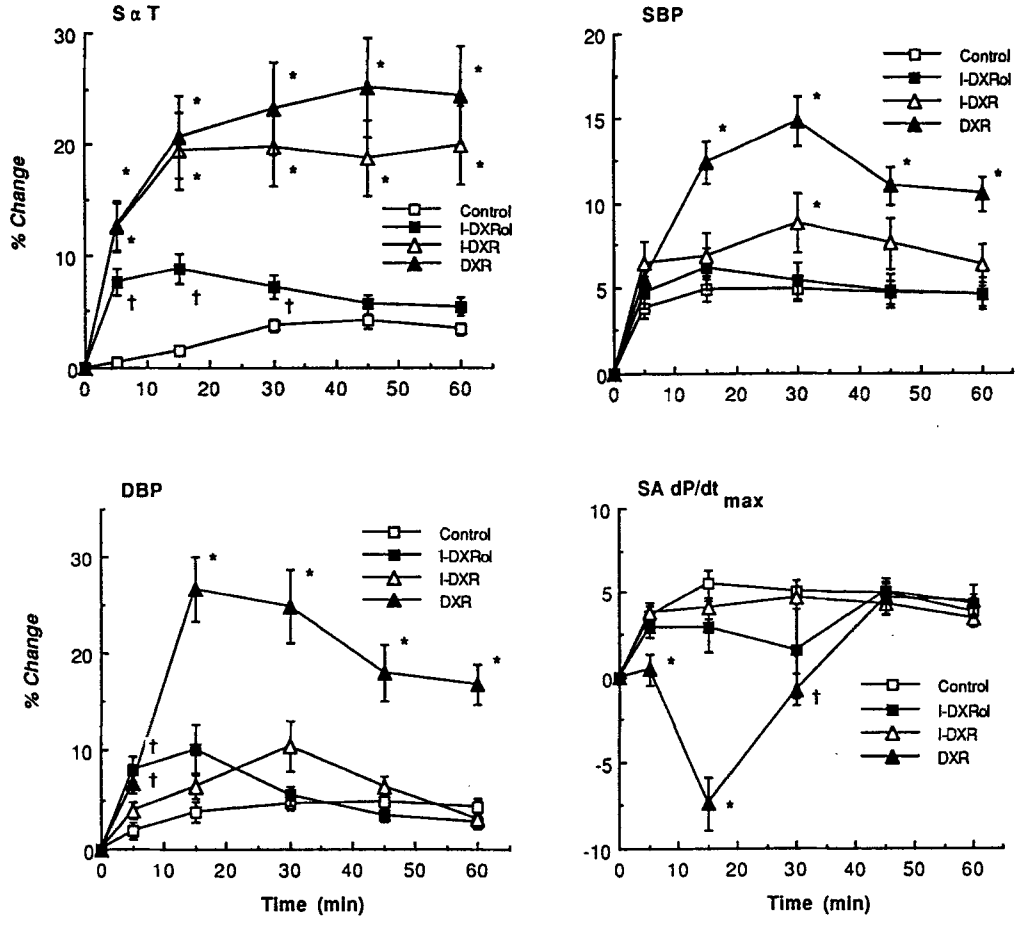

Fig. 1. Effect of the anthracyclines on the SαT segment duration, SBP, DBP, and SA dP/dt_{max} of anesthetized rats treated with 10 mg/kg DXR, 4 mg/kg I-DXR, or 4 mg/kg I-DXRol as indicated in Table 1. The changes in SαT segment, SBP, DBP, and SA dP/dt_{max} are expressed as the percentage of difference from baseline values (time 0). *Points*, mean; *bars*, SEM. * P <0.05 comparing DXR and I-DXR vs I-DXRol; † P <0.05 comparing DXR and I-DXRol vs controls

