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Novel antioxidants in anthracycline cardiotoxicity

Kálmán Hideg · Tamás Kálai

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Abstract It has been suggested nitroxides and their amine precursors prevent incidence of damage caused by superoxide and hydroxyl radicals formed during the oxidative metabolism of doxorubicin (DOX) and daunorubicin (DAU). Both doxorubicin and daunorubicin have been associated with cardiac toxicity in both adults and children. The authors herein suggest that cardioprotective molecules modified by nitroxides and their secondary amine precursors can prevent or diminish the anthracycline-induced cardiomyopathy by accumulating in cardiomiocytes.

Keywords Anthracyclines · Antioxidants · Cardiomyopathy · EPR · Free radicals · Nitroxides

Introduction

Anthracyclines are widely used for treating a range of malignances such as sarcomas, lymphomas, breast cancer, myeloma, small-cell lung cancer, bladder cancer and paediatric solid tumors. However, drugs such as DOX and DAU also cause oxygen-dependent DNA damage. Anthracyclines may also cause protein-associated breaks. These breaks are induced by the reaction of anthracyclines with topoisomerise II, which promotes DNA strand cleavage and reannealing [1].

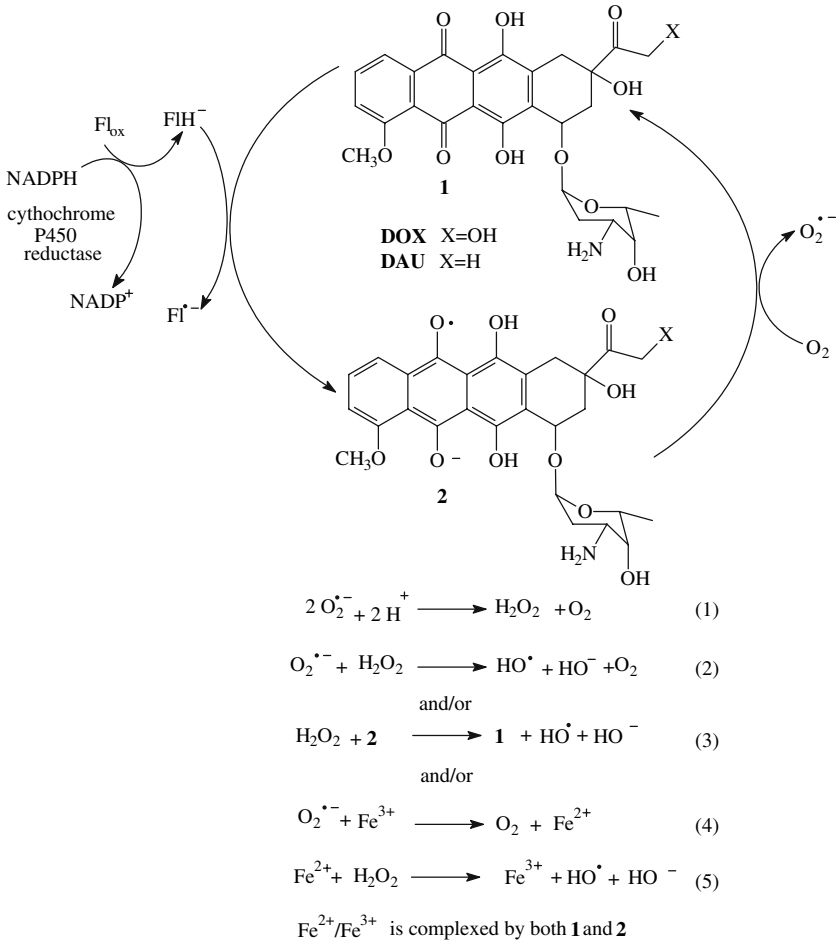
The DNA damage could also be attributed to electron transfer processes. A one-electron – reduction of anthracyclines, catalyzed by flavoenzymes produces the anthracycline semiquinone radical, which can be oxidized to anthracycline with a reduction of oxygen to superoxide anion radical ($O_2^{\cdot-}$). Both superoxide and semiquinone can generate hydroxyl radicals involved in a variety of deleterious biological processes, including DNA breaks (Scheme 1, Eqs. 1–3) [2]. The third possibility for DNA damage is the formation of a doxorubicin-ferric complex, which binds to DNA by a different mechanism than the intercalation of anthracyclines. This ferric complex is reduced by superoxide to yield a ferrous complex. The latter catalyses hydroxyl radical formation in a Fenton reaction near the DNA strands, cleaving them (Scheme 1, Eqs. 4&5) [3]. This latter process contribution has been confirmed by the fact that the addition of Dexrazoxane (an iron-chelator) prevents cardiac toxicity in humans [4, 5]. Beyond this, several other attempts has been made to reduce the cardiotoxicity of anthracyclines including special modes of administration, development of new formulations, liposome encapsulation [6], less cardiotoxic analogues or co-administration of antioxidant molecules such as flavones [7]. The results and ideas we present here are related to the latter concept. Nitroxides, and their amino precursors, can participate in one-electron processes and are capable of acting as multifunctional antioxidants.

