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Effects of three new anthracyclines and doxorubicin on the rat isolated heart

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The acute cardiac toxicity of three second-generation anthracycline analogues and doxorubicin was compared in a model of the rat isolated Langendorff perfused heart. The drugs, doxorubicin (DX), 4-epi-doxorubicin (4'EDX), 4-demethoxy-daunorubicin (4DMDR) and 4'-deoxy-doxorubicin (4'dxDX) were infused for 40 min at a concentration of 26 μM into the isolated hearts. All four compounds significantly reduced cardiac work and its first derivative. The time to 50% decrease in work (TW50) was respectively 36, 23, 9 and 7 min for DX, 4'EDX, 4'dxDx and 4DMDR. The three anthracycline derivatives, but not DX, significantly increased coronary resistance. Heart rate was reduced by all compounds compared with baseline, but not compared with controls. Rhythm disturbances were seen with all five hearts perfused with 4DMDR, which stopped beating before 40 min; 2/5 hearts in the 4'EDX group and 1/5 hearts in the 4'dxDX group also stopped before the end of perfusion. All the compounds reached concentrations in the myocardium 8 to 50 times higher than in the perfusing medium. The more cardiotoxic the compound, the higher was its myocardial concentration; a significant correlation was found for all four agents. Noradrenaline was never measurable in the perfusate of control and DX hearts; perfusion with the three anthracycline derivatives caused some release, but the pattern was not clearcut and the maximum concentrations attained in the perfusate were relatively low ($\leq 1{\cdot}6 \times 10^{-9}$ M). In conclusion, in the rat isolated perfused heart, the early cardiotoxicity induced by equimolar concentrations of the three anthracycline compounds was greater than that induced by DX and was directly related to drug accumulation in the myocardium. Catecholamines do not seem to have a major role in the development of toxicity in this model.

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The characteristics of anthracycline-induced cardiac damage, which often limit the use of such drugs in patients, were reviewed by Lenaz & Page (1976). The cardiotoxicity of the anthracyclines is best divided into acute and chronic (Bristow et al 1978a; Doroshow et al 1979). The acute cardiac toxicity of anthracyclines in man can appear within hours or days after single or multiple treatment and includes a pericarditis-myocarditis syndrome, left ventricular dysfunction and arrhythmias; chronic toxicity is a severe cardiomyopathy dependent on the cumulative dose. Acute toxic effects of doxorubicin (DX), such as increased coronary resistance, ventricular arrhythmias and a decrease in cardiac performance, have been observed in several animal species, in-vitro and in-vivo, and in patients (Bristow et al 1978b). As these cardiac lesions have some similarities, a common mechanism was proposed for the acute and chronic toxicity of DX, in which the release of vasoactive substances, like histamine and catecholamines, is believed to play a role (Bristow et al 1980, 1981).

4'-epi-Doxorubicin (4'EDX), 4-demethoxydaunorubicin (4DMDR) and 4'-deoxydoxorubicin (4'dxDX) are three second generation antitumour anthracyclines. All three have a broad spectrum of antitumour activity (Casazza 1979; Bristow et al 1981; Casazza et al 1983; Ganzina 1983; Giuliani et al 1983; Arcamone et al 1984; Barbieri et al 1984) and have been used in phase II clinical trials. Preliminary experimental data show that some of the analogues, at equivalent antitumour doses, are less cardiotoxic than DX (Zbinden et al 1978; Casazza 1979; Giuliani et al 1983; Barbieri et al 1984). The experimental model was the rat or mouse, treated with different regimens of the compounds. We used a simple model of rat retrogradely perfused isolated heart in order to compare DX and three new analogues, at equimolar concentrations, for their specific acute cardiac effects, their uptake into myocardium and their effects on catecholamine release by cardiac sympathetic nerve endings.

