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Doxorubicin cardiotoxicity may be caused by its metabolite, doxorubicinol

(adriamycin/anthracycline/cardiomyopathy/ion pumps)

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ABSTRACT **Doxorubicin (former generic name, adriamycin), a highly effective anticancer drug, produces cardiotoxicity, which limits its therapeutic potential. The mechanism of this cardiotoxicity has remained elusive. Our data suggest that this toxicity could involve doxorubicinol, the primary circulating metabolite of doxorubicin. Doxorubicinol was markedly more potent than doxorubicin at compromising both systolic and diastolic cardiac function. Similarly, doxorubicinol was much more potent than doxorubicin at inhibiting the calcium pump of sarcoplasmic reticulum [ATP phosphohydrolase (Ca^{2+}-transporting), EC 3.6.1.38], the Na^+/K^+ pump of sarcolemma [ATP phosphohydrolase (Na^+/K^+-transporting), EC 3.6.1.37], and the F_0F_1 proton pump of mitochondria [ATP phosphohydrolase (H^+-transporting, EC 3.6.1.34]. Our finding that this highly toxic metabolite was produced by cardiac tissue exposed to doxorubicin suggests that doxorubicinol could accumulate in the heart and contribute significantly to the chronic cumulative cardiotoxicity of doxorubicin therapy. Our observation that doxorubicin was more potent than doxorubicinol in inhibiting tumor cell growth *in vitro* suggests that the cardiotoxicity of doxorubicin is dissociable from its anticancer activity.**

Doxorubicin (former generic name, adriamycin), a highly effective anticancer drug, produces cardiotoxicity, which limits its therapeutic potential (1). Significant and persistent cardiac dysfunction develops in >60% of patients receiving maximal acceptable cumulative dosages of doxorubicin (430–600 mg/m^2) (1). The mechanism of this cardiotoxicity has remained elusive. According to a popular hypothesis, doxorubicin impairs cardiac function by generating oxygen free radicals that bind to and disrupt membrane proteins and phospholipids (2). However, from a practical viewpoint, this hypothesis has been disappointing because free radical scavengers and antioxidants have failed to prevent the cumulative doxorubicin cardiotoxicity (3, 4). The present study suggests another hypothesis based on a comparison of the effects (toxicities) of doxorubicin and its major metabolite, doxorubicinol (5–7). Our study demonstrates that doxorubicinol is more toxic to the heart than doxorubicin and points to doxorubicinol as a potential culprit in doxorubicin-induced cardiotoxicity.

MATERIALS AND METHODS

Preparation of Doxorubicinol. Doxorubicinol was synthesized from doxorubicin (Sigma; Adria Laboratories, Columbus, OH) according to the technique of Takahashi and Bachur (5). Identity and purity of doxorubicinol were confirmed by mass spectroscopy and HPLC as described (8).

Isolated Papillary Muscle Preparation. Right ventricular papillary muscles from New Zealand White rabbits (2.5–3.5 kg) were placed in a muscle bath containing Krebs-bicarbonate buffer (pH 7.4; 30°C) continuously bubbled with 95% O_2/5% CO_2 as described (9). Each muscle was affixed to a force transducer and electrically stimulated to contract isometrically 30 times per minute at its optimum length (L_{max}) to develop maximal tension. The maximal rate of force development per mm^2 of muscle tissue (dS/dt) and the force exerted by the muscle at rest per mm^2 of muscle tissue (resting stress) were determined from high-speed oscillographic tracings and used as indices of systolic and diastolic myocardial function, respectively. Doxorubicin or doxorubicinol was added to the muscle bath in cumulative fashion at 45-min intervals.