Results and discussion

It is well documented that TEMPOL (4-hydroxy-2,2,6,6-tetramethyl-piperidine), at 2.5 mM concentration, inhibits the DOX-induced membrane lipid peroxidation by free radical trapping in an isolated rat heart model perfused with 100 μg/ml DOX and by stabilizing the oxidized form of iron blocking the Fenton-reaction [8]. It has also been reported that TEMPOL has no effect on DOX induced cytotoxicity on Chinese hamster V79 cells [9]. These

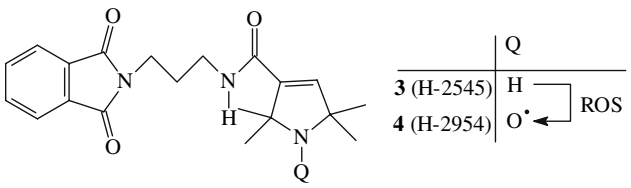
K. Hideg (✉) · T. Kálai
Department of Organic and Medicinal Chemistry, University of Pécs, P.O. Box 99, 7602 Pecs, Hungary
e-mail: kalman.hideg@aok.pte.hu

Scheme 1 Possible mechanism of ROS production during oxygen dependent metabolism of anthracycline antibiotics



observations prompted us to test our experimental drug H-2545 and its metabolite H-2954 (Scheme 2: compounds **3** and **4**) in order to determine whether they can prevent the acute deterioration of cardiac function following DOX administration [10]. It was reported that compound **3** (Fig. 1) could scavenge free radicals with the formation of compound **4** [11–13]. The hypothesis of preventing the DOX-induced cardiotoxicity with antioxidants **3** and **4** was proved with Langendorff-perfused rat heart experiments studying high-energy phosphate levels, contractile function, lipid peroxidation, protein oxidation and Akt phosphorylation [14].

From these experiments we determined that compounds H-2545 and H-2954 in 10 μM and 20 μM concentrations, had greater recovery of adenosine triphosphate (ATP) and creatine phosphate (PCr) levels when compared to DOX-only treated hearts, (Table 1) following ischemia/reperfusion (1 h). Protein oxidation and lipid peroxidation induced by doxorubicin administration, was inhibited by the experimental antioxidant drugs (**3, 4**). For instance, compound **3** in 20 μM concentration reduced the lipid peroxi-



Scheme 2 H-2545 and its oxidative metabolite H-2954

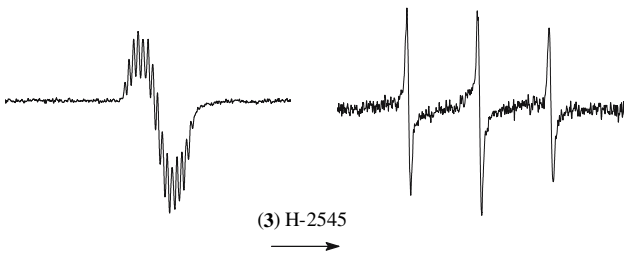


Fig. 1 EPR spectra of DOX-generated ROS trapping by compound **3**. The resultant EPR signal is characteristic of compound **4**

Table 1 Effect of H-2545 (**3**) and H-2954 (**4**) on creatine phosphate and ATP level