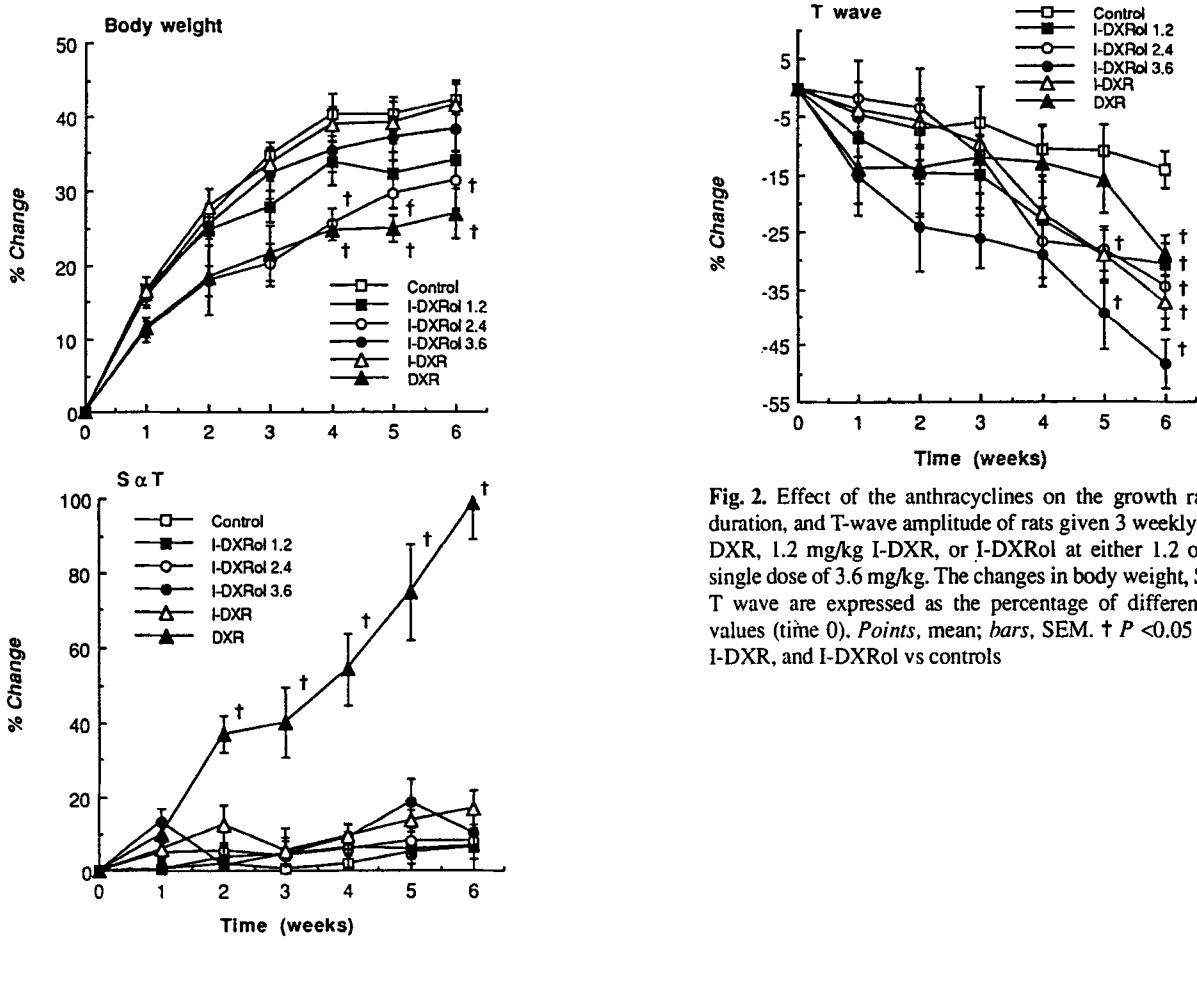

Fig. 2. Effect of the anthracyclines on the growth rate, SαT-segment duration, and T-wave amplitude of rats given 3 weekly doses of 3 mg/kg DXR, 1.2 mg/kg I-DXR, or I-DXRol at either 1.2 or 2.4 mg/kg or a single dose of 3.6 mg/kg. The changes in body weight, SαT segment, and T wave are expressed as the percentage of difference from baseline values (time 0). *Points*, mean; *bars*, SEM. † P <0.05 comparing DXR, I-DXR, and I-DXRol vs controls