Isolated Organelle and Ion-Pump Preparation. Cardiac muscle sarcoplasmic reticulum (SR) was prepared as described by Chamberlain *et al.* (10); enriched sarcolemma (SL) was prepared according to Frank *et al.* (11); bovine heart submitochondrial vesicles were prepared as described by Fleischer *et al.* (12); skeletal muscle light sarcoplasmic reticulum was prepared by a modification of Meissner *et al.* (13). Calcium-dependent ATPase [ATP phosphohydrolase (Ca^{2+}-transporting), EC 3.6.1.38] activity referable to the calcium pump of SR-enriched membrane fractions prepared from canine cardiac and rabbit skeletal muscle was determined at 37°C in imidazole buffer (20 mM; pH 7.4 at 25°C) (10). Ouabain-inhibited Na^+/K^+ ATPase [ATP phosphohydrolase (Na^+/K^+-transporting), EC 3.6.1.37] activity in sarcolemma-enriched membrane fractions from rabbit cardiac muscle was determined at 37°C in imidazole buffer (30 mM; pH 7.5 at 25°C) (14). Mg^{2+}-dependent ATPase activity referable to F_0F_1 reversible proton pump [ATP phosphohydrolase (H^+-transporting), EC 3.6.1.34] in submitochondrial vesicles prepared from bovine heart mitochondria was determined at 25°C in Tris·HCl buffer (65 mM, pH 7.5, at 25°C) (15). Calcium loading of membrane vesicles enriched in sarcoplasmic reticulum was determined at 25°C in a medium containing 100 mM potassium phosphate (pH 7.0), 7 mM NaCl, 50 μM $CaCl_2$, 1 mM $MgCl_2$, 0.2 mM antipyrylazo III, and ≈50 μg of protein per ml (10). Inorganic phosphate determinations were made according to Baginski *et al.* (16) and protein determinations were assessed by the method of Lowry *et al.* (17).

Tissue Incubation with Doxorubicin or Doxorubicinol. Small (≈100-mg) strips of atrial or ventricular cardiac tissues were

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Abbreviations: dS/dt, maximal rate of systolic force development per mm^2 of muscle tissue per unit time; SR, sarcoplasmic reticulum preparation; SL, enriched sarcolemma preparation.

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incubated with either doxorubicin or doxorubicinol at 30°C in Krebs-bicarbonate buffer bubbled with 95% O_2/5% CO_2. Cardiac tissues were exposed to doxorubicin (100 μg/ml) for either 45 or 180 min before removal for analysis of doxorubicin and doxorubicinol tissue concentrations. In other experiments, tissues were incubated with doxorubicinol for 45 min and then assayed for doxorubicinol.

Tissue Assay for Doxorubicin and Doxorubicinol. After incubation with doxorubicin or doxorubicinol, cardiac tissues (≈100 mg) were removed from the muscle baths, rinsed with normal saline, blotted dry, and weighed. Daunomycin (1 μg/ml) was added as an internal standard immediately before homogenization. Tissues were homogenized with a Polytron (Brinkman) for 30 sec in 4 ml of ice-cold normal saline. Homogenates were saturated with NH_4SO_4 before adding 4 ml of a 1:1 (vol/vol) mixture of chloroform/isopropanol (8). Next, the preparations were mixed using a Vortex mixer and centrifuged in a Beckman (model J-6B) at 1000 × *g* for 10 min. The organic phase from each preparation was removed, and the organic solvents were evaporated under N_2 at room temperature. The residue was resuspended in 250 μl of methanol and then was analyzed with a Waters HPLC system (equipped with M45 and 6000A pumps, a model 680 automated gradient controller, a WISP 710B automatic injector, and a Kratos Spectroflow 980 fluorescence detector; Applied Biosystem, Ramsey, NY). A Waters 4 μM phenyl Radial-Pak reversed-phase column was used to separate doxorubicin and doxorubicinol. The mobile phase was formic acid buffer (16 mM; pH 4.0) and acetonitrile (72:28 vol/vol), which was changed at 6.5 min to 66:35 and returned to 72:28 at 11.5 min. Doxorubicin and doxorubicinol were quantified using fluorescence detection (470 nm excitation and 550 nm emission wavelengths). Concentrations of doxorubicin and doxorubicinol (expressed as doxorubicin equivalents) were determined from doxorubicin standard curves.

