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PHARMACOLOGICAL ACTION OF A NEW SPIN TRAPPING COMPOUND, 2-PHENYL DMPO, IN THE ADRIAMYCIN-INDUCED CARDIOTOXICITY

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Adriamycin (ADR)-induced cardiotoxicity was adopted in this investigation as a reliable model of radical-dependent myocardial pathology allowing both quantitative studies of drug activity in the isolated organ and *in vivo* comparison of the cardio-protection vs. general toxicity. Since commercially available lipophilic spin trapping compounds were shown to develop significant protective activity, in this investigation a newly synthesized spin trap (2-phenyl-DMPO) was studied. In Langendorff rat heart, 200 μM ADR induced a significant impairment of contractile performance, while 2-phenyl-DMPO was not cardiotoxic up to the 5 mM concentration. By this dose, 2-phenyl-DMPO induced a significant protection against the ADR-induced contractile impairment. In *in vivo* experiments, ADR (9 mg/kg i.v.) produced a significant impairment of ECG, coronary flow and contractility. The continuous administration of 2-phenyl-DMPO i.p. by osmotic pump delivering 0.3 μmol/hr was unable to protect the animals against the cardiotoxic signs. Seven days after ADR administration, severe general toxicity (arrest of body weight increase) and myelotoxicity were also observed. 2-phenyl-DMPO was unable to protect the animals from these toxic signs. The present results confirm that lipophilic spin traps can be a new class of antiradical drugs, as confirmed by the experiments performed in the isolated heart with the 2-phenyl-DMPO; however, this last compound is probably metabolized *in vivo* to inactive derivatives.

INTRODUCTION

Numerous investigations have suggested that the generation of oxyradicals is causally involved in the development of different myocardial pathologies, including the postischemic reperfusion and the adriamycin (ADR)-induced cardiotoxicity. This last pathology represents a reliable experimental model for the study of antiradical interventions, since ADR is a typical radical-producing drug. In fact, ADR is reduced to the semiquinone by a one-electron transfer reaction catalyzed by NADPH-P_{450} reductase or, in myocardial tissue, by a specific NADH-dependent oxidoreductase.[1,2] ADR semiquinone in turn reacts with molecular oxygen to yield superoxide and hydroxyl radicals by a metal-catalyzed Fenton reaction.[3] Oxyradicals might initiate

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lipoperoxidation of the cell membranes,[4,5] which could account for the electrical and contractile impairment observed in ADR-treated heart. This view is supported by the observation that radical-generating systems can produce myocardial dysfunctions similar to that of ADR[6–9] and that an efficient cardioprotection was obtained by action of lipophilic spin traps, while hydrophilic compounds were inactive.[11–13]

Since the systems devoted to the detoxification of free radicals have a limited capacity in myocardium, the development of effective radical scavengers may be of paramount interest for cardioprotection. In the present investigation, pharmacological studies were performed on a newly synthetized lipophilic spin trap, 2-phenyl DMPO (2-Ph-DMPO).[14]

MATERIALS AND METHODS

Adriamycin (ADR) was kindly supplied by Farmitalia-C. Erba (Milano). 2-Phenyl-DMPO was synthetized in one of these laboratories.[14]

The partition coefficient chloroform/water was measured by a gravimetric assessment of the amount of substance present in the aqueous and in the organic phases, after achieving a steady state between the two phases and evaporating a volume of each to dryness.

In vitro studies Spontaneously beating atria were isolated from male Sprague Dawley rats of 150–170 g body weight. After 30 min equilibration in a modified Krebs Henseleit solution (mM composition: NaCl 137; KCl 5.4; $MgCl_2$ 1.2; $CaCl_2$ 1.8; $NaHCO_3$ 12.0; NaH_2PO_4 0.47; glucose 11) pH 7.4, saturated with O_2-CO_2 (95:5%) at 37°C, 2-Ph-MPO was added to a final concentration 5 mM, which was chosen for maximizing spin adduct formation while preserving myocardial function. ADR (20 μM) was added and the contractile response of the preparation continuously recorded for 60 min by means of an isometric transducer. The isometric tension (F) and the maximal rate of tension development (dF/dt) were measured, as well as the heart rate (HR). Groups of 6 organs were used.