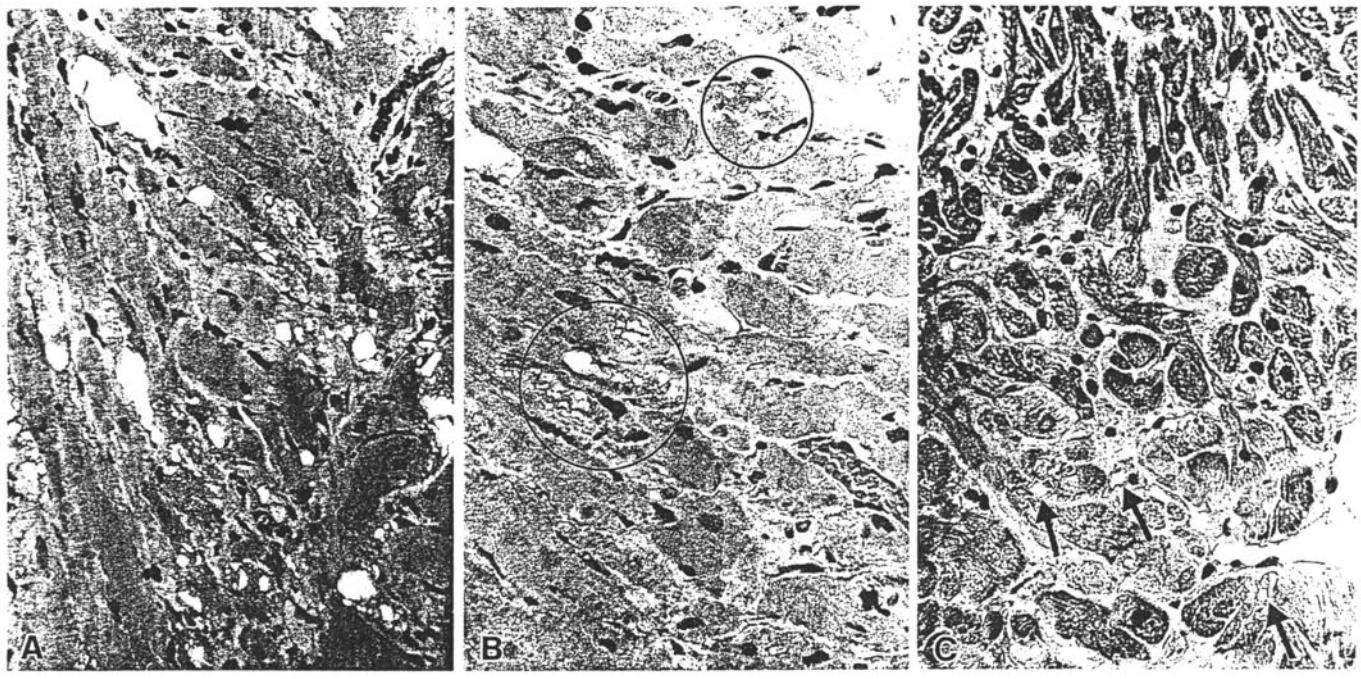

Fig. 3 A–C. Histological pictures of left ventricular sections performed at the end of the study. **A** Diffuse and severe damage to the myocardium in a DXR-treated rat; a large number of cell show extreme vacuolar degeneration. H&E, ×250. **B** Less evident degenerative changes in a rat treated with I-DXR; in *circles* are shown groups of cells with numerous microvacuolations. H&B, ×400. **C** Isolated areas of minimal alteration in a rat treated with 2.4 mg/kg I-DXRol; *arrows* indicate a few sarcoplasmic vacuolations. H&E, ×250

I-DXRol, whereas I-DXRol induced a significant but transient widening of this parameter (Fig. 1). DXR significantly increased SBP and DBP and decreased SA dP/dt_{max}, whereas I-DXR and I-DXRol increased DBP and SBP at one time point each (Fig. 1). A transient but nonsignificant reduction in SA dP/dt_{max} was induced by I-DXRol at 15 and 30 min after the infusion (Fig. 1). Heart rate was not significantly affected by the anthracyclines; however, a transient tachycardia was observed in animals receiving DXR (data not shown).

Body growth and survival

The increase in body weight of the groups studied is reported in Fig. 2. Both DXR and 2.4 mg/kg I-DXRol significantly impaired body growth; this effect was not significant in the groups receiving 1.2 and 3.6 mg/kg I-DXRol. Two deaths occurred among rats given DXR and those given a single dose of 3.6 mg/kg I-DXRol; one death was observed among the groups treated with I-DXR and 2.4 mg/kg I-DXRol.