***In Vitro* Cytotoxicity Studies.** The cytotoxicities of doxorubicin and doxorubicinol were evaluated in three pancreatic adenocarcinoma cell lines. Cell lines were from human (PANC-1) and hamster origin (PD PaCa and WD PaCa). PANC-1 cells (18) were obtained from the American Type Culture Collection, and the hamster cells were obtained through Scarpelli and Rao (19). Cells were adapted to tissue culture in our laboratory and maintained in culture using complete medium (RPMI 1640), supplemented with 10%

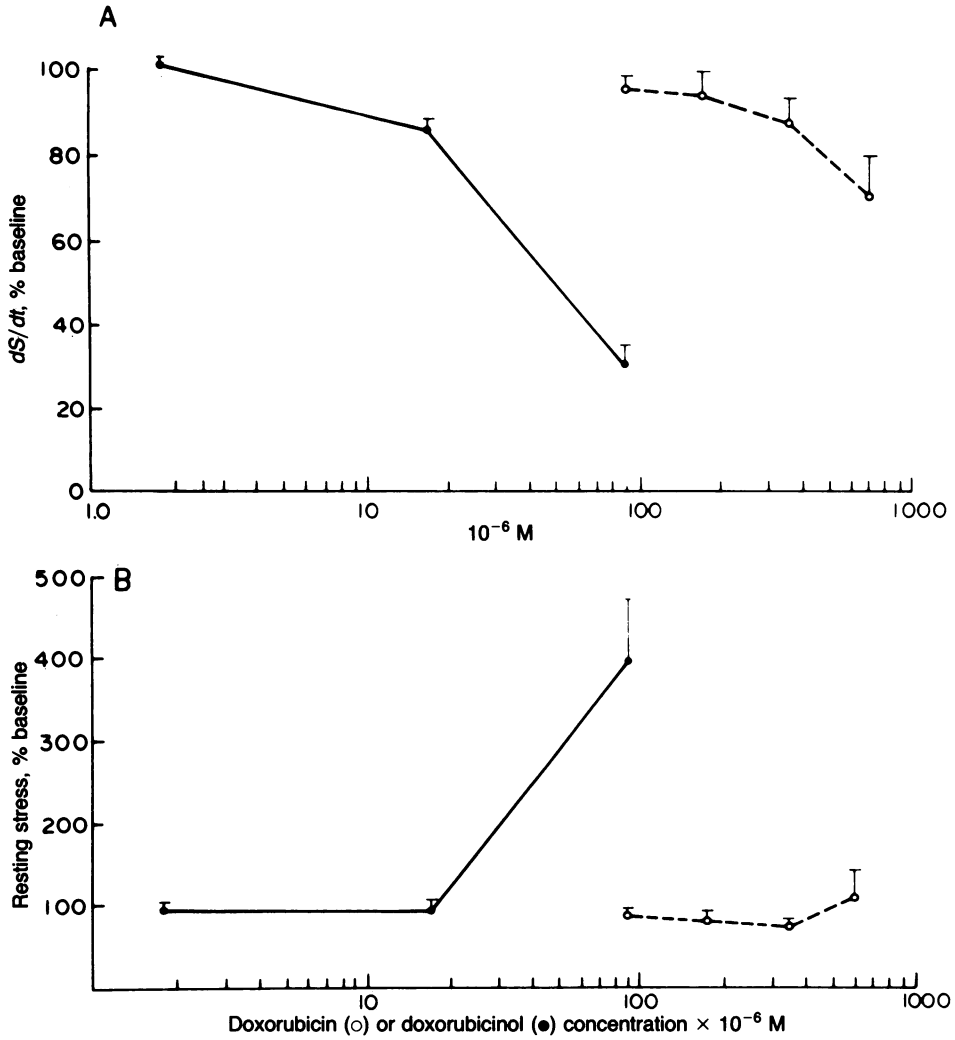

FIG. 1. Logarithmic concentration–response curve for doxorubicin and the primary metabolite doxorubicinol on contractility (*dS/dt*; *A*) and muscle stiffness (resting stress; *B*) expressed as the percentage of baseline predrug values in isolated heart muscle. Data are mean ± SEM for five observations. Doxorubicin responses (both *dS/dt* and resting stress) were significantly different ($P < 0.01$) from doxorubicinol responses at 90 μM as assessed by unpaired *t* test. Baseline predrug control values of *dS/dt* and resting stress did not differ significantly between the two groups. Baseline values (mean ± SEM) for the doxorubicin group were 7.62 ± 2.40 g per sec/mm² (*dS/dt*) and 0.232 ± 0.034 g/mm² (resting stress). Baseline values for the doxorubicinol group were 10.58 ± 2.03 g per sec/mm² (*dS/dt*) and 0.202 ± 0.013 g/mm² (resting stress).

heat-inactivated fetal bovine serum, glutamine, penicillin, and streptomycin (GIBCO) as described (20). Cells were harvested in late-logarithmic or early stationary-phase growth. Then they were washed and suspended in complete medium at a concentration of 1×10^6 cells per ml of medium (20). Cell suspensions were incubated for 1 hr at various concentrations of doxorubicin (0.1 μM–100 μM) or doxorubicinol (1 μM–100 μM). After the 1-hr incubation period, cells were washed twice with Hanks' balanced salt solution to remove doxorubicin or doxorubicinol. Cells were then resuspended for 7–14 days in complete medium containing 0.9% methylcellulose and 15% fetal bovine serum at 37°C in a humidified 95% air/5% CO_2 atmosphere. At the end of the 7- to 14-day growth period, the number of tumor colonies were counted. The number of colonies formed (a colony was defined as >50 cells) at each concentration of doxorubicin or doxorubicinol was compared with the number of colonies formed without doxorubicin or doxorubicinol (control groups). These comparisons were used to determine the concentration of doxorubicin or doxorubicinol required to inhibit colony formation by 50% (IC_{50}).