Materials and methods

CD-COBS male rats, 392 ± 3 g, supplied by Charles River (Calco, Italy) were used. Twenty-five rats were randomly divided into five groups. Animals were anaesthetized with diethyl ether, sodium heparin (500 u) was injected intravenously and, 1 min later, hearts were excised. The aorta was then connected to a modified Langendorff apparatus and perfused retrogradely at a constant flow rate of 10·0 mL min^{-1} with a perfusion medium equilibrated with 95% O_2 and 5% CO_2 at 37 °C. The composition of the perfusate (mM) was: NaCl, 118; KCl, 4·69; $CaCl_2.2H_2O$, 2·50; KH_2PO_4, 1·19; $MgSO_4.7H_2O$, 1·20; $NaHCO_3$; 25·0; glucose, 11·0. These constituents were dissolved in bidistilled water and filtered just before the experiment. The vertical apicobasal shortening of the hearts was measured with a displacement transducer loaded with 4 g (7006 Isotonic Transducer, Basile, Italy) connected to a 8-channel physiological recorder (Sistema, ESO 300VP, Battaglia Rangoni, Bologna, Italy). Work, first derivative of work (dW/dt), coronary perfusion pressure (pressure transducer, Gould P23 ID, USA) and heart rate were measured. The four parameters of cardiac function were recorded 10 and 15 min after connecting the heart to the Langendorff apparatus. At the end of the 15 min stabilization period, the drug solutions in 0·16 M glucose were infused into the perfusion medium to reach a final concentration of 26 μM (about 15 μg mL^{-1}). The hearts were perfused for another 40 min during which the parameters of cardiac function were recorded every 10 min. At 0, 1, 20 and 40 min the perfusate was collected and catecholamines released from the hearts were measured by HPLC, with electrochemical detection (Goldstein et al 1981). The concentrations of drugs in myocardium were measured at the end of the perfusion time, after homogenization of the whole heart, by HPLC methods described elsewhere (Israel et al 1978; Andrews et al 1980) and adapted to our conditions (Broggini et al 1980). Extraction recovery was similar for all compounds and averaged 85 ± 2%.

Results are expressed as mean ± standard deviation (s.d.). Data were analysed by analysis of variance, followed by Tukey's test for multiple comparisons; the program runs on an HP-85 (Hewlett Packard) desk computer (Rocchetti & Recchia 1982).

Results

The five groups of animals had similar baseline values of contractility and coronary resistance. Basal work was 1104 ± 90 erg in controls; 1152 ± 48 erg in DX; 1098 ± 96 erg in 4'EDX; 1152 ± 138 erg in 4DMDR; 1074 ± 198 erg in 4'dxDX. Perfusion pressure was 43 ± 4 mmHg in controls; 44 ± 2 mmHg in DX; 42 ± 4 mmHg in 4'EDX; 46 ± 6 mmHg in 4DMDR; 43 ± 9 mmHg in 4'dxDX. Perfusion pressure increased (Fig. 1) whereas work (Fig. 2) and its first derivative (dW/dt) decreased significantly starting from 10 min after the beginning of perfusion in 4'dxDX and 4DMDR groups and from 20 min in DX and 4'EDX groups. DX seemed to affect these variables less than the other compounds. The times to 50% decrease in work (TW50), calculated by graphic interpolation, were 36, 23, 7 and 9 min respectively for DX, 4'EDX, 4DMDR and 4'dxDX. TW50 of DX was significantly longer than all the others, 4DMDR and 4'dxDX TW50 were significantly lower than the others, but not different from each other.

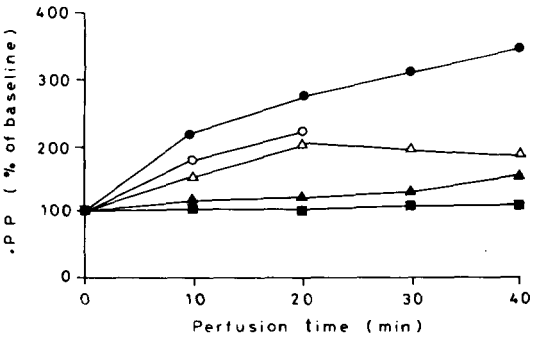

FIG. 1. Effect of anthracyclines on perfusion pressure (PP) of rat isolated heart (■——■ controls, ▲——▲ DX, ●——● 4'EDX, ○——○ 4DMDR, △——△ 4'dxDX).

