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INHIBITION OF LIPID PEROXIDATION BY ADRENOCHROME

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INTRODUCTION

Adrenochrome is an ortho-quinone derived from the autoxidation of adrenalin; it exhibits neurotoxic and cardiotoxic properties referable both to its interaction with sulfhydryl groups and to the production of oxygen reduced forms through a redox cycling process (Bindoli *et al.*,1989).

Several papers indicate that quinonoid compounds, particularly in their reduced form, can act as powerful antioxidants. Nevertheless, depending on the experimental conditions and on the specific properties of each considered quinone, they can either stimulate or inhibit lipid peroxidation; for instance anthracycline antibiotics, e.g. adriamycin stimulate lipid peroxidation (Goodman *et al.*, 1977), while menadione (Wills, 1972) and coenzyme Q (Beyer, 1988) act as powerful antioxidants. Adrenochrome was reported to exert its toxic effects through a peroxidative process (Gudbjarnason, *et al.*, 1978); in the present paper the effects of adrenochrome on different lipid peroxidation systems was examined and the effects are those of an antioxidant. Yet, this property does not exclude a toxic action through an oxidative stress-linked mechanism (depletion of GSH and production of oxygen reduced species).

METHODS

Microsomes (0.5 mg protein/ml) and liposomes (0.5 mg/ml) were incubated in 0.125 M KCl, 5 mM Tris, 15 mM Hepes (pH 7.4) at 30^{o} C with the peroxidizing systems indicated under the appropriate figures and tables. Lipid peroxidation was measured as malondialdehyde (MDA) with the thiobarbituric acid method.

RESULTS

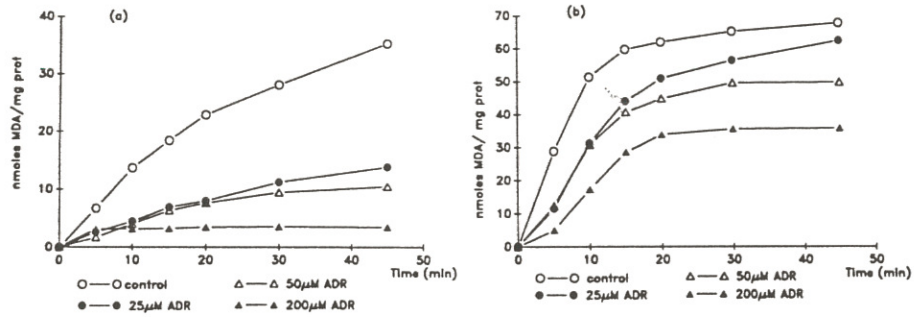
Microsomal lipid peroxidation is strongly stimulated by NADPH in the presence of iron ions, and depends on the concentrations of both NADPH and Fe^{2+}, while the presence of iron chelators appears also to play a fundamental role (Ursini *et al.*, 1989). In Table 1 the inhibitory effects of increasing concentrations of adrenochrome on the NADPH/Fe/-ADP-induced lipid peroxidation is reported. The extent of inhibition is directly proportional to the concentration of adrenochrome and is inversely related to that of added iron. The inhibitory action exerted by adrenochrome appears far more marked at relatively low concentrations of NADPH (0.1 mM) and Fe^{2+} (2 μM), where an IC_{50} of about 15 μM is apparent; at higher concentrations of both NADPH (2mM) and Fe^{2+}

(20 μM) an IC_{50} of about 150 μM was calculated. From the time course of the inhibition of the NADPH/Fe/ADP-induced lipid peroxidation by adrenochrome (Fig.1) it appears that relatively high concentrations of adrenochrome (200 μM) almost completely inhibited the lipid peroxidation induced by 0.1 mM NADPH/2 μM Fe^{2+}/ 20 μM ADP (Fig.1a) up to about 50 min (Fig. 1a), while in the presence of 2 mM NADPH/20 μM Fe^{2+}/ 0.2 mM ADP (Fig. 1b) the inhibition by increasing concentrations of adrenochrome is less evident and reaches a plateau.

Table 1.: Adrenochrome (ADR) inhibition of microsomal lipid peroxidation induced by NADPH/Fe^{2+}/ADP: **a**, **d**: 0.1 mM NADPH; **b**, **e**: 0.5 mM NADPH; **c**, **f**: 2mM NADPH. ADP:Fe^{2+} ratios were 10:1. Incubation time: 15 min. Values are % of the control (in brackets nmoles MDA/mg protein).