RESULTS

Doxorubicinol was nearly 30 times more potent than doxorubicin at depressing contractility (dS/dt), a measure of systolic function of isolated rabbit papillary muscles (Fig. 1*A*). Doxorubicinol, at 90 μM (50 μg/ml), decreased contractility by 69 ± 5% (mean ± SEM) of the basal predoxorubicinol value. In contrast, this same concentration of doxorubicin did not affect contractility. Doxorubicin, at 350 μM (200 μg/ml), depressed contractility by 11 ± 5% of basal values; the highest concentration of doxorubicin studied (700 μM or 400 μg/ml) decreased basal contractility by only 29 ± 10%. Thus, doxorubicinol is more potent than the parent compound at depressing systolic myocardial function in isolated heart muscle.

Impairment of diastolic myocardial function is another hallmark of the cardiotoxicity seen with doxorubicin therapy (3). In our isolated papillary muscle model, however, doxorubicin had little effect on myocardial resting stress (a measure of muscle stiffness and an index of diastolic myocardial function) even at a concentration of 700 μM. On the other hand, doxorubicinol, at a concentration lower by a factor of eight, increased resting stress to 395 ± 76% of basal values (Fig. 1*B*: $P < 0.0001$). Thus, doxorubicinol, rather than doxorubicin, depressed the diastolic function (i.e., increased resting stress) of isolated papillary muscles.

Because diastolic function is determined in part by sarcoplasmic calcium concentrations during rest (diastole) (21), the mechanism of the impaired diastolic function could involve perturbations of membranes that regulate sarcoplasmic calcium concentrations. Therefore, studies were conducted to evaluate effects of doxorubicin or doxorubicinol on calcium pump activity, defined as both rate of calcium loading and Ca^{2+} stimulated ATPase activity of sarcoplasmic reticulum vesicles. Doxorubicin, which failed to appreciably alter diastolic function, was not a potent inhibitor of calcium pump activity. In contrast, doxorubicinol, at a concentration that profoundly decreased diastolic function, almost totally abolished calcium pump activity (Table 1).

