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PROTECTIVE EFFECTS OF SPIN-TRAPPING AGENTS ON ADRIAMYCIN-INDUCED CARDIOTOXICITY IN ISOLATED RAT ATRIA

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Adriamycin (ADR) is known to exert a severe negative inotropic effect on isolated myocardial preparations; a role for free radical generation has been hypothesized. Spin-trapping of free radicals has been extensively exploited in ESR studies, both in cell-free systems and in intact tissues. The interaction between spin-traps and free radicals should in principle stop the reaction cascade leading to cellular damage. Based on this hypothesis, the possible cardioprotective action of three spin-trapping agents, 5,5-dimethyl-l-pyrroline-N-oxide (DMPO), N-*tert*-butyl-α-phenylnitrone (PBN) and α-(4-pyridyl l-oxide) N-*tert*-butylinitrone (POBN), was tested on isolated rat atria incubated in the presence of ADR; maximal non-cardiotoxic concentrations were used (50, 10 and 50 mM respectively) in order to achieve a maximal spin-trapping effect. A varying degree of protection was observed with the three compounds, directly correlated to their hydrophobicity, as assessed by chloroform/water partition coefficients. It is proposed that ADR-induced free radical generation is responsible for the acute cardiotoxic effects of the drug; this seems to be a site-specific mechanism restricted to one or more hydrophobic cellular compartment/s, since only lipophilic spin-trapping agents are able to prevent the development of the negative inotropic effect of ADR.

KEY WORDS: Adriamycin, cardiotoxicity, free radicals, spin-traps.

INTRODUCTION

It is generally accepted that the typical pathology acutely induced by adriamycin (ADR) in myocardial tissue, mainly consisting in a progressive impairment of the contractile force, is associated with oxygen free radical generation. According to most Authors, ADR is reduced to the corresponding semiquinone by a one-electron transfer reaction catalyzed by NADPH-P_{450} reductase or by a specific NADH-dependent oxidoreductase.[1,2] ADR semiquinone subsequently reacts with molecular oxygen to yield superoxide anion, which eventually leads to production of hydrogen peroxide and of hydroxyl radicals by a metal-catalyzed Fenton reaction (for a review see [3]). These active oxygen species might produce cardiotoxic effects by lipid peroxidation of the sarcolemmal membrane and/or of the membranes of intracellular organelles;[4–10] this would lead to disruption of the ionic balance which is necessary for excitation-contraction coupling in myocardial cells. However, it has not been definitely established as yet whether oxygen free radicals are cause or consequence of ADR-induced cardiotoxicity, or whether they merely represent a by-product devoid of pathological

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relevance. Investigations aimed to assess the protective effects of antioxidants and radical scavengers also failed to provide conclusive evidence on this topic, and conflicting results have been reported, possibly due to a wide variability in the experimental conditions and doses adopted. In the present study we investigate the effects of different spin-trapping agents on the ADR-induced acute cardiotoxic effects. These compounds can form stable adducts with radicals in intact tissues as well as in cell-free systems, thus allowing the detection of free radicals. On the other hand, this same reaction should stop the free radical flow through the reaction cascade leading to cellular damage, and therefore should afford some degree of protection. Spin-trapping agents may interact with most radical species generated by DXR activation, which may represent an advantage over the use of enzymatic antioxidants, such as superoxide dismutase, catalase or glutathione peroxidase, acting on strictly specific substrates.

In the experimental conditions adopted, the spin traps used were devoid of intrinsic pharmacological actions; therefore, the effects observed in ADR-treated organs are probably related to the trapping of the free radicals produced by the antibiotic. We studied the effects of three different spin-traps, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), N-*tert*-butyl-α-phenylnitrone (PBN) and α-(4-pyridyl 1-oxide) N-*tert*-butylnitrone (POBN), The widely different oil/water partition coefficients of the compounds tested might enable them to gain access to different cellular compartments, thus providing a useful clue to the specific site/s of ADR action involved in this effect.

MATERIALS AND METHODS

Adriamycin hydrochloride was kindly supplied by Farmitalia-C.Erba (Milano); DMPO, PBN and POBN were purchased from Aldrich Chemicals.