In vivo studies Groups of male Sprague Dawley rats of 150–170 g body weight were used. 2-Ph-DMPO was inserted into an osmotic pump (ALZET 2ML2: Charles River, Milano) at 50 mM concentration in water solution, thus delivering 0.3 μM mol/hr. This concentration was chosen because it is the highest dose which does not produce cardiac or general toxicity. The pump was implanted in the peritoneum in sterile conditions and left in place for 2 weeks; the animals received 1.8 μmol/hr per kg body weight. In the group receiving both ADR and the spin trap, this was administered by inserting the osmotic pump 3 days before iv injection of 9 mg/kg ADR. An ECG sign of cardiotoxicity (QαT duration)[15] was measured before and 7 days after ADR administration. At this time, animals were stunned and heart was isolated as previously described[13] and Langendorff-perfused under standard conditions. Groups of six animals were used. Left ventricular developed pressure and its rate of development (dp/dt), HR and the rate pressure product (RPP) as well as the coronary flow (CF) were measured.

Statistical analysis The experimental values were analyzed by ANOVA.

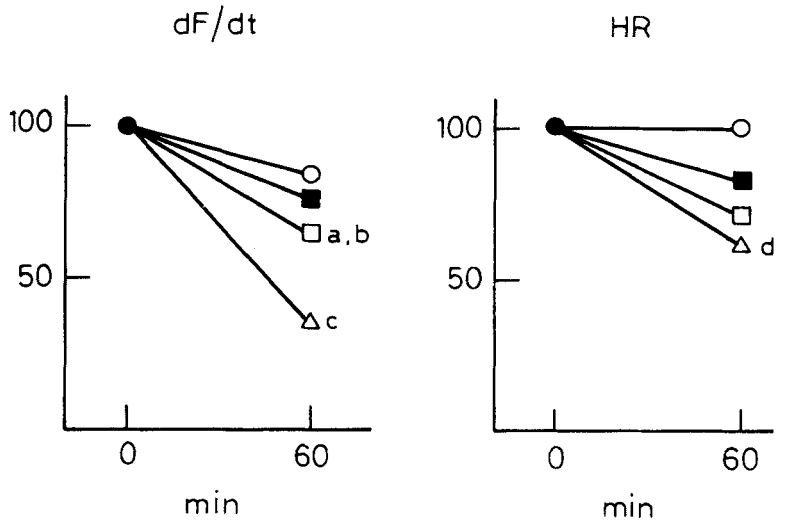

FIGURE 1 Effect of 2-Ph-DMPO on contractile parameters of isolated rat atria incubated with 200 μM ADR. a: $P < 0.05$ vs controls; b: $P < 0.05$ vs ADR; c: $P < 0.05$ vs all groups; d: $P < 0.05$ vs controls and 2-Ph-DMPO;
○ controls; △ ADR; ■ 2-Ph-DMPO; □ ADR+ 2-Ph-DMPO

RESULTS

Figure 1 shows the results of the experiments performed by incubating the organs *in vitro* at 5.0 mM concentration of 2-Ph-DMPO. Under these experimental conditions, HR and contractile performance can be considered to remain within a normal range. Higher concentrations were not extensively studied, because preliminary experiments showed that cardiotoxic effects would appear. ADR develops a dose-dependent and time-dependent inotropic and chronotropic effect. After 1 hr incubation, 200 μM ADR induced a significant decrease of HR and of contractile force (Figure 1), which were antagonized in part, but at a significant level, by 2-Ph-DMPO. In fact, in the presence of the spin trapping compound, dF/dt was significantly higher than that of the ADR group and also HR was (not significantly) better than the value of the ADR group.

The cardioprotective activity exerted by 2-Ph-DMPO was not observed when the compound was administered *in vivo*. ADR administered iv in the rat brought about significant signs of general toxicity, such as decrease of the body weight curve and of the peripheral leukocytes (Figure 2). Administration of 2-Ph-DMPO alone did not modify significantly the values of control animals, and was unable to antagonize significantly the effects of ADR (Figure 2). Also the cardioprotection, which was observed *in vitro* in the isolated rat atria, was not present after *in vivo* administration. This was apparent from the behaviour of non-invasive ECG parameters (QαT duration) as measured 7 days after treatment. According to previous data, ADR induced a significant enlargement of the Qαt interval, while the combination of 2-Ph-DMPO and ADR did not modify the pathological value of ADR (Figure 3). Also the mechanical performance of the heart isolated from animals treated with ADR was not improved by the combined treatment with 2-Ph-DMPO; some of the recorded parameters were even worse than following ADR alone.