Because doxorubicinol was such a potent inhibitor of the calcium pump of cardiac sarcoplasmic reticulum, we evaluated its effects on ATPases of other ion pumps. Again, there were dramatic differences between doxorubicin and doxorubicinol. For example, doxorubicinol (90 μM) completely inhibited bovine cardiac mitochondrial Mg^{2+}-ATPase activity referable to the F_0F_1 reversible proton pump, the proton pump responsible for ATP synthesis via oxidative phosphorylation (22). Doxorubicin at 700 μm had no effect on the ATPase of this proton pump. The ability of doxorubicinol to inhibit the ATPases of other ion pumps was not limited to cardiac tissue (which could partially explain other tissue toxicities associated with doxorubicin therapy). For example, doxorubicinol inhibited the Ca^{2+}-stimulated ATPase activity of skeletal muscle sarcoplasmic reticulum and abolished the Na^+/K^+-ATPase activities of sarcolemma from canine and rabbit heart preparations (Table 1). Doxorubicin had little or no effect on these activities even at 700 μM (Table 1).

Further experiments were conducted to measure cardiac tissue concentrations of doxorubicin and doxorubicinol because tissue concentrations may better predict cardiotoxicity than muscle bath concentrations. The tissue concentration of doxorubicin was 310 ± 111 μg/g of wet weight at a muscle bath concentration of 100 μg/ml (175 μM), whereas the tissue concentration of doxorubicinol was 15 ± 2 μg/g of wet weight at a muscle bath concentration of 50 μg/ml (90 μM) (Table 2). Thus, the ratio of tissue concentration to muscle bath concentration was 10-fold greater for doxorubicin than for doxorubicinol (i.e., for doxorubicin the ratio was 310/100 = 3.1; for doxorubicinol the ratio was 15/50 = 0.3). When viewed in this context, doxorubicinol was ten times more potent as a cardiotoxin than determined using muscle bath concentrations (see Fig. 1).

How do tissue concentrations of doxorubicinol in our study compare with cardiac tissue concentrations from animals

Table 1. Effects of doxorubicin and doxorubicinol on ion pump activities

Ion pump	Property assayed	Source	Species	Activity: Control	Activity: Doxorubicin, 700 μM	Activity: Doxorubicinol, 90 μM
Ca^{2+}	ATP-dependent Ca^{2+} loading*	Cardiac SR	Canine	0.13 ± 0.01 (3)	0.10 ± 0.01 (3)†	ND (2)†
		Skeletal SR	Rabbit	1.48 ± 0.15 (3)	1.09 ± 0.11 (3)†	ND (2)†
Ca^{2+}	Ca^{2+}-stimulated ATPase‡	Cardiac SR	Canine	2.47 ± 0.09 (2)	1.44 ± 0.03 (2)†	0.07 ± 0.04 (2)†
		Skeletal SR	Rabbit	6.60 ± 0.48 (4)	3.01 ± 0.26 (2)†	0.22 ± 0.10 (2)†
Na^+/K^+	Ouabain-inhibitable ATPase§	Cardiac SL	Canine	21.82 ± 6.41 (4)	20.81 ± 7.10 (2)	1.56 ± 1.19 (4)†
		Cardiac SL	Rabbit	0.92 ± 0.07 (2)	0.82 ± 0.03 (2)	ND (4)†
F_0F_1 proton	Mg^{2+}-stimulated ATPase‡	Cardiac mitochondria	Bovine	0.686 ± 0.062 (6)	0.761 ± 0.22 (2)	ND (4)†

Effects of doxorubicin and doxorubicinol on calcium loading of sarcoplasmic reticulum and ATPase activities of ion pumps. Numbers in parentheses after each activity represent the number of separate observations (done in duplicate) used to determine mean values. All activities are expressed as mean ± SEM. ND, no detectable activity at concentrations greater than 45 μM.

*μmol of Ca^{2+} per mg of protein per min.

†Significant differences from control activity at $P < 0.05$ assessed by one-way analysis of variance and Dunnett's procedure.

‡μmol of P_i per mg of protein per min. P_i, inorganic phosphate.