Spontaneously beating atria were isolated from female Sprague Dawley rats weighing 130–150 g and were allowed to equilibrate for 30 min in a modified Tyrode solution of the following mM composition: NaCl 137.0, KCl 5.4, $MgCl_2$ 0.51, $NaHCO_3$ 12.0, NaH_2PO_4 4.7, $CaCl_2$ 1.8, glucose 11.0, pH 7.4. The medium was thermostatted at 37°C and saturated with a O_2–CO_2 mixture (95:5%). At the end of the equilibration period, appropriate volumes of aqueous solutions of DMPO or PBN or POBN were added to the final concentrations of 50, 10 and 50 mM respectively; these concentrations were chosen to maximize spin adduct formation while preserving myocardial function. After 30 min pre-incubation with the spin-traps, ADR was added to the final concentration of 100 μg/ml. The contractile response of the preparations was continuously recorded for 60 min by means of an isometric force transducer; the isometric tension (F) developed by the preparations was measured, as well as the maximal rate of tension development and relaxation, $(\pm)\ dF/dt$.

The partition coefficients of the three agents were measured by a gravimetric assessment of the amount of substances present in the aqueous and chloroformic phases, after allowing the achievement of a steady state between the two phases.

Statistical analysis of the inotropic effects developed by the different agents tested was performed at selected time points (15, 30, 45 and 60 min after adding ADR to the incubation medium) by the analysis of variance, with Duncan's multiple range test for multiple comparisons (significance level: $p = 0.05$).

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RESULTS AND DISCUSSION

The contractile performance of isolated atria was measured both as the isometrically developed tension and as the maximal rate of tension development/relaxation. Similar alterations were observed for all these parameters following a 60 min incubation in the presence of ADR; therefore, only $(+)dF/dt$ values were analysed further and reported in the figures. $(+)dF/dt$ is considered a representative index of contractile processes, having been shown to parallel the myofibrillar Ca-ATPase activity[11] and the isomyosin pattern.[12]

Figure 1 shows that ADR induces a progressive impairment of the contractile processes, reducing $(+)dF/dt$ to 48.9% of the baseline value after 60 min. The same figure shows the results obtained with the three spin-traps tested. When used alone, they do not induce any significant alterations of the contractile performance observed in control preparations (data not shown). In the presence of ADR, DMPO does not prevent the impairment of contractile force induced by the anthracyline. In contrast, PBN is able to achieve a significant degree of protection against ADR-induced impairment of contractility throughout the observation period; by the end of the 60 min incubation in the presence of ADR, PBN-treated preparations retained an average 77.5% of the baseline contractility. POBN also develops a significant protective activity, albeit to a lesser extent as compared to PBN (63.4% residual contractility after 60 min).

Taken together, the results of the present experiments indicate that free radicals play a causal role in the development of the functional manifestations of acute DXR cardiotoxicity, since spin-trapping substances are able to prevent, at least in part, the specific pathology when used at concentrations in the range currently employed for ESR detection of radical adducts in tissues. Actually, a cardioprotective effect has been described against ischemia/reperfusion damage in the intact dog at PBN concentrations lower than those employed in this study;[13] such discrepancies may be accounted for by profound differences in the experimental models adopted.

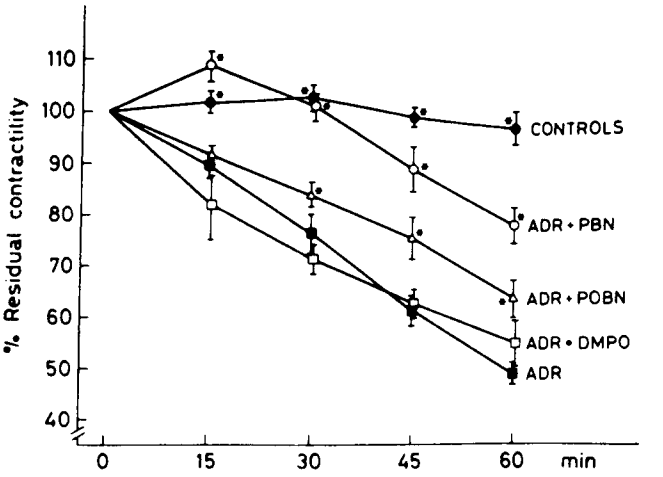

FIGURE 1 Effect of the spin-trapping agents DMPO (50 mM), PBN (10 mM) and POBN (50 mM) on the contractile impairment produced by ADR (100 μg/ml) on isolated rat atria. Each data point is the mean ± s.e. of 5–6 values. (*) $p < 0.05$ vs. ADR.