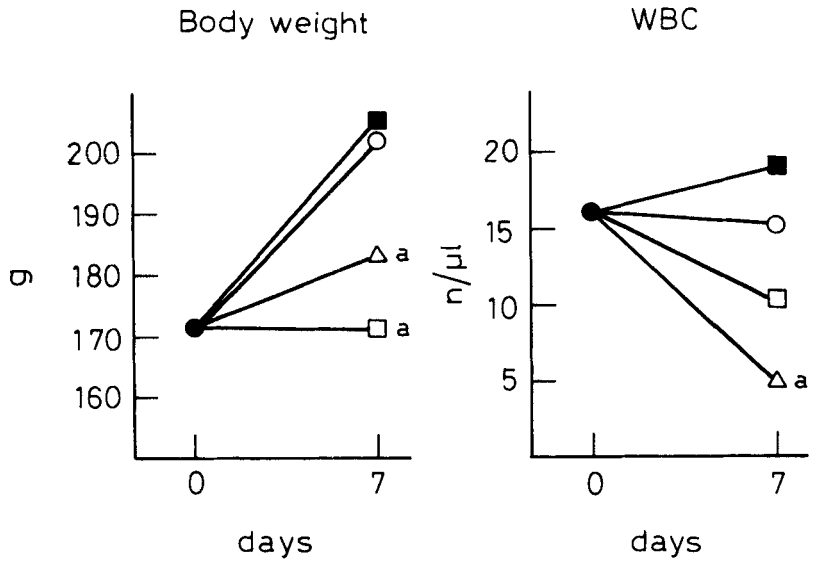

FIGURE 2 Effect of 2-Ph-DMPO on general toxicity of ADR. Values measured before and 7 days after ADR administration. a: $P < 0.05$ vs controls and 2-Ph-DMPO
○ controls; △ ADR; ■ 2-Ph-DMPO; □ ADR+ 2-Ph-DMPO

DISCUSSION

The present data confirm the results of previous investigations showing that a typical radical-dependent pathology, such as the ADR-induced cardiotoxicity, can be efficiently prevented or antagonized by spin traps. PBN was extensively studied. A significant protection was observed both in *in vitro* studies and after *in vivo* administration,[11,13] in acute cardiotoxicity as well as in a model of delayed ADR cardiotoxicity.[16] However, two conditions had to be respected for getting the protective effect against ADR cardiotoxicity. One of them is a general requirement and the other is of interest only in the case of *in vivo* treatment. First, the spin trapping compound must be able to cross the cell membrane and to penetrate into the cardiomyocytes thus reaching the intracellular site where ADR generates radicals. Previous studies showed[11] that the hydrophilic spin trap DMPO is devoid of cardioprotective activity against ADR cardiotoxicity and that a correlation exists between the protective effect *in vitro* and the chloroform/water partition coefficient of a series of three different spin trapping compounds. This is probably because DMPO resides in the cytosolic compartment of the cell,[12] while the lipophilic spin traps partition into the mitochondria, which is the most important site of radical production in the heart treated with ADR.[2] The second condition must be fulfilled when the protective compound is administered *in vivo*. Preliminary experiments showed that the injection of very high doses of PBN (up to 15 mg/kg × 2 i.p.: unpublished data) did not develop any protective effect, while the continuous administration of the spin trap (by an osmotic pump) was effective. This is related to the long residence time of ADR in myocardium (more than 2 weeks in the rat[17]) which enables ADR to generate radicals for a corresponding time span. Since spin trapping compounds are cleared from the body with a rate more rapid than

