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Short Communication

**EFFECTS OF PYRROLINE AND PYRROLIDINE NITROXIDES ON
LIPID PEROXIDATION IN HEART TISSUE OF RATS TREATED
WITH DOXORUBICIN**

ANETA KOCEVA-CHYŁA¹, KRZYSZTOF GWOŹDZIŃSKI²,
AGATA KOCHMAN³, ANETA STOLARSKA² and ZOFIA JÓŹWIAK¹
¹Department of Thermobiology and ²Department of Molecular Biophysics,
University of Łódź, Łódź, Poland, ³Department of Pathological Anatomy,
Medical University of Wrocław, Wrocław, Poland

Abstract: Protection from doxorubicin-induced lipid peroxidation *in vivo* by two pyrroline and pyrrolidine nitroxides, Pirolin, PL, and Pirolid, PD, was examined in the heart tissue of rats treated with this drug. The level of lipid peroxidation was estimated on the basis of MDA content. A considerable (three-fold) increase in the MDA amount was found in heart homogenates from rats injected with doxorubicin, whereas no significant changes in MDA content compared to control were observed in cardiomyocytes treated with the nitroxides (Pirolin or Pirolid) only. Pirolin injected simultaneously with doxorubicin showed antioxidative effect and markedly attenuated lipid peroxidation in the heart tissue caused by this drug. In contrast to Pirolin, structurally related Pirolid was ineffective in the protection of heart myocytes from DOX-induced lipid peroxidation.

Key Words: Doxorubicin, Nitroxides, Lipid Peroxidation, Cardiomyocytes

INTRODUCTION

One of the most severe side effects of doxorubicin, an effective anthracycline drug with a broad spectrum of antitumour activity, is its high cardiotoxicity, which very often becomes a limiting factor in anticancer therapy employing this drug [1]. It has been suggested that free radical-mediated damage to heart myocytes plays an important role in the development of serious cardiomyopathy in patients treated with anthracyclines [2]. These drugs were found to generate

Abbreviations used: Pirolin, PL - 3-carbamoyl-2,2,5,5-tetramethylpyrroline-1-oxyl; Pirolid, PD - 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl; DOX - doxorubicin; MDA – malondialdehyde.

free radicals and to induce the formation of lipid peroxidation products such as 4-hydroxynonenal, other unsaturated aldehydes and malondialdehyde [3, 4]. The direct involvement of free radicals in the mechanism of DOX toxicity as well as the ability of doxorubicin to bind to mitochondrial lipids were considered as the main factors in acute cardiotoxicity in patients treated with doxorubicin [5, 6]. Nitroxides, the nonimmunogenic antioxidants and stable radicals that readily react with oxygen free radicals, have been investigated in several studies as putative protectors of myocardium against anthracycline cardiotoxicity [7-9]. The objective of this study was to assess the protective effects of two five-membered pyrroline and pyrrolidine nitroxides, Pirolin, PL, and Pirolid, PD, on lipid peroxidation induced in heart myocytes of rats treated with doxorubicin.

MATERIALS AND METHODS

The animals (rats, Wistar strain of average weight 180-210 g) were obtained from the Central Animal Farm of the Medical University of Wrocław and maintained under the same water and food conditions (a standard diet containing 24 % of crude protein). The rats were divided into 6 groups (5 animals per group) and injected intraperitoneally with a drug (DOX), nitroxides (Pirolin/Pirolid) or with a combination of both, a drug and a nitroxide. The sixth group, comprising control animals, was given an injection of the same volume of physiological saline. Each animal received a single injection of 2 mg of DOX/nitroxide or both compounds mixed together in a total volume of 1 ml of sterile 0.9 % NaCl. On the fourth day after the injection the animals were anaesthetised, the hearts were removed, washed several times with 0.9 % NaCl and frozen at -700 C. Protein content in heart homogenates was determined by the Lowry method [10] and the amount of lipid peroxidation product MDA was estimated according to the method described by Monti *et al.* [7] with some modifications.

RESULTS AND DISCUSSION

Nitroxides Pirolin and Pirolid were investigated for their cardioprotective effects against oxidative stress induced by doxorubicin *in vivo*. The content of lipid peroxidation product MDA was estimated in heart homogenates obtained from rats injected with doxorubicin alone or with a combination of doxorubicin with Pirolin or Pirolid. For comparison some of the animals received Pirolin or Pirolid only. Since in the combined treatment both compounds (DOX and nitroxides) were injected mixed in solution together, we tested them for the possibility of chemical interaction. Using ESR spectroscopy we did not find any changes in the spectra of each of the nitroxides mixed with doxorubicin in comparison to the spectra of the nitroxides alone. This implies that the investigated compounds do not interact with each other in the redox reaction.

