Aging Increases the Cardiotoxicity of Daunorubicin and Daunorubicinol in the Rat

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This study examined effects of aging on the cardiac response in vitro to daunorubicin, a cancer chemotherapeutic agent that causes cardiotoxicity. Left ventricular trabeculae carnea were isolated from adult (aged 6–9 months) and old (aged 24–28 months) Fischer 344 rats and treated with daunorubicin (175 μM) or saline (controls) over a 210-minute study period. Daunorubicin-induced decline in contractility (ΔS) and Δd (d) was greater in old compared to adult myocardium (p < .02). Similarly, cardiac relaxation (90% relaxation time) was more impaired by daunorubicin in older preparations (p < .01). Although daunorubicin concentrations were unaffected by age, daunorubicinol concentrations in ventricular strips increased with time to a greater extent in the older group (p < .05). This study suggests that senescence increases the acute in vitro cardiotoxicity of daunorubicin and that the metabolite, daunorubicinol, may contribute to this toxicity.

ANTHRACYCLINES are widely used, highly effective antitumor agents whose principal side effect is a well-characterized, but poorly understood cardiotoxicity. Susceptibility to anthracycline cardiotoxicity appears to increase in elderly patients (Bristow et al., 1978; Von Hoff et al., 1979; Dresdale et al., 1983; Palmeri et al., 1986). The age-related increase in susceptibility cannot be simply attributed to coexisting cardiac disease (Von Hoff et al., 1979) and may relate to a decreased capacity of the sarcoplasmic reticulum (SR) to sequester Ca++ as has been demonstrated in rat heart (Froehlich et al., 1978; Narayan, 1981; Jiang and Narayan, 1990). Anthracyclines also diminish sarcoplasmic reticulum (SR) Ca++ availability by impairing Ca++ uptake (Boucek et al., 1987; Olson et al., 1988) and triggering Ca++ release from SR (Kim et al., 1989; Pessah et al., 1989; Mushlin et al., 1993). Thus, age-related impairments of Ca++ metabolism may amplify the toxic effects of anthracyclines on Ca++ homeostasis.

The primary metabolites (C-13 OH compounds) of doxorubicin and daunorubicin markedly depress myocardial function in vitro (Boucek et al., 1987; Olson et al., 1987). Doxorubicinol stimulates superoxide anion production in cardiac sarcomosomes and mitochondria (Gervasi et al., 1986) and inhibits SR Ca++ ATPase activity (Boucek et al., 1987; Olson et al., 1988). Furthermore, cardiac concentrations of doxorubicinol rather than daunorubicin correlate with contractile dysfunction in preparations isolated from rabbits given a single dose of daunorubicin (Cusack et al., 1993). At the cardiac concentrations observed in that study, daunorubicinol significantly decreases SR Ca++-ATPase activity in vitro, suggesting a role for the C-13 OH metabolites in the cardiotoxicity of anthracycline therapy.

The present report addresses the hypothesis that aging increases sensitivity to the cardiotoxic effects of anthracyclines, by comparing daunorubicin-induced impairments of contractile function to basal control values. Ventricular preparations isolated from young adult (6–9 month) and old (24–28 month) Fischer 344 rats were treated with daunorubicin (175 μM) or saline (controls) over a 210-minute study period. Daunorubicin-induced decline in contractility (ΔS) and Δd (d) was greater in old compared to adult myocardium (p < .02). Similarly, cardiac relaxation (90% relaxation time) was more impaired by daunorubicin in older preparations (p < .01). Although daunorubicin concentrations were unaffected by age, daunorubicinol concentrations in ventricular strips increased with time to a greater extent in the older group (p < .05). This study suggests that senescence increases the acute in vitro cardiotoxicity of daunorubicin and that the metabolite, daunorubicinol, may contribute to this toxicity.
the bath, and the bath was washed three times with fresh buffer. Next, Krebs-bicarbonate buffer containing 3.5 mM Ca\textsuperscript{2+} was added to the bath, and contractile function was evaluated after a 20-min stabilization period. This process was repeated, using replacement buffers with declining Ca\textsuperscript{2+} concentrations (2.5, 1.5, and 1.0 mM). Following each replacement, the preparation was allowed to equilibrate for 20 minutes before determining cardiac function.