ADR	a	b	c	d	e	f
(μM)	2 μM Fe^{2+}			20μM Fe^{2+}		
0	100 (4.6)	100 (8.2)	100 (16.1)	100 (29.9)	100 (38.8)	100 (54.8)
5	62	90	96	92	96	94
10	55	75	87	66	80	90
25	30	39	75	48	70	77
50	18	36	66	46	59	73
100	16	25	42	34	48	60
200	14	18	23	27	38	35

Fig. 1. *Time course of the inhibition of microsomal lipid peroxidation induced by increasing concentrations of adrenochrome.*

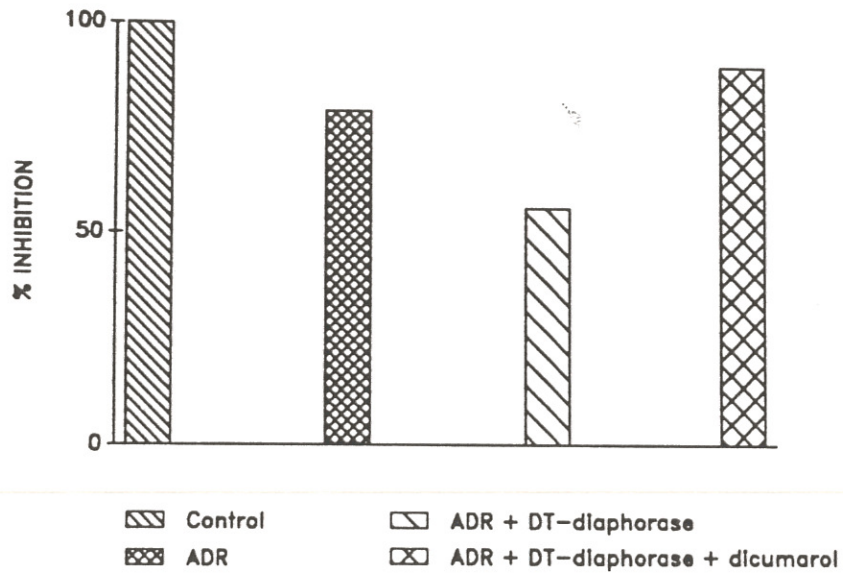


In a previous paper (Bindoli *et al*, 1988) it was shown that adrenochrome can be reduced by the mitochondrial and microsomal electron transport chain giving rise to the corresponding semiquinone that autoxidizes with the formation of the superoxide anion subsequently dismutating to H_2O_2. These reduced forms of oxygen might have a role in the induction of

lipid peroxidation, particularly in the presence of metal catalysts. On the other hand the addition of superoxide dismutase and catalase does not substantially increase the inhibitory effect exerted by adrenochrome on lipid peroxidation (not shown) indicating that O_2^- and H_2O_2 are not involved in the induction of lipid peroxidation. In addition, this indicates that the inhibition of lipid peroxidation by adrenochrome is not dependent on a depletion of oxygen from the reaction vessel due to the redox cycling of adrenochrome. Both adrenochrome and ferric ions are reduced by the flavoprotein of the microsomal electron transport chain, but they do not appear to compete in the reduction process. Consequently, the inhibition of lipid peroxidation by adrenochrome is not referable to an interference with the iron-reduction process.

Cumene hydroperoxide induces microsomal lipid peroxidation with a process dependent on cytochrome P-450 and independent of iron ions catalysis. In the absence of NADPH there is a scarce inhibition of lipid peroxidation by adrenochrome, while this inhibition, even though not large, is increased by addition of NADPH (data not shown), indicating that the reduced form of adrenochrome is more effective than the oxidized one in inhibiting lipid peroxidation. Adrenochrome, similarly to several other quinones can be reduced by the enzyme DT-diaphorase in a two-electrons process. In Fig. 2 the inhibition by adrenochrome of lipid peroxidation induced in liposomes by cumene hydroperoxide and hemin is reported. Adrenochrome *per se* is scarcely effective (20% inhibition), while the addition of DT-diaphorase increases its inhibitory effect to about 50%.

Fig. 2. *Effect of DT-diaphorase on adrenochrome-induced inhibition of MDA formation in liposomes.*



Liposomes were incubated for 15 minutes in the presence of 1 mM cumene hydroperoxide, 12µg/ml hemin and 0.5 mM NADPH. Other additions: 200 µM adrenochrome (ADR), 100 µM dicumarol and 1mg prot of DT-diaphorase obtained as dialysed cytosol after 105.000 x g centrifugation of a rat liver homogenate.

CONCLUSION

From the reported results it appears that the toxicity of adrenochrome is not referable to a peroxidative process. On the contrary, adrenochrome inhibits lipid peroxidation, induced with three different systems either dependent or independent of iron ions. Consequently, the toxicity of aminochromes should essentially be referred to arylation of nucleophilic groups and to redox cycling that brings about the formation of reactive semiquinones and oxygen reduction products. The reduced forms of adrenochrome (leucoadrenochrome or the corresponding semiquinone) are particularly efficient in eliciting the antioxidant effect; nevertheless a partial antioxidant effect is also exhibited by the oxidized form.

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