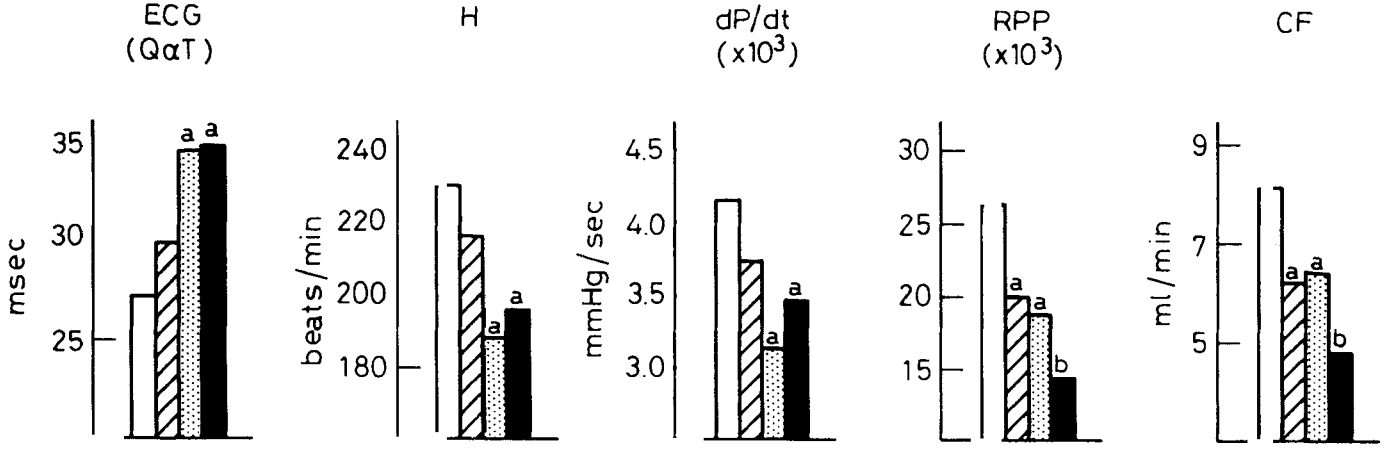

FIGURE 3 Effect of 2-Ph-DMPO on functional parameters f myocardial performance measured 7 days after ADR administration. a: $P < 0.05$ vs controls; b: $P < 0.05$ vs all groups
□ controls; □ ADR; □ 2-Ph-DMPO; ■ ADR+ 2-Ph-DMPO

ADR (t½ for PBN about 2.5 hrs[18]) they can counteract the ADR effects only if they are administered by a schedule allowing their continuous presence during the residence time of ADR in the myocardium. This experimental condition allows mimicking *in vivo* the situation occurring in the isolated organ, in which ADR and the spin trap are mutually interacting throughout the experimental time.

In the isolated myocardial preparation used in this study, 2-Ph-DMPO was able to develop a cardioprotective effect about as potent as PBN.[11] This is in line with the lipophilic properties of the two compounds: the chloroform/water partition coefficient is 199 for PBN and 8 for 2-Ph-DMPO. Presumably, 2-Ph-DMPO might be more active than PBN, if it could be administered at the same concentration. However, the 5-member ring structure present in 2-Ph-DMPO apparently adds more toxicity to the molecule than the aliphatic tail of PBN. At variance from PBN, which was shown to produce a significant cardioprotection also in *in vivo* experiments,[13,16] 2-Ph-DMPO was completely inactive after continuous administration of a comparable dose by i.p. route, and even some of the measured parameters evidenced the presence of a cardiotoxic effect. The most likely explanation for the different behavior of PBN and of 2-Ph-DMPO is that PBN either is not metabolized by the rat, or it generates active, non-toxic metabolites; on the contrary, 2-Ph-DMPO apparently generates inactive and toxic metabolites.

A further outcome of this investigation is that the need for lipophilic properties for obtaining a protective effect on the contractile impairment in radical-related myocardial pathologies is a general requirement which applies, in addition to the ADR cardiotoxicity, also to the contractile failure of ischemic and reperfused heart. This was observed by using PBN or DMPO in Langendorff ischemic and reperfused rat heart and was confirmed in this study. In fact, 2-Ph-DMPO develops a significant cardioprotective effect in ischemic and reperfused hearts (data not shown); DMPO was inactive,[19] while PBN developed a significant effect.[20]

On the whole, the present study and the literature data support the view that lipophilic spin traps might be a new class of drugs for the prevention of radical-related pathologies.