To determine the effect of daunorubicin treatment on cardiac concentrations of both daunorubicin and daunorubicinol, we prepared thin strips from the right ventricular free wall (100–200 mg; < 1.0 mm in thickness), and then incubated the strips with daunorubicin (175 \mu M; Krebs-bicarbonate buffer) for 30, 90, 150, or 210 minutes. At each time point, strips were removed, immediately placed in a freezer at −80 °C, and stored until assayed for daunorubicin and daunorubicinol.

Cardiac function studies. — Left ventricular trabeculae carneae were affixed to force transducers (Kulite BG25; Leonia, NJ) and stimulated to contract isometrically by square wave pulses (Grass S8; Quincy, MA) at 10% above threshold voltage. Trabeculae carneae were stretched to the length at which they developed maximum tension (T\textsubscript{max}). Preparations were stabilized at a contraction frequency of 15/min. Data were recorded using a Gould 2400S physiological recorder and a Buxco (Troy, NY) pulsatile analyzer. The cardiac parameters measured included resting stress (RS), developed stress (DS), the maximum first derivative of stress development (dS/dt), time to development of peak stress (TTPS), and 90% relaxation time (90% RT). To obtain stress, parameters of force were normalized for cross-sectional area. This entailed blotting each muscle on a paper towel, weighing the muscle and calculating stress, assuming that the muscle was cylindrical in shape, with a specific gravity of one. Baseline and post-treatment functional parameters in preparations were measured at stimulation rates of 15 and 90 contractions per minute (cpm) and immediately after a 2-min rest period.

Analytical methods. — Cardiac tissue samples were assayed for daunorubicin and daunorubicinol concentrations, using doxorubicin as an internal standard (Cusack et al., 1993). Standard curves for daunorubicin and daunorubicinol were prepared by adding these agents to naive rat tissue or expired human plasma.

The daunorubicin standard was obtained from homogenized rat kidney that had been incubated for 8 hours in a bath containing 5 \mu g/ml daunorubicin (Cusack et al., 1995). The mean value (± SE) in heart tissue for the slopes of standard curves for daunorubicin was 524 ± 68 and for daunorubicinol was 460 ± 67. The limit of detection of daunorubicin and daunorubicinol was 0.2 ng injected directly onto the HPLC column (Waters; Milford, MA).

Data analysis. — Effects of daunorubicin and age on parameters of function including developed stress, dS/dt, time to peak stress and 90% relaxation time were compared using analysis of covariance. This analysis, with adjustment for baseline, compared the mean of the seven post-baseline measures of each parameter between the age and treatment groups. This analysis allowed detection of age effects on parameters, drug effects on parameters, and also an Age by Drug interaction signifying that the drug effect was different between age groups.

The effect of different buffer Ca\textsuperscript{2+} concentrations on DS in both age groups was tested in a repeated measures ANOVA with age and drug treatment as factors. Comparisons between age and treatment groups at baseline (Tables 1 and 2) were made using one-way ANOVA with Bonferroni’s post hoc test. Comparisons of tissue daunorubicin and daunorubicinol concentrations in both age groups were made by repeated measures ANOVA with Student-Neuman-Keuls test. The null hypothesis was rejected when p < .05.