Chronic cardiotoxicity study

DXR induced a marked increase in the duration of the SαT segment (Fig. 2); no significant differences were observed in SαT values among I-DXR- and I-DXRol-treated groups and controls. A significant T-wave flattening was induced by the anthracyclines (Fig. 2); this was more evident in the groups treated with I-DXR and 3.6 mg/kg I-DXRol. The majority of DXR-treated hearts showed a marked cardiac atrophy and dilation, particularly in the left ventricle. Animals treated with I-DXR or I-DXRol exhibited only faint atrophy and/or modest cardiac dilation. The cardiac histology in the control rats was unremarkable, whereas the hearts of DXR-treated animals demonstrated the most severe histological damage, with sarcoplasmic macrovacuolations and myolysis (Table 2, Fig. 3A). The cardiac lesions in animals treated with I-DXR and 2.4 mg/kg I-DXRol were significantly more severe than those in controls (Table 2, Fig. 3B, C), whereas doses of 1.2 and 3.6 mg/kg I-DXRol induced the lowest degree of cardiotoxicity (Table 2).

Table 2. Degree of myocardial lesions in animals treated once a week for 3 weeks with DXR, I-DXR, and I-DXRol[a]

Treatment (mg/kg)		Score (mean ±SEM)	*n*	*P*
Controls	–	0	6	
DXR	3	2.70±0.25	4	<0.05 vs all groups
I-DXR	1.2	1.40±0.24	5	<0.05 vs controls and DXR group
I-DXRol	1.2	0.67±0.21	6	<0.05 vs DXR group
	2.4	0.83±0.30	5	<0.05 vs DXR group and controls
	3.6	0.75±0.25	4	<0.05 vs DXR group

[a] See Materials and methods for the scoring system used
n Number of animals examined from which a mean ±SEM score was calculated

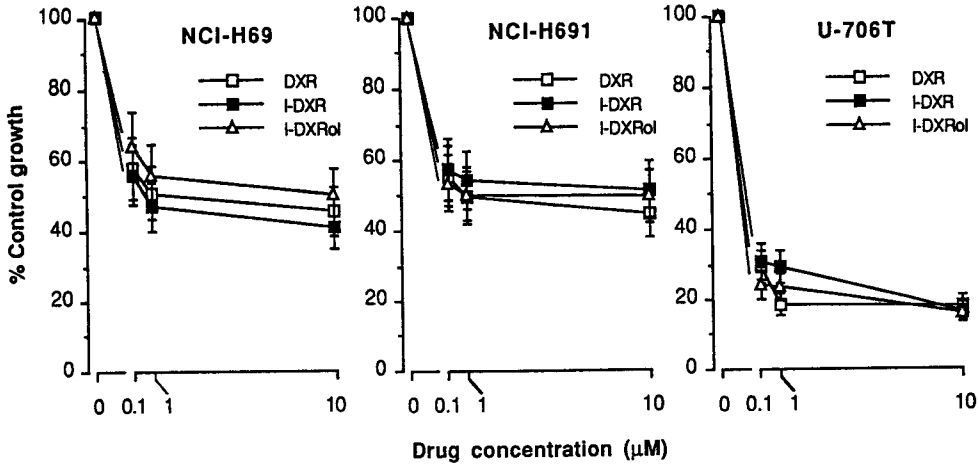

Fig. 4. Antiproliferative effect of different concentrations of DXR, I-DXR, and I-DXROl on NCI-H69, NCI-H691, and U-706T cells treated for 144 h. Data are expressed as the percentage of control growth [22]. *Points*, mean of 3 experiments; *bars*, SEM

In vitro cytotoxicity

DXR, I-DXR and I-DXROl inhibited in a dose-dependent manner the growth of the three cell lines tested, with U-706T being the most sensitive (Fig. 4). No significant difference in the cytotoxicity of the three compounds was observed (Fig. 4).