References

1. P.J. Thornalley and N.J.F. Dodd (1985) Free radical production from normal and adriamycin-treated rat cardiac sarcosomes. *Biochemical Pharmacology*, **34**, 669–674.
2. H. Nohl (1988) Identification of the site of adriamycin activation in the heart cell. *Biochemical Pharmacology*, **37**, 2633–2637.
3. C.E. Myers (1988) Role of iron in anthracycline action. In *Organ directed toxicity of anticancer drugs* (eds. M.P. Hacker, J.S. Lazo and T.R. Tritton), Martinus Nijhoff Publ., Boston, pp. 17–30.
4. P.K. Singal, C.M.R. Deally and L.E. Weinberg (1987) Subcellular effects of adriamycin in the heart: a concise review. *Journal of Molecular and Cellular Cardiology*, **19**, 817–827.
5. E.A. Griffin-Green, M.M. Zaleska and M. Erecinska (1988) Adriamycin-induced lipid peroxidation in mitochondria and microsomes. *Biochemical Pharmacology*, **37**, 3071–3077.
6. K.P. Burton, J.M. McCord and G. Ghai (1984) Myocardial alterations due to free radical generation. *American Journal of Physiology*, **246**, H776–783.
7. K.P. Burton (1988) Evidence of direct toxic effects of free radicals on the myocardium. *Free Radical Biology and Medicine*, **4**, 15–24.
8. A.S. Blaustein, L. Schine, W.W. Brooks, B.L. Fanburg and O.H.L. Eing (1886) Influence of exogenously-generated oxidant species on myocardial function. *American Journal of Physiology*, **250**, H595–599.
9. C.V. Jackson, J. Mickelson, T.K. Pope, P.S. Rao and B.R. Lucchesi (1986) Oxygen free radical-mediated myocardium and vascular dysfunction. *American Journal of Physiology*, **251**, H1225–1231.

10. F. Villani, M. Galimberti, E. Monti, F. Piccinini, E. Lanza, A. Rozza, L. Favalli, P. Poggi and F. Zunino (1990) Effect of glutathione and N-acetylcysteine on *in vitro* and *in vivo* cardiac toxicity of doxorubicin. *Free Radical Research Communications*, **11**, 145–151.
11. E. Monti, L. Paracchini, G. Perletti and F. Piccinini (1991) Protective effects of spin trapping agents on adriamycin-induced cardiotoxicity in isolated rat atria. *Free Radical Research Communications*, **14**, 41–45.
12. D. Cova, L. De Angelis, E. Monti and F. Piccinini (1992) Subcellular distribution of two spin trapping agents in rat heart: possible explanation for their different protective effects against doxorubicin-induced cardiotoxicity. *Free Radical Research Communications*, **15**, 353–360.
13. A. Jotti, L. Paracchini, G. Perletti and F. Piccinini (1992) Cardiotoxicity induced by doxorubicin *in vivo*: protective activity of the spin trap alpha-phenyl-tert-butyl nitrone. *Pharmacological Research*, **26**, 143–150.
14. E.G. Janzen, Y-K Zhang and D.L. Haire (1994) Synthesis of a novel nitrone, 2-phenyl-5,5-dimethyl-1-pyrroline-N-oxide-nitronyl-^{13}C for enhanced radical addend recognition and spin adduct persistence. *Journal of the American Chemical Society*, **116**, 3738–3743.
15. F. Villani, E. Monti, F. Piccinini, L. Favalli, E. Lanza, A. Rozza and P. Poggi (1986) Relationship between ECG changes and myocardial morphological alterations induced by adriamycin in rat. *Tumori*, **72**, 339–344.
16. L. Paracchini, A. Jotti, G. Bottiroli, E. Prosperi, R. Supino and F. Piccinini (1993) The spin trap *alpha*-phenyl-*tert*-butyl nitrone protects against myelotoxicity and cardiotoxicity of adriamycin while preserving the cytotoxic activity. *Anticancer Research*, **13**, 1607–1612.
17. E. Monti, F. Piccinini, F. Villani and L. Favaili (1986) Myocardial contractility and heart pharmacokinetics of adriamycin following a single administration in the rat. *Cancer Chemotherapy Pharmacology*, **18**, 289–291.
18. B. Chen, T.M. Bray, E.G. Janzen and P.B. McCay (1990) Excretion metabolism and tissue distribution of a spin trapping agent, *alpha*-phenyl-*tert*-butyl nitrone (PBN) in rats. *Free Radical Research Communications*, **9**, 317–323.
19. S. Bradamante, A. Jotti, L. Paracchini and E. Monti (1993) The hydrophilic spin trap, 5,5-dimethyl-1-pyrroline-1-oxide, does not protect the rat heart from reperfusion injury. *European Journal of Pharmacology*, **234**, 113–116.
20. S. Bradamante, E. Monti, L. Paracchini, E. Lazzarini and F. Piccinini (1992) Protective activity of the spin trap *tert*-butyl-*alpha*-phenyl nitrone (PBN) in reperfused heart. *Journal of Molecular and Cellular Cardiology*, **24**, 375–386.

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