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EFFECT OF GLUTATHIONE AND N-ACETYLCYSTEINE ON *IN VITRO* AND *IN VIVO* CARDIAC TOXICITY OF DOXORUBICIN

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The effects of two sulfhydryl compounds, glutathione (GSH) and N-acetylcysteine (NAC), on the cardiotoxicity of doxorubicin (DXR) were tested on *in vitro* and *in vivo* models. DXR was administered to rats as 4 weekly i.v. doses of 3 mg/kg. GSH (1.5 mmoles/kg), given i.v. 10 min before and 1 hr after DXR, was found to prevent the development of the delayed cardiotoxic effects of DXR, as assessed by electrocardiographic and mechanical parameters, as well as by histological examination of left ventricular preparations. In contrast, equimolar oral doses of NAC (1 hr before and 2 hrs after DXR) were found to be ineffective. Both GSH and NAC prevented the negative inotropic effect produced by DXR on isolated rat atria. A good correlation exists between the cardioprotective effects of the two agents and their ability to enhance the non-protein sulfhydryl group content of the myocardium. Differences observed *in vivo* between GSH and NAC might be accounted for by pharmacokinetic factors.

KEY WORDS: Doxorubicin, cardiotoxicity, glutathione, N-acetylcysteine.

INTRODUCTION

The clinical utility of doxorubicin (DXR) in the treatment of many types of cancer is limited by the occurrence of a dose-dependent, life-threatening congestive cardiomyopathy.[1,2] A large body of evidence supports the oxidative nature of this injury. According to this mechanism, DXR would initiate a reaction cascade leading to oxygen free radical generation in the myocardium,[3-6] while inhibiting the limited capacity of the heart to detoxify reactive oxygen metabolites,[7,8] thus resulting in extensive peroxidative damage. Since sulfhydryl groups play an important role in promoting the nonenzymatic detoxification of free radicals, and since a drop in the reduced glutathione (GSH) pool was found in the myocardium of DXR-treated animals,[9,10] treatments increasing myocardial sulfhydryl group content might prevent the effects of exposure to DXR.

Individual reports concerning the administration of GSH[10,11] and N-acetylcysteine (NAC)[12,13] in DXR-treated mice suggest an improvement of drug-induced car-

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diotoxicity. However, differences in the treatment schedules adopted in these studies do not allow a comparative evaluation of the two sulfhydryl compounds; in addition, no functional parameters were examined by the authors.

The present investigation was designed to evaluate the effect of both GSH and NAC on DXR-toxicity using the same *in vivo* rat model. The model was extensively characterized in previous papers,[14,15] and was shown to allow the evaluation of the delayed cardiotoxic effects of the drug. The effects of GSH and NAC on isolated rat atria exposed to DXR were also studied to gain a better insight into the mechanism of action of these drugs.

METHODS

In Vivo Experiments

Female Sprague Dawley rats received 4 weekly i.v. administrations of DXR at the dose of 3 mg/kg. GSH was given as 0.5 g/kg i.v. injections 10 min before and 2 hrs after each DXR administration; the choice for the dose of GSH (1.5 mmoles/kg, corresponding to 0.5 mg/kg) was based on the results of preliminary experiments. The route for the administration of GSH and NAC was chosen according to current clinical protocols. Since in the rat the a.u.c. after oral administration of NAC was not found to differ significantly from the value obtained after parenteral injections,[16] the dose of NAC was equimolar with respect to GSH (1.5 mmoles/kg, corresponding to 0.24 g/kg). The time schedule for NAC administration was slightly modified as compared to GSH (1 hr before and 2 hrs after DXR), to allow for the oral pharmacokinetics of the drug. The development of cardiac toxicity was monitored by biweekly measurements of a predictive ECG parameter (QαT interval), up to 8 wks after the beginning of DXR treatment; QαT intervals were not corrected for changes in the heart rate according to the findings of some authors.[17] At the end of the observation period the animals were sacrificed and left ventricular fragments were processed as previously described[15] and examined by light and electron microscopy. The incidence and severity of morphological lesions were quantitated according to a score system modified

TABLE 1

Morphological evaluation of DXR cardiotoxicity: effect of glutathione and N-acetylcysteine (Mean ± S.E. of 6–12 preparations).

Score Levels
0 normal
0.2 very few (< 5) isolated vacuolated myocytes
0.5 more than 10 isolated vacuolated myocytes
1 small clusters of vacuolated myocytes
1.5 multiple clusters of vacuolated myocytes
2 diffuse myocyte damage

Treatment	Score
Controls	0.06 ± 0.04
DXR	0.77 ± 0.16*
GSH	0.10 ± 0.05
DXR + GSH	0.56 ± 0.09*^{0}
NAC	0.015 ± 0.01
DXR + NAC	1.19 ± 0.22*

* $p < 0.05$ vs Controls; ^{0} $p < 0.05$ vs DXR

from Billingham[18] and reported in Table I. The myocardial contractile performance was also assessed on atria isolated from treated animals.

