Mechanism of Acute Anthracycline Cardiotoxicity in Isolated Rat Hearts: Carminomycin versus Daunomycin

Selva Saman, Peter Jacobs, and Lionel H. Opie

MRC-UCT Ischaemic Heart Disease Laboratory, and the Departments of Medicine [S. S., L. H. O.] and Haematology [P. J.], University of Cape Town, Cape Town, South Africa

ABSTRACT

Cardiotoxicity limits the use of anthracyclines which are potent anticancer agents. In the isolated rat heart, we investigated the mechanism of acute anthracycline cardiotoxicity and compared a new anthracycline, carminomycin, with daunomycin which is in established use. Daunomycin $1.75 \times 10^{-6}$ M produced a fall in cardiac output $(36 \pm 2 \text{ versus } 58 \pm 1 \text{ ml/min}; p < 0.01)$, left ventricular power production $(9 \pm 0.7 \text{ versus } 16 \pm 0.3 \text{ mJ/sec/g}; p < 0.01)$, and efficiency of heart work $(3.3 \pm 0.2 \text{ versus } 6.3 \pm 0.2 \text{ mJ/sec/ml } O_2; p < 0.01; \text{ mean } \pm \text{ S.E. } 40 \text{ min after daunomycin})$. Carminomycin $(1.75 \times 10^{-6} \text{ M})$ produced a greater fall in cardiac output than equimolar daunomycin $(26 \pm 2 \text{ versus } 36 \pm 2 \text{ ml/min}; p < 0.01)$. Daunomycin did not reduce coronary flow rate, heart rate, or oxygen consumption. From the preceding data, we inferred that, since afterload and preload were constant in this model, heart failure was due to a depressed inotropic state. Procedures that increased cytosolic calcium relieved heart failure namely, pretreatment with digoxin $(62.4 \mu g)$, isoproterenol $(10^{-6} \text{ M})$, and increased perfusate Ca$^{2+}$ $(5 \text{ mM versus } 2.5 \text{ mM})$ all prevented carminomycin-induced fall in cardiac output $(41 \pm 1, 47 \pm 5$, and $52 \pm 1$, respectively, versus $26 \pm 2 \text{ ml/min}; p < 0.01$). Acute anthracycline contractile failure was also associated with a fall in high-energy phosphate compounds which could also have contributed to the decreased inotropic state. We conclude that carminomycin is more cardiotoxic than daunomycin in equimolar concentrations and that a lowered cytosolic calcium and decreased energy stores might cause the contractile failure. The cytosolic calcium and high-energy phosphate compounds were lowered by separate mechanisms.

INTRODUCTION

Anthracyclines are potent antimitotic agents $(29)$. Their use may be limited by cardiotoxicity of which 2 types have been identified. (a) Acute clinical cardiotoxicity with a fall of ejection fraction can be found within 4 to 24 hr of administration of anthracycline in patients $(3, 26)$. (b) With cumulative doses of anthracycline, a chronic toxic cardiomyopathy may occur $(17)$. There may be a common mechanism for the acute and chronic cardiotoxicity because the myocardial lesions were similar in one study $(31)$. This paper investigates the mechanism of acute contractile failure in an isolated heart model. We also compare daunomycin with equimolar concentrations of carminomycin $(6)$, a new analogue reported to be less cardiotoxic $(7)$. Previously contractile failure in blood perfused hearts has been explained by a decreased coronary flow rate $(19)$. However, the finding of ATP depletion in cultured cells exposed to Adriamycin $(25)$ argues against a simple effect on coronary flow and suggests a direct toxic effect on energy production $(10)$. Another hypothesis is that anthracyclines might cause myocardial cell membrane damage and thereby cause enzyme release $(9, 22)$. Another possible hypothesis is that there is a decreased availability of cytosolic calcium, because anthracyclines may block membrane Na$^+$/Ca$^{2+}$ exchange $(5)$ or antagonize calcium-dependent slow-response action potentials $(1)$. Cardiac glycosides are reported to prevent acute contractile failure caused by anthracyclines $(4, 33)$, although they neither compete with anthracyclines for membrane binding sites $(27)$ nor prevent myocardial uptake of anthracyclines $(2)$. Because cardiac glycosides are thought to increase cytosolic calcium $(14, 20)$ indirectly, these observations support the proposed role of a decreased cytosolic calcium in acute contractile failure.