RESULTS

Baseline functional parameters in trabeculae carneae contracting at a frequency of 15 per minute (cpm) are shown in Table 1. Baseline values did not differ significantly between groups within each age cohort. There were, however, some age-related differences in baseline values. For example, the older groups exhibited a lower resting stress (RS) and a significant prolongation of both TTPS and 90% RT compared to young groups (p < .05). Compared with control, daunorubicin treatment produced a significantly greater decline in dS/dt (p < .001; Figure 1). Daunorubicin-induced decreases in dS/dt, relative to controls, were greater in the old preparations, with a significant interaction of age and drug in mean post-baseline dS/dt compared to baseline (p < .02; Figure 1). In old preparations, controls declined from 9.6 ± 0.9 to 8.6 ± 1.0 g/sec/mm\textsuperscript{2}, whereas daunorubicin-treated muscles decreased from 8.9 ± 1.7 to 4.9 ± 0.9 g/sec/mm\textsuperscript{2}. In young trabeculae carneae, controls declined from 8.0 ± 1.3 to 6.8 ± 1.2 g/sec/mm\textsuperscript{2}, and daunorubicin-treated preparations decreased from 8.0 ± 0.8 to 4.8 ± 0.5 g/sec/mm\textsuperscript{2}. The decline in DS during treatment with daunorubicin over 210 minutes also was significantly greater in older than younger preparations; the mean post-baseline DS in old daunorubicin-treated trabeculae carneae showed a greater decline compared to controls than in young muscles (RT < .01; data not shown). In old preparations, controls declined from 0.74 ± 0.07 to 0.66 ± 0.06 g/mm\textsuperscript{2}, and daunorubicin-treated muscles decreased from 0.71 ± 0.14 to 0.46 ± 0.08 g/mm\textsuperscript{2}. In young trabeculae carneae, controls declined from 0.57 ± 0.09 to 0.46 ± 0.08 g/mm\textsuperscript{2}, and daunorubicin-treated preparations decreased from 0.56 ± 0.06 to 0.37 ± 0.04 g/mm\textsuperscript{2}.

Baseline TTPS and 90% RT were more prolonged in old compared to young preparations in both control and daunorubicin-treated groups (Table 1; p < .05). In addition, analysis of covariance indicated that daunorubicin treatment prolonged both TTPS (p < .001; data not shown) and 90% RT (Figure 2; p < .001). There was also a significant Age by Drug interaction, indicating that, in older rat trabeculae carneae, the effect of daunorubicin on both TTPS (p < .001) and 90% RT (Figure 2; p < .01) was increased. In older animals, the 90% RT in control preparations decreased from a baseline of 175 ± 5 to 169 ± 4 msec (mean of post-baseline values), whereas 90% RT in daunorubicin-treated trabeculae carneae increased from 177 ± 11 to 225 ± 17.
Table 1. Pretreatment (Baseline) Values of Cardiac Mechanical Function in Trabeculae Carneae From Young and Older Fischer 344 Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>RS (g/mm²)</th>
<th>DS (g/mm²)</th>
<th>dS/dt (g/sec/mm²)</th>
<th>TTPS (msec)</th>
<th>90% RT (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young controls 15 cpm</td>
<td>0.41 ± 0.03</td>
<td>0.57 ± 0.10</td>
<td>8.1 ± 1.3</td>
<td>103.9 ± 1.9</td>
<td>137.0 ± 3.5</td>
</tr>
<tr>
<td>Young daunorubicin 15 cpm</td>
<td>0.44 ± 0.04</td>
<td>0.62 ± 0.08</td>
<td>8.7 ± 1.1</td>
<td>108.2 ± 1.8</td>
<td>136.5 ± 3.1</td>
</tr>
<tr>
<td>Old controls 15 cpm</td>
<td>0.33 ± 0.02*</td>
<td>0.74 ± 0.07</td>
<td>9.6 ± 0.9</td>
<td>125.4 ± 2.0*</td>
<td>175.7 ± 5.2*</td>
</tr>
<tr>
<td>Old daunorubicin 15 cpm</td>
<td>0.33 ± 0.04*</td>
<td>0.71 ± 0.14</td>
<td>8.9 ± 1.7</td>
<td>124.6 ± 3.0*</td>
<td>177.5 ± 10.6*</td>
</tr>
</tbody>
</table>

Notes. RS = resting stress; DS = peak developed stress; dS/dt = maximum first derivative of stress development; TTPS = time for contraction to reach peak stress; 90% RT = time for peak contraction to relax by 90%; cpm = contractions per minute.