In Vitro Experiments

The contractile performance of spontaneously beating isolated rat atria was assessed after 1 hr exposure to 10 μg/ml of DXR, with or without 1.5 mM GSH or NAC. Contractile responses were expressed as percent of the corresponding baseline values.

Determination of Non-Protein Sulfhydryl Concentration

Non-protein sulfhydryl (NP-SH) groups in rat myocardium were measured by the method described by Sedlak and Lindsay.[19] To determine tissue NP-SH concentrations, 7–8 animals were given NAC or GSH and/or DXR as described for the *in vivo* cardiotoxicity studies; an equal number of controls received an equivalent volume of physiological saline. The animals were killed 1 hour after the last drug administration; atria from the same treatment groups were pooled and processed as described.[19] Triplicate determinations were performed on each pool.

NP-SH concentration was also measured in myocardial tissue after *in vitro* exposure to 1.5 mM GSH and NAC. For these determinations, pools of 7–9 atria were incubated for 60 min in the presence of the agents and were subsequently processed according to the same method. Triplicate determinations were performed on each pool.

Statistical analysis. Differences in QαT at the end of the observation period and in dF/dt of *in vitro* treated preparations after 1 hour incubations with DXR and/or GSH or NAC were assessed by oneway analysis of variance with Duncan's multiple range test at a confidence level of 0.05. Semi-quantitative histological data were analysed by Kruskal-Wallis non parametric analysis of variance.

RESULTS AND DISCUSSION

Figure 1 shows the time course of QαT duration in rats treated with DXR. By the end of the experimental period (8 weeks after beginning of the treatment), a significant enlargement of the QαT interval was observed in DXR-treated animals, in line with the results of previous investigations.[14] GSH and NAC *per se* did not modify the ECG pattern. The DXR-GSH combination was found to significantly reduce QαT duration, as compared to DXR alone, thus suggesting a protective action against anthracycline-induced cardiomyopathy. In contrast, NAC was unable to modify the effect of DXR on this parameter. No significant alterations of heart rate were observed in any of the experimental groups.

These observations are consistent with morphological findings (Table I). The average score attributed to preparations from DXR-treated animals was found to be significantly higher than the average value for control samples. Again, GSH administration (1.5 mmoles/kg) significantly reduced the lesions produced by DXR, whereas an equimolar dose of NAC did not affect the morphological score. The same pattern was observed for the evaluation of the contractile force developed by atria isolated

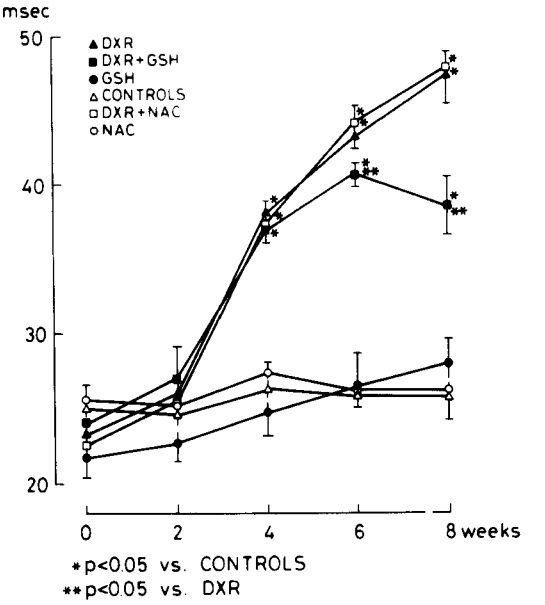

FIGURE 1 Time course of QαT duration in rats treated with doxorubicin (4 weekly i.v. administrations of 3 mg/kg). Effect of glutathione (1.5 mmoles/kg i.v. 10 min before and 1 hr after DXR) and N-acetylcysteine (1.5 mmoles/kg p.o. 1 hr before and 2 hrs after DXR). Means ± S.E.M. of 6–8 values.

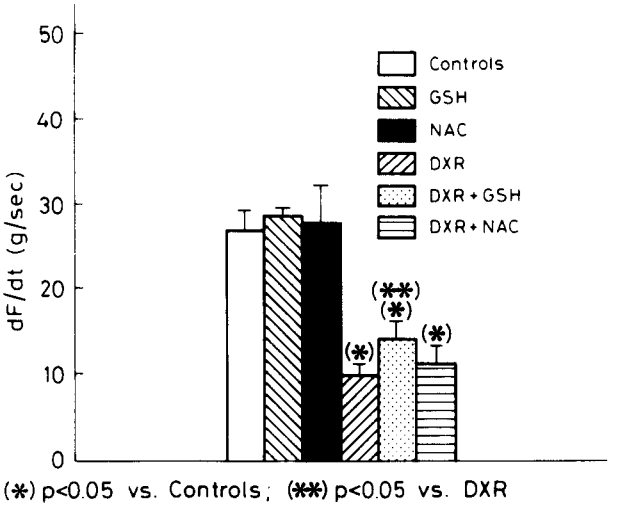

FIGURE 2 Contractile responses developed by atria isolated from doxorubicin-treated rats (4 weekly i.v. administrations of 3 mg/kg). Effect of glutathione (1.5 mmoles/kg i.v. 10 min before and 1 hr after DXR) and N-acetylcysteine (1.5 mmoles/kg p.o. 1 hr before and 2 hrs after DXR). Means ± S.E.M. of 6–8 values.