We explored the mechanism of acute anthracycline cardiotoxicity by the following procedures: (a) the role of coronary flow was studied by relating coronary flow rates to myocardial mechanical performance; (b) the possible role of myocardial energy metabolism was evaluated by measuring the tissue content of high-energy phosphates and of cyclic AMP; (c) the possible toxic effect of anthracycline on mitochondria was indirectly studied by observing the effects of coenzyme Q$_{10}$, an agent thought to stabilize the mitochondrial membrane $(18)$; (d) release of lactate dehydrogenase into the perfusing medium was used as a nonspecific index of cell membrane damage; and (e) the possible role of calcium ions was studied by altering the perfusate calcium concentration or by administration of isoproterenol or digoxin.

MATERIALS AND METHODS

Isolated Working Heart. Hearts (excised from male Long-Evans rats; weight, 250 to 300 g) were rapidly arrested in Krebs-Henseleit buffer at 4°C. The hearts were preperfused via the aorta by the Langendorff method at a hydrostatic pressure of 65 cm H$_2$O for 15 min and were thereafter perfused by the left atrium $(21)$ for a further 75 min at a filling pressure of 10 cm H$_2$O, working against a hydrostatic pressure of 100 cm; 100 ml of perfusing medium were recirculated. The perfusate was a Krebs-Henseleit $(16)$ bicarbonate buffer equilibrated with 95% O$_2$ and 5% CO$_2$, with 11 mm glucose as external substrate, and with an ionic composition of NaCl, 118.5 mm; KCl, 4.7 mm; CaCl$_2$, 2.50 mm; KH$_2$PO$_4$, 1.19 mm; MgSO$_4$, 1.19 mm; and NaHCO$_3$, 25 mm. CaCl$_2$ $(5.0 \text{ mm})$ was obtained by doubling the CaCl$_2$ added to the solution. Aortic pressure was measured by a Statham P23DB pressure transducer and monitored on a Device M2 direct writer, Welwyn Garden City, Hertfordshire, United Kingdom. Heart work $(W)$ or power production $(13)$ was measured as the sum of pressure and kinetic power by:

\[
\text{Pressure power} = W_p = 0.002222 \times P_s \times CO
\]

\[
\text{Kinetic power} = W_k = \frac{1}{432 \times 10^2} \times \frac{\partial (CO)^2}{A^2} \times \frac{T}{T_0} \times (\frac{I}{I_0})^2
\]

Received February 24, 1982; accepted December 12, 1983.
where $W_p$ is pressure power, $P_s$ is peak systolic pressure, $CO$ is cardiac output, $W_k$ is kinetic power; $d$ is density of perfusate; $A$ is internal cross-sectional area of aortic cannula, $T$ is cycle time, and $T_e$ is ejection time.

Units are milliwatts (mJ/sec) for kinetic power, mm Hg for pressure power, ml/min for cardiac output, g/cu cm for density, and sq cm for area. The perfusate density was taken as 1 g/cu cm.

The efficiency of mechanical work was the total power production (pressure plus kinetic) divided by the oxygen uptake.

Release of lactate dehydrogenase from the heart was measured as the arteriovenous difference (8) at 5, 15, 30, and 60 min of left atrial perfusion.

The required concentrations of daunomycin and carminomycin ($1 \times 10^{-5} \text{ M and } 1.75 \times 10^{-5} \text{ M}$) were obtained in the perfusate by addition of a solution to the atrial perfusion at 20 min of working heart. Solutions were prepared from the mannitol-stabilized powder of daunomycin HCl and carminomycin HCl. In one series, rats were pretreated with coenzyme QIC (10 Mg/kg) via the tail vein at 2 hr, and again at 30 min before sacrifice, in 2 equally divided doses. In another series, rats were pretreated with 62.4 µg digoxin via the tail vein at 1 hr before sacrifice (4), when the mean serum concentration was $20 \pm 2.8 \text{ nmol/liter}$. Isoproterenol ($10^{-5} \text{ M}$) was added to the perfusate at 10 min before anthracycline was added.

At the end of the experiments, the hearts were freeze-clamped by aluminum tongs (34), cooked to the temperature of liquid nitrogen, and analyzed for ATP and phosphocreatine (23). Cyclic AMP was assayed by the method of Tovey et al. (30). Results were expressed in terms of the initial wet weight.

Drugs. Daunomycin and carminomycin were obtained from Maybaker, Port Elizabeth, South Africa, and Bristol-Myers Co., New York, NY, respectively.

Statistical Analysis. Results were expressed as mean ± S.E. Statistical analysis was by one-way analysis of variance or 2-way analysis of variance as indicated; $p$ values greater than 0.05 were considered not significant.

RESULTS

Control Working Heart. In control hearts, cardiac output and coronary flow rates were stable during the 75-min perfusion period that hearts were made to work (Chart 1). At 60 min, left ventricular power production and efficiency were the same as 15-min values (Chart 2). Heart rate was 244 ± 5 at 5 min but stabilized at between 220 and 230/minute after 15 min. Left ventricular stroke volume was 0.24 ± 0.01 ml at 5 min and stabilized at 0.26 ± 0.01 ml after 15 min. Peak aortic systolic pressure was not changed for the duration that hearts worked.