*p < .05 old vs corresponding young group by one-way ANOVA and Bonferroni post hoc test.

Figure 1. Time-related effects of daunorubicin (175 µM) on dS/dt in trabeculae carneae from young (n, 15) and old (n, 15) Fischer 344 rats. Preparations were stimulated to contract at 15 cpm (.25 Hz). Line graphs show baseline (time 0 min) and seven post-baseline values for DS. Each inset shows baseline (solid bar) and mean of seven post-baseline values (open bar) for DS in control (C) and daunorubicin-treated (D) groups. Data are shown as mean ± SE. Adjusting for baseline, using analysis of covariance, there was a significant effect of age on the negative inotropic effect of daunorubicin on mean post-baseline dS/dt (p < .02).

Figure 2. Time-related effects of 175 µM daunorubicin on 90% RT in trabeculae carneae from young (n, 15) and old (n, 15) Fischer 344 rats. Preparations were stimulated to contract at 15 cpm (.25 Hz). Line graphs show baseline (time 0 min) and seven post-baseline values for 90% RT. Each inset shows baseline (solid bar) and mean of seven post-baseline values (open bar) for 90% RT in control (C) and daunorubicin-treated (D) groups. Data are shown as mean ± SE. Adjusting for baseline, using analysis of covariance, there was a significant effect of age on the effect of daunorubicin on mean post-baseline 90% RT (p < .01). Data are shown as mean ± SE.

msec (Figure 2). In young rats, the 90% RT in control muscles decreased from 137 ± 4 to 131 ± 3 msec, and in drug-treated preparations 90% RT increased from 136 ± 3 to 150 ± 6 msec.

Following the 210-min study with daunorubicin, the bath was rinsed three times to wash out the daunorubicin. The parameters of contractile function were then measured at Ca²⁺ concentrations ranging from 3.5 mM (Table 2) to 1.0 mM. At 3.5 mM Ca²⁺ in the older group, as compared to the younger one, resting stress was lower, and both TTPS and
90% RT were prolonged ($p < .05$; Table 2). There were no age-related differences in either DS or dS/dt at this concentration of Ca\(^{2+}\) (3.5 mM). The effect of daunorubicin on contractile parameters persisted after removal of the drug from the bath; daunorubicin-treated preparations had significantly lower DS (Ca\(^{2+}\) concentration by drug treatment interaction; $p < .002$) compared with controls both in young and old hearts (Figure 3).

Over the range of Ca\(^{2+}\) concentrations in the buffer from 1.0 mM to 3.5 mM, DS increased (Figure 3). This positive inotropic effect of Ca\(^{2+}\) on DS (Figure 3) and on dS/dt (data for the latter not shown) occurred both in daunorubicin pretreated and control groups. However, the magnitude of the inotropic effect of Ca\(^{2+}\) on DS was significantly attenuated by daunorubicin treatment compared to control ($p < .01$). This effect of daunorubicin treatment, moreover, was altered by age, with an increased effect of daunorubicin treatment compared to control in the aged preparations ($p < .05$).

It should also be noted that DS was significantly increased by age ($p < .05$) at each individual Ca\(^{2+}\) concentration except 3.5 mM (Figure 3). This may be due, at least in part, to the prolongation of TTPS with age ($p < .01$) at corresponding Ca\(^{2+}\) concentrations. It does not appear due to altered dS/dt which was not increased in older preparations at matching buffer Ca\(^{2+}\) concentrations.