Baseline heart rate values were similar in all groups: 292 ± 11 beats min^{-1} in controls: 330 ± 29 beats min^{-1} in DX; 308 ± 11 beats min^{-1} in 4'EDX; 301 ± 19 beats min^{-1} in 4DMDR and 311 ± 24 beats min^{-1} in 4'dxDX. Heart rate decreased slightly (10%), but not significantly in the control group during perfusion, whereas significant changes from baseline, but not from controls, were found as early as 10 min after perfusion with DX and 20–30 min after with the other compounds. Some hearts stopped beating before the end of the

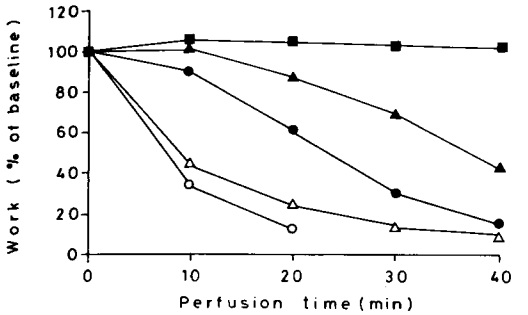

FIG. 2. Effect of anthracyclines on work of rat isolated heart (symbols as in Fig. 1).

Table 1. Noradrenaline concentrations* (pg mL^{-1}) in the perfusate outflowing from the hearts.

	Perfusion time (min)			
Drug	0	1	20	40
C	n.m.	n.m.	n.m.	n.m.
DX	n.m.	n.m.	n.m.	n.m.
4DMDR	n.m.	n.m.	104·54 ± 14·1**	***
4'dxDX	n.m.	n.m.	37·3 ± 32·9	29·92 ± 29·24
4'EDX	31·7 ± 27·5	45·8 ± 54·9	21·4 ± 37·1	33·2 ± 29·0

* Release rates (pg min^{-1} g^{-1}) can be calculated from a constant flow of 11 mL min^{-1} and an average heart weight of 1·37 ± 0·05 (s.d.) g.
** Mean ± s.d.
*** 2 out of 3 hearts stopped before 40'.
n.m. = <20 pg mL^{-1}.

perfusion: 5/5 for 4DMDR, 2/5 for 4'EDX and 1/5 for 4'dxDX. Rhythm disturbances were observed in all hearts perfused with 4DMDR, within 10–20 min, shortly before they stopped beating.

Mean myocardial concentrations of the four compounds at the end of perfusion were 0·2 ± 0·01 μmol g^{-1} (100 μg g^{-1}) of wet tissue for DX; 0·3 ± 0·003 μmol g^{-1} (150 μg g^{-1}) for 4'EDX; 1·3 ± 0·2 μmol g^{-1} (652 μg g^{-1}) for 4DMDR and 0·5 ± 0·2 μmol g^{-1} (240 μg g^{-1}) for 4'dxDX. Myocardium/perfusion medium partition ratios ranged from 7·7 to 50·4 for DX and 4DMDR, respectively. Myocardial concentrations of anthracyclines were inversely correlated with the heart work measured after 20 min of perfusion ($r^2 = 0·75$, $P < 0·0003$) (Fig. 3).

Measurable concentrations of noradrenaline (NA) (31–246 pg mL^{-1}) were found as early as 1 min after starting drug perfusion, in the effluent from hearts perfused with the three anthracycline derivatives, but not in the DX group or controls (NA concentration < 20 pg mL^{-1}). NA was also measurable during the predrug period in 3/15 experiments; adrenaline was never measurable (Table 1).

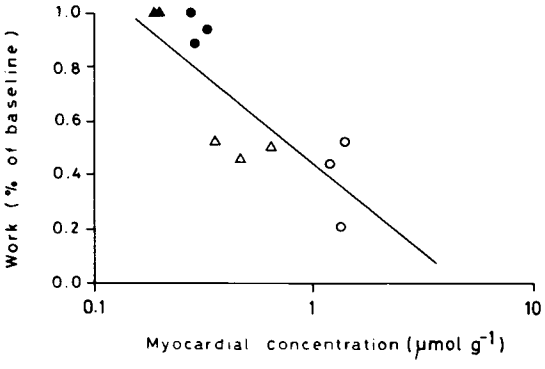

FIG. 3. Correlation between work at 20 min and anthracycline concentration in rat perfused heart (symbols as in Fig. 1); $r^2 = 0·75$, $P < 0·0003$.