Oxygen consumption was 166 ± 4, 164 ± 6, and 153 ± 4 units/g/min at 15, 30, and 60 min. Phosphocreatine, ATP, and cyclic AMP values were 5.1 ± 0.8 µmol/g, 4.7 ± 0.2 µmol/g, and 0.40 ± 0.02 nmol/g, respectively (Table 1), at the end of the perfusion period (75 min).

Effect of Daunomycin and Carminomycin. Daunomycin and carminomycin, which were added 20 min after the onset of heart work, produced mechanical heart failure which was concentration related (Chart 1). Fifty-five min after daunomycin and carminomycin ($1.75 \times 10^{-5} \text{ M}$), cardiac output was reduced by 44 and 56% respectively. Left ventricular stroke volume was reduced by 42 and 56%, and peak aortic systolic pressure was reduced by 12 and 16%, respectively (Table 1). Heart rate did not fall, so that the decreased cardiac output was caused by a fall in the stroke volume. At 2 equimolar concentrations, carminomycin produced greater fall in cardiac output ($p < 0.01$) than daunomycin. Coronary flow rates were not reduced. Left ventricular power production and efficiency of work were reduced by...
Table 1

Effect of anthracyclines (55 min) and added CoQ10 on myocardial energy metabolism and mechanical function

<table>
<thead>
<tr>
<th></th>
<th>ATP (μmol/g, wet wt)</th>
<th>Phosphocreatine (μmol/g, wet wt)</th>
<th>Cyclic AMP (μmol/g, wet wt)</th>
<th>Peak systolic pressure (% of predrug value)</th>
<th>Stroke volume (ml)</th>
<th>Heart rate (beats/min)</th>
<th>Cardiac output (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.7 ± 0.2</td>
<td>5.1 ± 0.8</td>
<td>0.40 ± 0.04</td>
<td>101 ± 1</td>
<td>0.26 ± 0.01</td>
<td>223 ± 5</td>
<td>57.1 ± 0.8</td>
</tr>
<tr>
<td>Daunomycin</td>
<td></td>
<td></td>
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<tr>
<td>(n = 8-16)</td>
<td>5.2 ± 0.3</td>
<td>5.5 ± 0.4</td>
<td>0.40 ± 0.02</td>
<td>102 ± 2</td>
<td>0.24 ± 0.01</td>
<td>228 ± 10</td>
<td>52.9 ± 1.3</td>
</tr>
<tr>
<td>10^-6 x 1 M</td>
<td>3.1 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>0.35 ± 0.02</td>
<td>88 ± 3</td>
<td>0.15 ± 0.01</td>
<td>220 ± 9</td>
<td>32.0 ± 2.4</td>
</tr>
<tr>
<td>10^-5 x 1.75 M</td>
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<td></td>
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<tr>
<td>Carminomycin</td>
<td></td>
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<tr>
<td>(n = 8-16)</td>
<td>3.3 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>0.36 ± 0.02</td>
<td>97 ± 2</td>
<td>0.20 ± 0.01</td>
<td>219 ± 3</td>
<td>44.3 ± 1.6</td>
</tr>
<tr>
<td>10^-6 x 1 M</td>
<td>2.9 ± 0.2</td>
<td>4.6 ± 0.4</td>
<td>0.38 ± 0.02</td>
<td>86 ± 2</td>
<td>0.14 ± 0.01</td>
<td>196 ± 11</td>
<td>26.7 ± 2.0</td>
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<tr>
<td>10^-5 x 1.75 M</td>
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<tr>
<td>Coenzyme Q10 +</td>
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</tr>
<tr>
<td>carminomycin</td>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^-6 x 1.75 M</td>
<td>4.2 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>0.36 ± 0.02</td>
<td>84 ± 2</td>
<td>0.12 ± 0.01</td>
<td>214 ± 12</td>
<td>24.7 ± 2.3</td>
</tr>
</tbody>
</table>

* p < 0.05 versus control.
** p < 0.01 versus control.
*** p < 0.001 versus control.
^ p < 0.0001 versus control.
~ p < 0.001 versus Carminomycin 10^-6 x 1.75 M.
* p < 0.01 versus Carminomycin 10^-5 M at one.
- absence of data.