Discussion

The reduction of the C_{13}-carbonyl group of DXR is a metabolic step that seems to play an important role in the cardiotoxicity induced by the parent drug [3, 5, 7, 21]. The results of the present study demonstrate that in the rat, I-DXROl is characterized by a lower degree of both acute and chronic cardiotoxicity as compared with DXR. In the acute study, I-DXROl induced significantly less severe changes in SαT segment, SBP, DBP, and SA dP/dt_{max} than those caused by DXR; I-DXR treatment was associated with significant changes in SαT segment and SBP as compared with I-DXROl, whereas no differences were observed between changes in DBP and SA dP/dt_{max} induced by I-DXR and I-DXROl.

In the chronic study, only DXR significantly enlarged the SαT segment; however, the cardiotoxic effect induced by DXR, I-DXR, and I-DXROl was demonstrated by the development of a T-wave flattening. The reduction in T-wave amplitude, an expression of altered repolarization of cardiac muscle [6], was more evident in animals treated with I-DXR and I-DXROl at the three doses given. Repeated treatment with I-DXR or I-DXROl did not significantly alter the SαT segment, which is the most reliable parameter for monitoring DXR cardiotoxicity [6]. The alterations of cardiac histology were also significantly less severe than those induced by DXR. The low cardiotoxic potential of I-DXR has also been documented in previous studies [2] and may depend on the low cardiotoxicity of its principal metabolite I-DXROl, as demonstrated in the present study, on its lower reactivity at the subcellular level [14], and on its pharmacokinetics, which is characterized by a higher elimination rate as compared with DXR [11]. Furthermore, I-DXR tissue levels are higher than those of DXR in the tumor and small intestine of tumor-bearing mice, and the $AUCs_{0\rightarrow24}$ of I-DXR in these tissues are 2.3 and 2.6 times that of DXR respectively.

The ECG results were in agreement with the cardiac histological picture in the different groups; severe cardiotoxicity was observed in the hearts of animals treated with DXR, whereas less severe cardiac effects were observed in rats treated with I-DXR and 2.4 mg/kg I-DXROl. The animals treated with 1.2 and 3.6 mg/kg I-DXROl showed the lowest and most localized histological lesions. These results further support previous findings of a relationship between the cardiac histological damage induced by anthracyclines and the ECG changes [6, 10] and provide evidence that the low cardiotoxicity of I-DXROl could be an additional reason for the low cardiotoxicity of I-DXR.

Much of the interest in the relationship between DXR metabolism and cardiotoxicity, with implications for the development of newer anthracyclines, has derived from pharmacokinetic data showing that a single dose of DXR in the rat resulted in high cardiac concentrations of DXR and DXROl [23]; moreover, repeated DXR administration was accompanied by a marked rise in cardiac DXROl concentrations, although the plasma and pulmonary levels of the latter did not change significantly [10]. The accumulation of DXROl in the heart may depend on the intracardiac metabolism of DXR to DXROl by aldoketoreductases, which have been detected in this organ [17, 24]. Moreover, in agreement with these findings, we have demonstrated that the isolated rat heart metabolizes DXR to DXROl and aglycones [25].

Toxicological data have shown that like the parent drug, DXROl is cardiotoxic, since rats exposed to repeated doses of exogenous DXROl developed ECG alterations and histological cardiac damage similar to those induced by DXR [7]. In addition, biochemical studies have shown that DXROl stimulates superoxide anion and lipid peroxidation in cardiac sarcosomes and mitochondria [13], a step that is widely considered to be important in cardiac toxicity [13, 20]. Other authors have demonstrated some peculiar characteristics of the metabolite; it was shown that the acute cardiotoxic effect of DXROl in vitro is considerably greater than that of the parent drug [3]. In other experiments, the activity of DXROl was found to be several times that of DXR in inhibiting cardiac contractility in rabbit papillary muscle [19, 21]. The mechanism of cardiac dys-

function may be related to ATPase inhibition, as DXRol is a potent inhibitor of Ca^{2+}-Mg^{2+}-ATPase in the sarcoplasmic reticulum, of Mg^{2+}-ATPase in mitochondria, and of Na^{+}-K^{+}-ATPase activity in the sarcolemma [3].

In conclusion, our results suggest that the conversion of I-DXR to I-DXRol is not a metabolic step that leads to a cardiotoxic metabolite, which provides further evidence for the low cardiotoxicity of I-DXR. On the basis of our data, further studies in other models are needed to improve our understanding of the toxicological profile of I-DXRol and to investigate its possible use as an anticancer drug.

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