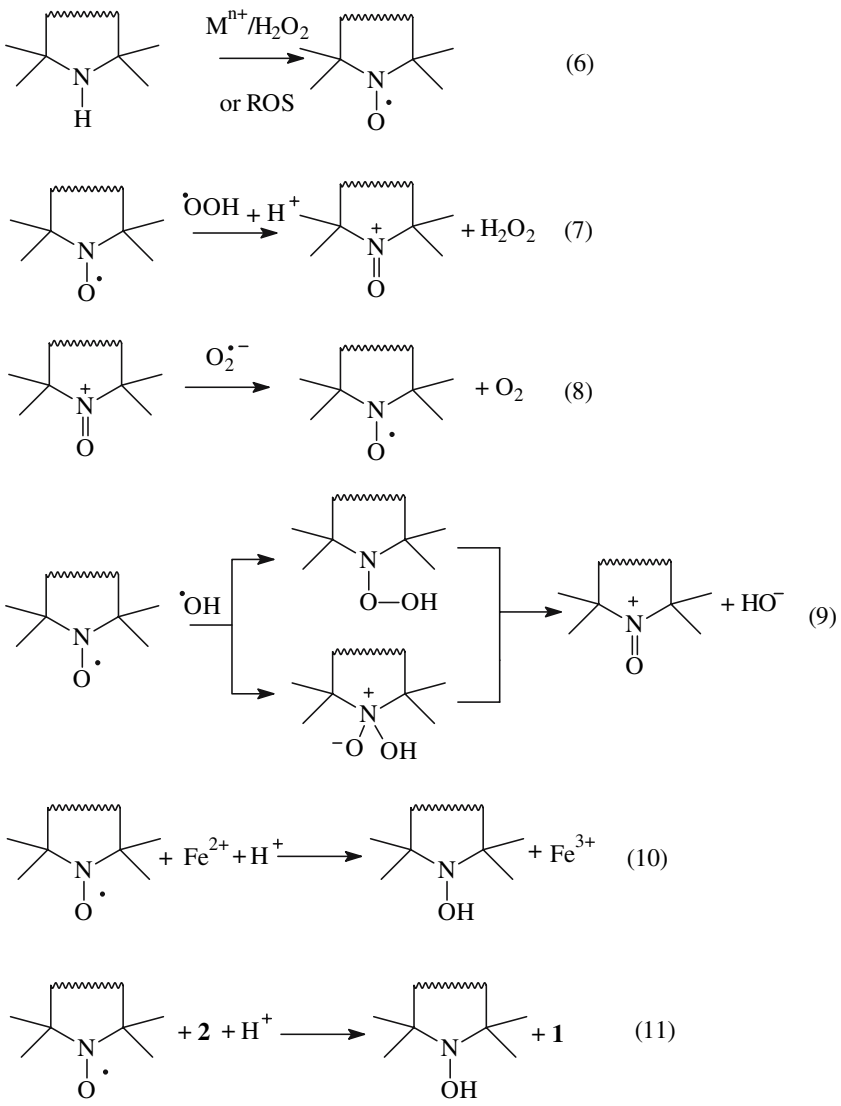
	Control	Dox	**3** (10 μM)	**3** (20 μM)	**4** (10 μM)	**4** (20 μM)
PCr (% recovery)	81 ± 9	20 ± 4	48 ± 7	62 ± 7	40 ± 6	61 ± 10
ATP(% recovery)	85 ± 4	15 ± 10	47 ± 8	61 ± 7	41 ± 9	60 ± 8

Table 2 Effect of H-2545 (**3**) and H-2954 (**4**) on lipid peroxidation and protein oxidation

	Control	Dox	**3** (10 μM)	**3** (20 μM)	**4** (10 μM)	**4** (20 μM)
Lipid peroxidation (nM/gwt)	40 ± 1	110 ± 5	80 ± 5	50 ± 5	81 ± 5	58 ± 9
Protein oxidation (nM/gwt)	1350 ± 100	2100 ± 150	1650 ± 70	1500 ± 100	1620 ± 100	1550 ± 120

dation value almost to the control level (Table 2). The concentration dependence of antioxidant activity suggests that compounds **3** and **4** participate not only in catalytic, but in stoichiometric radical scavenging processes as well. These compounds reduced the Akt phosphorylation by preventing activation of the Akt kinase cascade, and improved the contractile function when compared to DOX-only perfused hearts. The co-administration of antioxidants **3** and **4** with DOX did not alter the anticancer properties verified on malignant cell cultures (HeLa, PANC-1, HEPG-

Scheme 3 Possible mechanism of detoxification of ROS formed during DOX metabolism



2). Although free radicals participate in the DOX anticancer effect, DOX can intercalate between DNA strands and inhibit topoisomerase II, blocking DNA and RNA synthesis. Presumably, this mechanism is not involved in cardiotoxicity because in some cases heart failure developed after doxorubicin administration [15].