Table 2

Oxygen consumption and lactate dehydrogenase release related to cardiac output 40 min after anthracycline was added

<table>
<thead>
<tr>
<th></th>
<th>Oxygen consumption (μg/min)</th>
<th>Lactate dehydrogenase release (milliunits/g/min)</th>
<th>Cardiac output (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>153 ± 6</td>
<td>8.7 ± 1.3</td>
<td>57.5 ± 1.0</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>168 ± 3</td>
<td>7.6 ± 1.3</td>
<td>36 ± 2.6</td>
</tr>
<tr>
<td>10^-6 x 1 M</td>
<td>147 ± 8</td>
<td>5.0 ± 1.2</td>
<td>25.9 ± 2.1</td>
</tr>
</tbody>
</table>

* n = 13 to 16.  
# Mean ± S.E.
\% p < 0.001 versus control, statistical analysis by one-way analysis of variance.

Effect of Interventions. The following interventions were tested: (a) a higher perfusate calcium ion concentration (5 mM versus 2.5 mM); (b) addition of isoproterenol to the perfusate; and (c) pretreatment of rats with either digoxin or coenzyme Q10. The aim was to prevent the fall in cardiac output caused by carminomycin (1.75 x 10^-6 M decreased cardiac output to 44% of the control value at 55 min). Chart 3 shows that the cardiac output was restored towards normal by a high calcium, isoproterenol, and digoxin pretreatment, but not by pretreatment with coenzyme Q10. Of the successful interventions, calcium was the most effective with a cardiac output 91% of control value, fall in ATP was 34 or 38%, and the fall in phosphocreatine was 37 or 10% (not significant), respectively.

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whereas digoxin pretreatment gave 74% of the control. The high calcium treatment also partially prevented the fall in left ventricular power production and efficiency of work caused by carminomycin (Chart 4).

**Release of Lactate Dehydrogenase.** Low levels of enzyme release were found at 60 to 75 min after the onset of atrial perfusion; higher values in the first 15 min were probably related to the trauma of excision and mounting of the hearts (8). Neither daunomycin nor carminomycin increased enzyme release over the whole time course of perfusion; results at 75 min are shown in Table 1.

**DISCUSSION**

An early hypothesis for the acute myocardial contractile failure caused by anthracyclines was that of coronary vasosconstriction. Mhatre et al. (19) proposed that an anthracycline metabolite, which required blood for its production, was responsible for contractile failure. Yet, when their isolated hearts were perfused with a blood-free solution, the coronary perfusion pressure did not change. In our hearts perfused by an artificial medium, there was contractile failure even though the coronary flow did not fall (Chart 1). Since the preload (left atrial pressure) and afterload (height of perfusion column) were unchanged, and heart rate did not fall (Table 1), the acute heart failure could be ascribed to a reduced inotropic state rather than to any coronary vasoconstriction.

A disturbance of cellular energy stores could play a role, as suggested by recent studies with nuclear magnetic resonance in isolated rabbit hearts (12). Anthracyclines might impair the production of high-energy components by acting on the mitochondrial (24). In our hearts, the contractile failure produced by treatment with anthracyclines was associated with a fall in ATP, but it is unlikely that fall in ATP was the only factor causing the contractile failure, since coenzyme Q10 restored the high-energy phosphate compounds towards normal but did not improve mechanical function (Table 1).

Anthracyclines may depress the inotropic state by modulating the ambient cytosolic calcium concentration. This hypothesis was supported by our finding that procedures that increased cytosolic calcium relieved heart failure, namely, higher perfusate calcium and isoproterenol, or digoxin. A higher perfusate calcium could increase the inward flux of calcium and thereby elevate the cytosolic calcium ion concentration. Isoproterenol might do this by enhancing calcium entry by the slow inward current (28), thereby increasing the cytosolic calcium ion concentration. It is unlikely that anthracyclines altered calcium flux by modulating cyclic AMP, because the myocardial levels of the latter were unchanged.

We conclude that, in our model, anthracyclines lowered cytosolic calcium ion concentration and impaired the production of high-energy phosphates by separate mechanisms. It must be emphasized that our conclusions, based on the acute effects of anthracyclines on an in vitro heart preparation, may not necessarily be relevant to the situation in patients receiving anthracyclines.

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10. Doxorubin, J. H. Effect of anthracyclines in the production of high-energy components by acting on the mitochondria (24). In our hearts, the contractile failure produced by treatment with anthracyclines was associated with a fall in ATP, but it is unlikely that fall in ATP was the only factor causing the contractile failure, since coenzyme Q10 restored the high-energy phosphate compounds towards normal but did not improve mechanical function (Table 1).

11. Doroshow, J. H. Effect of anthracyclines on an in vitro heart preparation, may not necessarily be relevant to the situation in patients receiving anthracyclines.

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Cancer Res 1984;44:1316-1320.

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