§μmol of P_i per mg of protein per hr.

Table 2. Relation between doxorubicin and doxorubicinol cardiac tissue concentrations and myocardial function

	Doxorubicin	Doxorubicinol
Bath conc.	100 μg/ml	50 μg/ml
Cardiac conc.	310 ± 111 μg/g of wet wt.	15 ± 2 μg/g of wet wt.
dS/dt, % basal value	95 ± 5%	31 ± 5%
Resting stress, % basal value	80 ± 3%	395 ± 76%

Cardiac tissue concentrations, cardiac contractility (*dS/dt*), and resting stress were determined 45 min after adding doxorubicin (175 μM) or doxorubicinol (90 μM) to muscle baths. *dS/dt* and resting stress are expressed as percentages of basal predrug values. Basal values of *dS/dt* (7.62 ± 2.40 g per sec per mm^2 for doxorubicin and 10.58 ± 2.03 g per sec per mm^2 for doxorubicinol) and resting stress (0.232 ± 0.034 g per mm^2 for doxorubicin and 0.202 ± 0.013 g/mm^2 for doxorubicinol) did not differ between the two groups. Data are mean ± SEM. n = 5 and 6 for cardiac tissue concentrations of doxorubicin and doxorubicinol, respectively.

injected with doxorubicin? There are no reports that relate cardiac doxorubicinol concentrations to alterations in cardiac function. Tissue concentrations of doxorubicinol have been measured in rats after a single injection of doxorubicin at 6 mg/kg (23), but this dose was not associated with acute cardiac dysfunction (24). The doxorubicin and doxorubicinol tissue concentrations in our study were greater than those reported in the above-mentioned study (23). Note that the relatively high cardiac tissue concentration of doxorubicin in our study (310 ± 111 μg/g of wet weight) failed to produce significant alterations in systolic (*dS/dt*) or diastolic (resting stress) function (Table 2). In contrast, a relatively low cardiac tissue concentration of doxorubicinol (15 ± 2 μg/g of wet weight) was associated with a 69% reduction in *dS/dt* and 395% increase in resting stress (Table 2).

Doxorubicinol accumulates in cardiac tissue in a time-dependent fashion after parenteral administration of doxorubicin (6, 7). Because intramyocardial metabolism of doxorubicin to doxorubicinol could explain this accumulation, we designed experiments to determine whether the heart can convert doxorubicin to doxorubicinol. Cardiac tissue was exposed to doxorubicin (175 μM) for 45 or 180 min (Fig. 2). Exposure to doxorubicin generated substantial quantities of doxorubicinol within 45 min (0.91 ± 0.5 μg/g of wet weight). The concentration of doxorubicinol was significantly higher (5.8 ± 2.5 μg/g of wet weight) when muscles were exposed to doxorubicin for 180 min. These observations suggest that intracardiac conversion of doxorubicin to doxorubicinol may be an important mechanism responsible for cardiac accumulation of doxorubicinol.

Because doxorubicinol was more potent than doxorubicin in inhibiting contractile function, studies were conducted to determine the potency ratios of doxorubicin and doxorubicinol as cytotoxic agents to cancer cells. Cytotoxicities of doxorubicin and doxorubicinol were compared in three cancer cell lines. As shown in Table 3, to decrease colony formation by 50% (IC_{50}) always required a greater concentration of doxorubicinol than of doxorubicin. Thus, in our system, doxorubicin was 5 to 28 times more potent than doxorubicinol as an antitumor agent.