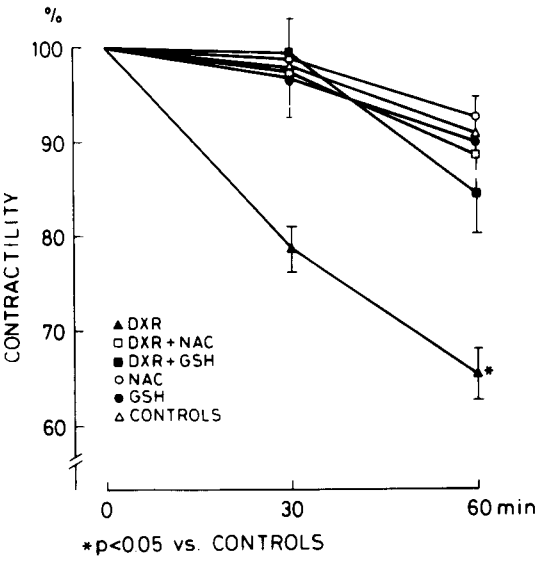

FIGURE 3 Contractile responses developed by isolated rat atria during 1 hr exposure to doxorubicin (10 μg/ml). Effect of glutathione (1.5 μmoles/ml) and N-acetylcysteine (1.5 μmoles/ml). Means ± S.E.M. of 4–6 values.

from treated rats (Figure 2), with a significant impairment with DXR alone and a significant protection with GSH. The present results are consistent with the studies of Tsan *et al.*,[20] showing that, although GSH is unable to enter the cells, enhanced cellular levels of GSH may occur as a consequence of breakdown at the cell surface and internal resynthesis of the molecule. In contrast, no significant effects were observed *in vivo* with NAC. NAC is supposed to enhance the intracellular NP-SH content either directly, via intracellular conversion to GSH, or indirectly, by promoting cystine uptake by the cells.[21] Since differences in the *in vivo* behaviour of GSH and

TABLE 2

Non-protein sulfhydryl group concentration in rat myocardial cells (μmoles/g fresh tissue): effect of glutathione (GSH) and N-acetylcysteine (NAC).

	in vitro[1]	*in vivo*[2]
Controls	1.11 ± 0.034	1.23 ± 0.084
DXR	0.09 ± 0.008*	1.15 ± 0.029
GSH	1.325 ± 0.024*^{0}	1.39 ± 0.027*^{0}
GSH + DXR	1.09 ± 0.029^{0}	1.35 ± 0.04 ^{0}
NAC	1.455 ± 0.035*^{0}	1.22 ± 0.045
NAC + DXR	1.29 ± 0.030*^{0}	1.23 ± 0.021

* $p < 0.05$ vs controls; ^{0} $p < 0.05$ vs DXR

[1] Means ± S.E.M. of triplicate determinations performed on pools of 7–9 rat atria after 1 hr exposure to GSH or NAC (1.5 mM) and/or DXR (10 μg/ml).

[2] Means ± S.E.M. of triplicate determinations performed on pools of 7–8 atria excised from rats treated with GSH or NAC (1.5 mmoles/kg) and/or DXR (3 mg/kg, see Methods) 1 hr after the last drug administration.

NAC are not explained by their presumptive mechanisms of action, further *in vitro* investigations were performed.

In *in vitro* experiments, DXR was found to develop a significant negative inotropic effect on isolated rat atria following 1 hr exposure (Figure 3). Both GSH and NAC (1.5 μmol/ml) significantly affected the cardiotoxic action of DXR on these preparations, restoring the contractile performance to near-control values. As already observed for *in vivo* experiments, heart rate was unaffected by the treatments.

Data obtained for NP-SH determinations in myocardial tissue are consistent with the achieved degree of cardioprotection (Table II): in fact, both NAC and GSH significantly enhanced intracellular concentration of NP-SH groups in preparations treated *in vitro*, whereas only GSH was found to affect this parameter in hearts excised from *in vivo* treated animals. Besides the dose adopted for cardiotoxicity studies, a very large dose of NAC, corresponding to the maximal tolerated oral dose (2.0 g/kg)[17] was also administered, but no significant modifications of NP-SH concentration were observed (data not shown).

According to the results of the present experiments, a good correlation exists between the protective activity of GSH and NAC and their ability to restore the myocardial NP-SH levels. This is supported by both *in vitro* and *in vivo* data and suggests that a supply of exogenous sulfhydryl groups may enable the myocardium to withstand the oxidative injury initiated by DXR. The differences observed *in vivo* between GSH and NAC are probably due to pharmacokinetic factors; however, since NAC proved to be ineffective on this parameter even at very high doses, its clinical utility as a protective agent against DXR-induced cardiotoxicity is doubtful.

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