Lengthening the stimulus interval between contractions (force-interval relation) invariably increased baseline DS in all groups (Figure 4). The interval-related increases in DS, however, were less pronounced in daunorubicin-treated than in vehicle-treated preparations. For example, the mean post-baseline DS in young controls increased by 158% from .26 ± .04 g/mm\(^2\) at 90 cpm to .67 ± .12 g/mm\(^2\) following 2 min rest, whereas in young daunorubicin-treated muscles, DS increased by 68% from .25 ± .02 g/mm\(^2\) at 90 cpm to .42 ± .05 g/mm\(^2\) following 2-min rest. In old animals, mean post-baseline DS increased by 81% from .43 ± .05 g/mm\(^2\) at 90 cpm to .78 ± .07 g/mm\(^2\) following 2 min rest, while in daunorubicin-treated preparations DS increased by 53% from .32 ± .05 g/mm\(^2\) to .49 ± .09 g/mm\(^2\). These data also showed that a significant age-related difference (Age by Treatment interaction) in the effect of daunorubicin occurred at 90 cpm ($p < .01$) and 15 cpm ($p < .005$) but not following a 2-min interval.

Daunorubicin and daunorubicinol concentrations in right ventricular muscle strips progressively increased in both age groups during the 210-min experiment. At all time points, ventricular concentrations of daunorubicin far exceeded those of daunorubicinol (Figure 5). There were no age-related differences in concentrations of daunorubicin. By contrast, daunorubicinol concentrations were significantly higher in old vs young ventricular strips treated with daunorubicin for 150 and 210 minutes (Figure 5). Similarly, daunorubicin-treated trabeculae carneae (removed after the Ca\(^{2+}\) concentration-response study was completed in drug-free buffer) revealed comparable concentrations of daunorubicin in the two age groups (513 ± 42 in young vs 474 ± 30 μg/g in old), whereas concentrations of daunorubicinol were 67% higher in old than in young preparations (58 ± 10 vs 36 ± 3 μg/g; $p < .05$). Thus, differences in daunorubicin and daunorubicinol concentrations between age groups in trabeculae carneae reflected those in right ventricular strips.

Following daunorubicin treatment, the decline in DS and dS/dt (Figure 1) compared with controls was greater in old
than in young trabeculae carneae. Was this age difference due to increased sensitivity to the effect of anthracycline or due to increased concentration of daunorubicin or daunorubicinol? The data suggest an increased sensitivity of old trabeculae to anthracycline toxicity, since at equivalent time points, concentrations of daunorubicin were not increased in older preparations. Likewise, at ventricular concentrations of daunorubicinol that were similar in the two age groups (at 210 in young vs 150 min in old), the decrease in dS/dt was greater in the older preparations (p < .05) despite the shorter duration of drug exposure in the older group (Figure 6).

DISCUSSION
Clinical studies have shown that old age is a risk factor for chronic anthracycline cardiotoxicity (Bristow et al., 1978; Von Hoff et al., 1979; Palmeri et al., 1986). For example, Palmeri et al. (1986) showed that age was an independent predictor of final LVEF after completion of doxorubicin therapy. This age-related effect is not simply due to an increased incidence of coronary heart disease in older cancer patients (Von Hoff et al., 1979). These observations in humans are consistent with prior reports of increased toxicity of doxorubicin in 24-month versus 6-week-old rats (Colombo et al., 1989). In that study, older rats suffered more weight loss and mortality at lower cumulative doses of doxorubicin, but direct measures of cardiotoxicity were not performed. Another investigation compared effects of 15 mg/kg doxorubicin (over 2 weeks) in 1-2 month vs 6-7-month-old Sprague-Dawley rats, showing greater effects in the older rats on indices of cardiotoxicity, such as contractile dysfunction, histopathology, and lipid peroxidation (Weinberg & Singal, 1987). That study reflects changes with maturity rather than aging per se, however.