DISCUSSION

Doxorubicin and other anthracyclines with a C-13 carbonyl functional group are metabolized to the C-13 alcohols (25). Before this study, little was known about the relationship of these C-13 alcohol metabolites and the cardiotoxicity of anthracyclines. The present data suggest that doxorubicinol, the C-13 alcohol metabolite of doxorubicin, could play an important role in the cardiotoxicity seen with doxorubicin therapy. For example, doxorubicinol was much more potent

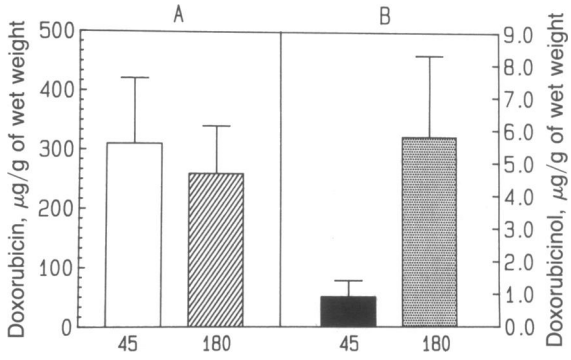

FIG. 2. Cardiac tissue concentrations of doxorubicin (*A*) and doxorubicinol (*B*) after 45 and 180 min of exposure to doxorubicin (175 μM). Data are mean ± SEM expressed as μg of doxorubicin or doxorubicinol per gram of wet weight of cardiac tissue.

than doxorubicin as an inhibitor of both systolic and diastolic cardiac function (Fig. 1). Furthermore, this metabolite was a more potent inhibitor than doxorubicin of three ion pumps (Table 1). Moreover, doxorubicinol abolished the calcium-loading activity of cardiac sarcoplasmic reticulum vesicles, whereas doxorubicin had little effect on this activity (Table 1).

The present study suggests a hypothesis that will require further investigation. This hypothesis centers on the observation that doxorubicin accumulates in cardiac tissue in a time- and dose-dependent fashion (6, 7, 23). Consistent with this idea, the present study demonstrated that the heart can convert doxorubicin to doxorubicinol (Fig. 2). When intracellular levels of doxorubicinol reach toxic limits, inhibition of ion pumps (Table 1) would cause cardiac dysfunction. Such a hypothesis could explain the chronic cumulative nature of doxorubicin cardiotoxicity.

In contrast to relative potencies as cardiotoxic agents, doxorubicin was more potent than doxorubicinol at inhibiting cancer cell growth *in vitro* (Table 3). Thus, our data suggest a potential approach for enhancing the safety and efficacy of doxorubicin therapy. Inhibition of the aldoketo reductases that catalyze the C-13 carbonyl reduction of doxorubicin to doxorubicinol would result in more doxorubicin to kill cancer cells but less doxorubicinol to compromise cardiac function. Clearly, studies are necessary to determine the effects of aldoketo reductase inhibitors on the antineoplastic activity and the cardiotoxicity of doxorubicin therapy.

In summary, doxorubicinol, the primary circulating metabolite of doxorubicin, profoundly compromised both systolic and diastolic function of isolated rabbit papillary muscles. Relatively low (compared with doxorubicin) muscle

Table 3. Cytotoxicity of doxorubicin and doxorubicinol in tumor cells

Cell line	Doxorubicin, IC_{50} in μM	Doxorubicinol, IC_{50} in μM	DMF, $IC_{50\text{-ol}}/IC_{50\text{-in}}$
PANC-1	1.4 ± 0.2	35.4 ± 4.7	25
PD PaCa	1.6 ± 0.2	44.5 ± 0.5	28
WD PaCa	9.8 ± 1.5	49.5 ± 1.1	5

Cytotoxicity of doxorubicin or doxorubicinol in three pancreatic adenocarcinoma cell lines. Cell lines were from human (PANC-1) and hamster origin (PD PaCa and WD PaCa). IC_{50} is the concentration of doxorubicin or doxorubicinol required to inhibit the number of colonies formed in each cell line by 50% compared with untreated control cells. The results using doxorubicin were mean ± SEM of seven experiments; those for doxorubicinol were mean ± SEM of four experiments. DMF (dose-modifying factor) is the ratio of IC_{50} for doxorubicinol to the IC_{50} for doxorubicin. The IC_{50} values for doxorubicin and doxorubicinol differed significantly ($P < 0.05$) for each cell line.

bath and cardiac tissue concentrations of doxorubicinol nearly abolished calcium loading and inhibited by >90% the ATPase activities of sarcoplasmic reticulum, mitochondria, and sarcolemma. Conversely, doxorubicin, at concentrations as high as 700 μM, had little effect on systolic or diastolic function and did not substantially inhibit calcium loading by sarcoplasmic reticulum vesicles or ATPase activities of the three ion pumps. Also, the heart can metabolize doxorubicin to doxorubicinol, which could result in the intramyocardial accumulation of this highly toxic metabolite. Thus, doxorubicinol may be important in the chronic cumulative cardiac toxicity associated with doxorubicin chemotherapy.