In the rats injected with Pirolin or Pirolid alone, a little (20 %), but not statistically significant increase in lipid peroxidation in comparison with control was observed (Fig. 1).

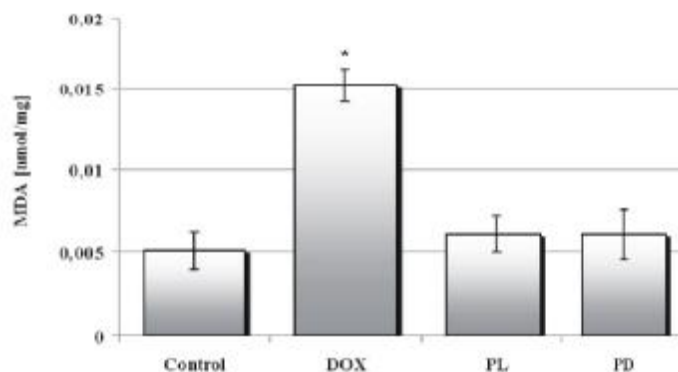


Fig. 1. Lipid peroxidation in the hearts of rats after a single injection of doxorubicin or nitroxide. * $p < 0.01$

Heart homogenates from the rats injected with DOX showed a significant degree of lipid peroxidation with a three-fold increase in MDA content, as compared to control (Fig. 1). Pirolin inhibited this effect and significantly (two-fold) lowered the level of lipid peroxidation (amount of MDA product) induced by DOX (Fig. 2). This indicates that Pirolin can protect heart myocytes against the oxidative stress induced by DOX and confirms the earlier findings showing that induction of oxidative stress and significant lipid peroxidation are among the basic mechanisms responsible for the toxicity of DOX in heart cells [2-5, 11].

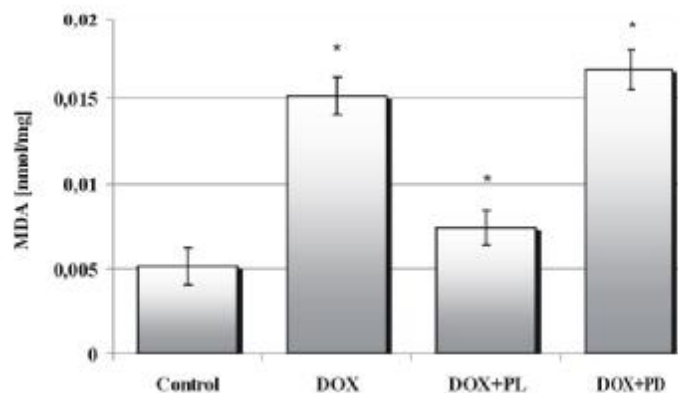


Fig. 2. The effect of nitroxides Pirolin (PL) and Pirolid (PD) on lipid peroxidation induced in vivo by doxorubicin in rat cardiomyocytes. * $p < 0.01$

In contrast to Pirolin, structurally related Pirolid did not display any cardioprotective effect. The animals receiving DOX, regardless of absence or presence of this nitroxide, showed a similar level of MDA products (Fig. 2). This implies a different activity of both nitroxides in cell protection against oxidative stress induced by doxorubicin.

All nitroxides bear a >N-O group, which plays a key role in their antioxidative properties. However, the structure of the rings (6-membered or 5-membered) and the substituents decide of both hydrophobic and hydrophilic properties of the compounds as well as of their reactivity as antioxidants. We suggest that different actions of Pirolin and Pirolid, which possess the same substituents, could be connected with different conformations of their heterocyclic rings: Pirolin is more planar than Pirolid. It is possible that the different structures of nitroxide rings can condition different penetration of these nitroxides through the plasma membrane and thus different reactivity with reactive oxygen species inside the cell. These findings imply the importance of the ring structure for antioxidant activity of nitroxides. Previously we found, using piperidine nitroxides, that substituents at position 4 of the nitroxide ring are important structural factors for the antioxidant and prooxidant activity of these compounds [12, 13]. The results of this study clearly show that beside ring substituents, lipoficity and intracellular localisation, another structural diversity, the heterocyclic ring, can decide about antioxidant properties of nitroxides [14].

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