In the present study, daunorubicin treatment caused a greater decrement in contractile function (DS and dS/dt) in trabecular carneae from older vs younger rats (Figure 1), suggesting that senescence increases the acute cardiotoxicity of anthracyclines. What might be the cause of the daunorubicin-induced contractile dysfunction? Under normal physiologic conditions, the magnitude of contractile
Figure 5. Concentrations of daunorubicin (upper panel) and daunorubicinol (lower panel) in right ventricular strips from young (n, 14) and old (n, 14) Fischer 344 rats. Strips were incubated in 175 μM daunorubicin and removed for assay at times indicated on the x-axis. Data are expressed as mean ± SE. *p < .05, young vs old using repeated measures ANOVA and Student-Neuman-Keuls test.

stress (DS) in the rat heart is closely related to the peak Ca^{2+} concentration achieved in the cytosol (Orchard & Lakatta, 1985). In the rat, the peak Ca^{2+} concentration achieved in heart muscle depends more upon Ca^{2+} release by the sarcoplasmic reticulum (SR) (Kort & Lakatta, 1983) than on transcellular Ca^{2+} influx (Willerson et al., 1978). Thus, daunorubicin may depress DS by disturbing SR Ca^{2+} loading or recycling. In addition, C13-OH anthracyclines, such as doxorubicinol (Boucek et al., 1987, Olson et al., 1988) and daunorubicinol (Cusack et al., 1993), are potent inhibitors of Ca^{2+} loading of SR, and exert striking negative inotropic effects in isolated cardiac preparations (Boucek et al., 1987; Olson et al., 1988). Doxorubicin also triggers Ca^{2+} release from SR (Kim et al., 1989; Mushlin et al., 1993), which could further deplete SR Ca^{2+} stores and impair contractile function, as occurs with high concentrations of caffeine (Mushlin et al., 1993).

The effect of age on daunorubicin-induced reduction of contractility (dS/dt, data not shown and DS; Figure 4) was rate-dependent. For example, the reduction of DS by daunorubicin was greatest following 2-minute rest and was similar in both age groups (Figure 4). The depressant effect of daunorubicin on DS was less at 15 and especially 90 cpm. This negative inotropic effect of daunorubicin was increased in older preparations both at 15 cpm (p < .005) and 90 cpm (p < .01). The greater effect of daunorubicin in the rat myocardium at slower rates of contraction (Figure 4) is the converse of observations in the rabbit (Mushlin et al., 1993). Borzak et al. (1991) noted that rate-force relationship in ventricular cells from the young adult rat is highly dependent on Ca^{2+} release from the SR at rates of stimulation below, but not above 1 Hz (60 cpm). This accords with the observation that the increase in peak force of contraction in ventricular myocytes at low frequencies was offset by drugs (caffeine) that decrease Ca^{2+} accumulation in the SR but not by antagonists that block inward Ca^{2+} current (Schouten and ter Keurs, 1991). Thus, the rate-dependence of daunorubicin effect on DS, at least in young rats, is consistent with an effect on SR Ca^{2+} handling. This is similar to the effect of ryanodine in rat atria (Stemmer and Aker, 1986). Thus, there may be a common mechanism of action of daunorubicin, caffeine, and ryanodine in reducing peak force in the rat cardiocyte that relates to effects on SR Ca^{2+} metabolism.

The age difference in the effect of daunorubicin on DS is consistent with the idea that systolic contraction in older myocardium is more dependent than young muscle on SR Ca^{2+} transport at higher rates of contraction.