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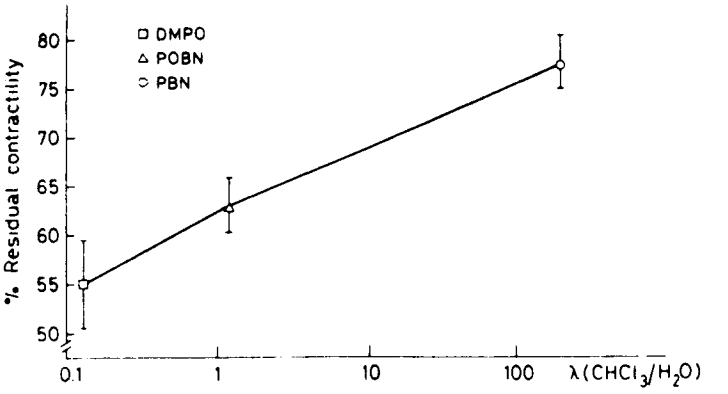

FIGURE 2 Relationship between residual contractility (% of baseline values) of isolated rat atria after 60′ incubation with ADR (100 μg/ml) and DMPO (50 mM) or PBN (10 mM) or POBN (50 mM) and the chloroform/water partition coefficient (λ) of the three spin-trapping agents. ADR alone reduced the contractile response to 48.9% of the baseline value after 60′ incubation (see Figure 1). Each data point is the mean ± s.e. of 5–6 determinations.

A tentative explanation for the marked differences observed in the protective activity displayed by the different compounds might be provided by their different chloroform/water partition coefficients. A value of 0.13 was found for DMPO and of 199.0 for PBN, whereas POBN showed an intermediate value of 1.19. Figure 2 shows that a good correlation exists between cardiac protection and hydrophobic nature of the molecule ($r = 0.963$).

The present findings might provide a novel interpretation for the observations reported by other authors,[14] who found DMPO-radical adducts in the perfusate of ADR-treated isolated hearts. The ineffectiveness of DMPO as a cardioprotective agent observed in the present study, in spite of the active spin-trapping effect reported at the concentration adopted (50 mM), might be explained assuming that DMPO, due to its hydrophilic nature, is able to interact with a population of oxygen radicals which is located in the aqueous (cytosolic) cell compartment and which probably does not play a pathogenetic role in ADR-induced effects. In contrast, the lipophylic properties of POBN and especially PBN would enable them to exert their radical-trapping effects at membrane sites of ADR action, which might be responsible for the observed cardiotoxicity.

Based on the present findings and on data obtained by other investigators on cultured embryonic chick heart myocytes[15] and on isolated rat heart,[16] it can be proposed that ADR and the protective spin-traps PBN and POBN share a common site of action, which is probably located in or around nuclei or mitochondria. Further experiments are required to obtain a better insight into this problem.

On the whole, the present investigations provide the first direct evidence of a site-specific generation of free radicals in ADR cardiotoxicity. A site-specific mechanism might also explain the high spin-trap concentrations required to observe a cardioprotective effect in this experimental model. Marked differences seem to exist between different radical-induced (or radical-producing) cardiac pathologies. In fact, a protective effect has been described for both PBN and DMPO against reperfusion arrhythmias in isolated rat hearts,[17,18] whereas DMPO proved completely devoid of effect in our model.

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As to ADR-induced acute cardiotoxicity, the highly artificial experimental settings adopted for the present study do not allow any definite extrapolation to the "*in vivo*" situation. However, these findings might serve as guidelines for further investigations on the "*in situ*" heart; should these observations be confirmed in more physiological experimental conditions, the use of new spin-trapping compounds, designed to be more cell permeant, more lipophylic and less toxic than those which are now available, might be suggested as a novel approach to the prevention of the cardiotoxicity induced by DXR.

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