Discussion

The acute cardiac toxicity of DX, 4'EDX, 4DMDR and 4'dxDX were comparatively evaluated in the rat isolated heart, retrogradely perfused; this model has already been used under similar experimental conditions (de Wildt et al 1985). We perfused the hearts at a constant flow and not at a constant pressure in order to study the anthracyclines' direct effects on the heart, not mediated by relative hypoxia due to the progressive increase in coronary resistance caused by these compounds, as has been shown for DX and daunomycin in dog (Vick & Herman 1971) and rat isolated perfused hearts (Julicher et al 1986). The concentration of anthracyclines in the perfusate (26 μM, corresponding to 15 μg mL^{-1}) was chosen from our preliminary experiments with DX, which indicated significant, but not maximal toxicity. Similar, or even higher, concentrations have been used to perfuse rat hearts with carminomycin and daunomycin (Saman et al 1984) and DX (Julicher et al 1986). Equimolar concentrations were used to compare the intrinsic specific toxicity of the four compounds, independently of their action on other systems, which may not parallel the cardiac effects. At this concentration, all the anthracyclines studied caused cardiac toxicity. Using TW50 as a semiquantitative index of cardiac damage, 4DMDR and 4'dxDX were the more toxic compounds. Their toxicity appeared to be related to a higher uptake by the heart.

In fact, we found a very good correlation between myocardium concentrations of all four anthracyclines and decrease in cardiac work (Fig. 3). The partition ratio between myocardium and perfusate in-vitro is similar to the myocardium/plasma ratio observed in mice at peak concentration after a single i.v. bolus (Broggini et al 1980, 1984; Formelli et al 1981). The lower absolute values found in-vivo are probably due to plasma protein binding, occurring in-vivo but not in-vitro.

These results indicate that the affinities for myocardium of the four compounds tested are different, being highest for 4DMDR, and that these differences may well explain differences in cardiac toxicity obtained

using equimolar medium concentrations, as is usually done in experimental pharmacology. Therefore, cardiac toxicity can be confidently considered similar if different myocardial concentrations are taken into account. This is important, in order to interpret the data obtained in-vivo in relation to ours. In fact the effective antitumour doses commonly used in mice and rats for 4DMDR and 4'dxDX are 3 to 8 times lower than those of DX and 4'EDX (Casazza 1979; Casazza et al 1983; Giuliani et al 1983; Broggini et al 1984). This might explain the better benefit/risk ratio in animals (Casazza 1979) and possibly in man (Berman et al 1983; Arcamone 1984) found by some authors for 4'dxDX and 4DMDR, usually expressed in a higher ratio between cardiotoxic and antitumour doses.

NA release rates of the hearts perfused with the three anthracycline derivatives ranged from a minimum of 242 to a maximum of 2054 pg min^{-1} g^{-1} of wet tissue. The pattern of release was highly variable and not even consistent within each experiment. Higher release rates corresponded to the development of toxic effects (cardiac arrest or marked reduction in contractility). In any case, NA concentrations in the perfusate were never higher than $1{\cdot}6 \times 10^{-9}$ M, which should be sub-threshold for cardiac effects in our model. NA release thus appears to be a consequence, more than a cause or mediator, of the observed cardiac toxicity. Data in the literature about the role of catecholamines in mediating acute cardiac toxicity of DX are contrasting. Bristow et al (1981) showed early, statistically significant rises in arterial NA concentrations in rabbits after a single i.v. bolus of DX. Two criticisms can be made of this finding: first, the rises reported, from 300–400 to 400–500 pg mL^{-1} were statistically but not physiologically signifcant and, second, the increase in NA could be due to non-cardiac effects of DX. Our findings are in agreement with those of Herman et al (1972), who could not prevent the acute cardiac toxicity of DX by pretreating dog isolated hearts with β-blockers. Data by Jackson et al (1984) who found no acute changes of catecholamine content in myocardium of rabbits treated with a single bolus of DX also back our conclusions.

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