One of the main changes in cardiac function that occurs with age in rat heart is prolongation of contraction with delayed relaxation (Froehlich et al., 1978; Wei et al., 1984). In our study, the times to peak stress (TTPS) and 90% relaxation time (90% RT) were significantly prolonged in trabeculae carnea of older rats (Table 1). This is considered to be due to the prolonged Ca^{2+} transient that occurs during contraction in aged myocardium (Froehlich et al., 1978) resulting, at least in part, from a reduced rate of Ca^{2+} uptake by the SR (Jiang and Narayanan, 1990). In addition, the lengthened contraction in ventricular muscle may ensue from the prolonged duration of the action potential in aged Fischer 344 and Wistar rats (Capasso et al., 1983; Wei et al., 1984). Incubation with daunorubicin significantly protracted both TTPS (data not shown) and 90% RT (Figure 2) in trabeculae carnea in young and old rats, consistent with the inhibitory effect of anthracyclines and their alcohol metabolites on SR Ca^{2+} sequestration (Boucek et al., 1987; Cusack et al., 1993). In this regard, it is significant that in this study, aging altered the prolongation of TTPS (p < .002) and 90% RT (Figure 2, p < .01) by daunorubicin, suggesting that SR Ca^{2+} in older rat myocardium may have increased sensitivity to the effects of anthracycline.

Effect of buffer Ca^{2+} concentration on DS is presented in the order of ascending Ca^{2+} in Figure 3. In trabeculae carnea beating at 15 cpm, DS in control and drug-treated preparations varied directly with buffer Ca^{2+} concentrations (Figure 3). Daunorubicin treatment reduced the inotropic effect of Ca^{2+} both in young and old preparations. The inhibition of Ca^{2+}-induced inotropic by daunorubicin occurred to a greater extent in old than in young rats (p < .05). In previous studies, increased external bath Ca^{2+} concentra-
Figure 6. Relationship between concentrations of daunorubicin (left panels) or daunorubicinol (right panels) in right ventricular strips and DS (upper panels) and dS/dt (lower panels) in trabeculae carneae from young (n, 14) and old (n, 14) Fischer 344 rats. Both DS and dS/dt are presented as percent of baseline with correction for percent baseline in corresponding controls. At specific times (30, 90, 150, and 210 min) after beginning treatment with 175 μM daunorubicin, the relation between contractile function (DS and dS/dt) in trabeculae carneae and concentrations of daunorubicin or daunorubicinol in ventricular strips was assessed. Data are expressed as mean ± SE.

ctions offset the negative inotropic effect of anthracyclines both in acute (Saman et al., 1984) and chronic (Jensen, 1986) models of cardiac cardiotoxicity. Anthracyclines have been considered to increase Ca2+ accumulation in cardiac cells, possibly by inhibition of sarcolemmal ATP-dependent Ca2+ transport, which helps transport Ca2+ out of the cell (Harada et al., 1990), or by causing channel-mediated influx of Ca2+ (Combs et al., 1985). However, the present data do not suggest myocardial Ca2+ overloading since, in daunorubicin-treated trabeculae carneae, (a) resting force was not increased compared to controls (Table 2), and (b) increased external Ca2+ concentrations increased rather than decreased DS and dS/dt. Thus, reduced inotropy of Ca2+ in daunorubicin-treated preparations may be better explained by impairment of SR Ca2+ uptake or release. If so, the greater impairment by daunorubicin pretreatment of Ca2+ inotropic function in old compared with young animals (Figure 3) may be due to an increased effect of anthracycline on aged SR function.