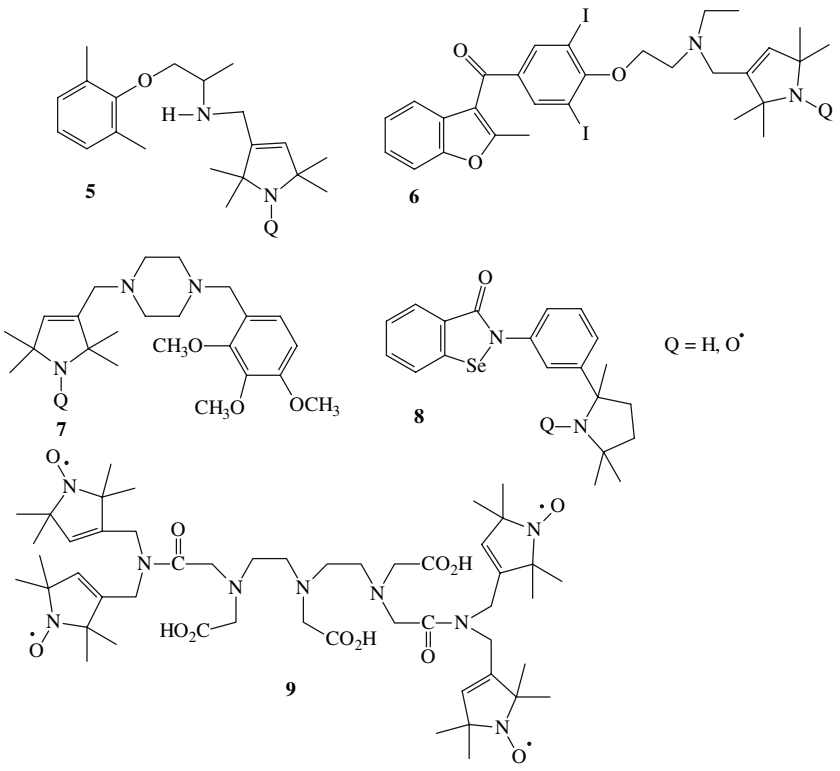
The nitroxides and their sterically hindered secondary amine precursors can be regarded as multifunctional non-enzymatic antioxidants. The sterically hindered amine reacts with hydrogen peroxide (H_2O_2) in the presence of transition metal ions to yield non-toxic nitroxides. However, other reactive oxygen species (ROS) (for example $^1O_2$) also oxidize them to nitroxide (Eq.6) (Scheme 3) [16]. This process was proven in an in vitro EPR experiment by adding compound **3** to a solution of compound **1** (DOX) in a buffer (pH = 8), resulting in a loss of DOX signal (one band with a fine structure) and the appearance of an isotropic triplet signal, characteristic of nitroxide free radicals such as compound **4.** This triplet appears within 5 min, and it remains consistent over a period of 24 h. (Scheme 1)(Belagyi, and Hideg, unpublished results).

The superoxide dismutase activity of nitroxides is well known (Eqs. 7 and 8) [17]. The process is acid catalyzed, but the reaction is rather slow at physiological pH values. Thus the superoxide dismutating activity, presumably, is not the only cause of antioxidant activity of compound **4** (H-2954) [18]. The nitroxides scavenge hydroxyl radicals (Eq. 9), oxidise transition metals such as Fe^{2+} (Eq. 10), pre-empting their participitation in Fenton reaction (Eq. 5) [19], and oxidize semiquinones to quinones (Eq. 11) without oxygen consumption or superoxide radical formation in a manner analogous to the juglone semiquinone radical oxidation (Scheme 3) [20]. It is interesting to note that nitroxides are also efficient scavengers of $NO_2^{\cdot}$ and $CO_3^{\cdot-}$. Although these species do not form during the oxidative metabolism of DOX, hydroxyl radicals formed during the metabolism may react further with NO_2^- to yield $NO_2^{\cdot}$ or with CO_3^{2-} to give $CO_3^{\cdot-}$**.** Considering that both anions are possible putative parts of biological systems, this scavenging capability may be important [18].

Conclusions and outlook

Our results indicate that nitroxides or their amine precursors play multiple roles in elimination of ROS formed during DOX-oxidative metabolism without reducing their anticancer effect. The target-oriented nitroxide **4** or nitroxide precursor **3** are proven to be effective in the prevention of the DOX-induced cardiotoxicity. Modified cardioactive compounds such as mexiletine derivative **5**, [21] amiodarone analog **6,** [22] trimetazidine derivative **7,** [23] ebselen analog **8** [24] and complex forming compound **9** [25], which presumably also have a protective effects (Scheme 4). Compounds **5–7** are advantageous in that they accumulate in membranes of cardiomyocites and can

Scheme 4 Potentially active nitroxides and amine precursors in prevention or decrease of DOX-induced cardiotoxicity



detoxify ROS in statu nascendi by both stoichiometric and catalytic pathways. Compounds **8,** as an ebselen analog, and compound **9,** as a paramagnetic MRI agent, may not be target specific, however beyond the catalytic and stoichiometric ROS detoxifying capability of nitroxides they have glutathion-peroxidase-like activity and metal complex forming capability, respectively. Further investigations of compounds **5–9** are in progress.

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