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1. Dresdale, A., Bonow, R. D., Wesley, R., Palmer, S. T., Barr, L., Mathison, D., D'Angelo, T. & Rosenberg, S. A. (1983) *Cancer* **52,** 51–60.
2. Myers, C. E., McGuire, W. P., Liss, R. H., Ifrim, I., Grotzinger, K. & Young, R. C. (1977) *Science* **197,** 165–167.
3. Unverferth, D. V., Leier, C. V., Balcerzak, S. P. & Hamlin, R. L. (1985) *Am. J. Cardiol.* **56,** 157–161.
4. Myers, C., Bonow, R., Palmeri, S., Jenkins, J., Corden, B., Locker, G., Doroshaw, J. & Epstein, S. (1983) *Semin. Oncol.* **10,** Suppl. 1, 53–55.
5. Takahashi, S. & Bachur, N. R. (1976) *Drug Metab. Dispos.* **4,** 79–87.
6. Del Tacca, M., Danesi, R., Ducci, M., Bernardini, C. & Romanini, A. (1985) *Pharmacol. Res. Commun.* **17,** 1073–1084.
7. Peters, J. H., Gordon, G. R., Kashiwase, D. & Acton, E. M. (1981) *Cancer Chemother. Pharmacol.* **7,** 65–60.
8. Brenner, D. E., Galloway, S., Cooper, J., Noone, R. & Hande, K. R. (1985) *Cancer Chemother. Pharmacol.* **14,** 139–145.
9. vanBoxtel, C. J., Olson, R. D., Boerth, R. C. & Oates, J. A. (1978) *J. Pharmacol. Exp. Ther.* **207,** 277–283.
10. Chamberlain, B. K., Levitsky, D. O. & Fleischer, S. (1983) *J. Biol. Chem.* **258,** 6602–6609.
11. Frank, J. S., Philipson, K. B. & Beydler, S. (1984) *Circ. Res.* **54,** 414–423.
12. Fleischer, S., Meissner, G., Smigel, M. & Wood, R. (1974) *Methods Enzymol.* **31,** 292–299.
13. Meissner, G., Conner, G. E. & Fleischer, S. (1973) *Biochim. Biophys. Acta* **298,** 246–269.
14. Seiler, S. & Fleischer, S. (1982) *J. Biol. Chem.* **257,** 13862–13871.
15. Fleischer, S. & Fleischer, B. (1967) *Methods Enzymol.* **10,** 406–433.
16. Baginski, E. S., Foa, P. P. & Zak, B. (1967) *Clin. Chim. Acta* **15,** 155–158.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
18. Lieber, M., Mazzetta, J. A., Nelson-Rees, W., Kaplan, M. & Todaro, G. (1975) *Int. J. Cancer* **15,** 741–747.
19. Scarpelli, D. G. & Rao, M. S. (1979) *Cancer Res.* **39,** 452–458.
20. Chang, B. K. & Gutman, R. (1982) *Cancer Res.* **42,** 2666–2670.
21. Fabiato, A. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41,** 2238–2244.
22. Mitchell, P. (1979) *Science* **206,** 1148–1159.
23. Rossini, L., Monti, E., Cova, D. & Piccinini, F. (1986) *Arch. Toxicol.*, Suppl. 9, 474–478.
24. Monti, E., Piccinini, F., Villani, F. & Favalli, L. (1986) *Cancer Chemother. Pharmacol.* **18,** 289–291.
25. Young, C. W. & Raymond, V. (1986) *Cancer Treat. Rep.* **70,** 51–63.