Could age-related differences in anthracycline cardiotoxicity be attributable to differences in cardiac daunorubicin or daunorubicinol concentrations? The C-13 OH metabolites of anthracyclines, including doxorubicinol and daunorubicinol, are produced in myocardium and other tissues including liver and kidney by the action of ubiquitous carbonyl reductases (Bachur and Gee, 1976). Daunorubicin concentrations tended to be lower in older rat right ventricular tissue at different times during the study (Figure 5) and in working trabeculae carneae removed at the end of the study (working trabeculae carneae could not be used to measure drug concentrations throughout the study because of the inordinate number of hearts and studies required). However, in older rats, daunorubicinol concentrations were significantly higher in ventricular tissue at 150 and 210 min (Figure 5), and in
working trabeculae carneae when compared with young rats. Nevertheless, it seems unlikely that elevated heart concentrations of daunorubicin totally explain the increased cardiotoxicity of daunorubicin treatment in older rat trabecular carneae preparations. For example, dS/dt was impaired to a greater extent in older than younger preparations at equivalent tissue daunorubicin concentrations (at 210 min in young vs. 150 min in old preparations; Figure 6). This suggests that senescent myocardium is more sensitive to the negative inotropic effects of daunorubicin treatment. However, the time-related effect of daunorubicin and/or daunorubicinol may not simply be a result of time-dependent accumulation of drug or metabolite in the myocardium; the effect may also be due to progressive toxicity of the drug or metabolite over time in this preparation such as accumulation of free radicals or progressive perturbation of Ca\textsuperscript{2+} transport mechanisms. While comparison of drug effect at a steady-state tissue concentration of daunorubicin and daunorubicinol would best characterize the concentration-related toxicity of these compounds, achieving steady state using this model is not possible. For example, in our preparation, despite prolonged in vitro exposure to daunorubicin (210 min), both daunorubicin and daunorubicinol concentrations continued to rise (Figure 5). This caveat does not, however, detract from the conclusion that the toxicity of daunorubicin exposure is increased in senescent myocardium.

Doxorubicinol and daunorubicinol impair myocardial function in vitro (Boucek et al., 1987; Olson et al., 1987, 1988; Mushlin et al., 1993). In addition, doxorubicinol inhibits Ca\textsuperscript{2+} ATPase activity of SR with an IC\textsubscript{50} of 4 \mu M (Boucek et al., 1987; Olson et al., 1988). Daunorubicinol, at 10 \mu M, has been reported to produce a 39\% inhibition of Ca\textsuperscript{2+} by isolated cardiac SR (Cusack et al., 1993). This concentration of daunorubicinol was reported to occur in the rabbit heart 72–120 h following a single intravenous injection of daunorubicin (15 mg/kg). Moreover, the depression of contractility in papillary muscles was significantly related to ventricular daunorubicinol but not daunorubicin concentrations (Cusack et al., 1993). Mushlin et al. (1993) reported that in acute doxorubicin cardiotoxicity in vitro, impairment of both systolic and diastolic function was related to increases of both doxorubicin and doxorubicinol concentrations. De Jong et al. (1993) also observed cardiotoxicity in mouse atria from addition of alcohol metabolites including doxorubicinol, daunorubicinol, and epirubicinol to the incubation buffer. The atrial tissue IC\textsubscript{50} concentrations for these alcohol metabolites were lower than for their parent drugs. Thus, alcohol metabolites such as daunorubicinol may be important in the causation of acute or subacute anthracycline cardiotoxicity. Data from the present study support this hypothesis.

Our study provides the first demonstration that senescence enhances acute anthracycline cardiotoxicity in the rat. Acute cardiotoxicity, although uncommon, also has been reported in humans within hours of anthracycline administration, featuring pericarditis, histological changes, altered contractile function, and possibly drug-related sudden death (Bristow et al., 1978; Wortman et al., 1979; Freiss et al., 1985; Brown et al., 1989). Mitochondria and sarcoplasmic reticulum in human heart undergo acute swelling within 4 hours of doxorubicin administration (Unverferth et al., 1981). These histological changes are consistent with early dysregulation of Ca\textsuperscript{2+} metabolism, as suggested in the present study. Acute histology is similar to that seen in chronic anthracycline cardiomyopathy that also heavily involves the SR and mitochondria (Bristow et al., 1978). Moreover, the acute changes in myocardial function shown in this study (with impaired contractility and prolonged relaxation) are similar to those observed in chronic cardiotoxicity in the rat (Jensen, 1986), further suggesting that long-term toxicity may share mechanisms with the acute form. Thus, acute anthracycline toxicity has clinical relevance because of its direct effects and likely relationship with chronic cardiomyopathy. The present study, demonstrating an age-dependent acute cardiotoxicity, therefore suggests that chronic cardiotoxicity might also increase in senescence.

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