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SHORT COMMUNICATION

Matusik et al: Apelin-13 and oxidative damage caused by DOX

Differential effects of apelin-13 on lipid peroxidation and DNA oxidation in doxorubicin-treated rats: A preliminary study

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ABSTRACT

Doxorubicin-induced cardiotoxicity is closely associated with oxidative stress (OS), and apelin-13 has been proposed as a potential cardioprotective peptide. However, its effects on specific oxidative stress (OS) markers remain poorly understood. This preliminary study aimed to evaluate the impact of apelin-13 on oxidative stress markers in rats chronically treated with doxorubicin (DOX). Male rats received DOX with or without apelin-13 (40 µg/kg body weight/day). The levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) were measured as indicators of oxidative DNA damage and lipid peroxidation, respectively. The DOX treatment resulted in increased MDA levels, which were unaffected by apelin-13. Conversely, 8-OHdG levels decreased with DOX alone but returned to baseline levels in the presence of DOX and apelin-13. In conclusion, while apelin-13 did not mitigate DOX-induced lipid oxidative damage, it may selectively influence nuclear OS markers. This suggests a complex and context-dependent role of apelin-13 in modulating oxidative stress associated with DOX treatment.

Keywords: Apelin-13, doxorubicin, oxidative stress.

INTRODUCTION

Doxorubicin (DOX), a member of the anthracycline group, is one of the most effective anticancer drugs, but its clinical use is severely limited by its cardiotoxicity, associated with increased oxidative stress (OS) in myocardial cells [1]. The toxic effects of DOX are associated with, among others, the overproduction of reactive oxygen species (ROS), which leads to lipid peroxidation, DNA damage, and mitochondrial dysfunction [2]. Indicators of such damage include: malondialdehyde (MDA), a marker of lipid peroxidation, and 8-hydroxy-2'-deoxyguanosine (8-OHdG), a product of oxidative DNA modification [3].

Apelin, an endogenous ligand of the APJ receptor, plays an important role in regulating cardiovascular function and metabolism [4]. In recent years, it has been shown that the apelin isoform, apelin-13, can exert a protective effect on the heart by reducing DOX-induced oxidative and apoptotic damage [5, 6]. However, the mechanisms of this protection are not fully understood, especially in the context of its effects on OS markers, such as MDA and 8-OHdG.

The aim of this study was to evaluate the effect of apelin-13 on markers of OS in rats treated with DOX.

MATERIALS AND METHODS

Animals

The study involved 24 male Sprague-Dawley rats (12 weeks old, 250–300 g). All procedures were approved by the II Local Animal Ethics Committee (No. WAW2/087/2021, dated 02.06.2021). The animals were bred and housed at the Central Laboratory of Experimental Animals (Medical University of Warsaw) under standard conditions (22–24 °C, 12 h light/dark cycle, 45–65% humidity) with ad libitum access to food and water.

Experimental design

The animals were randomly divided into 3 groups (n = 8). For 28 days, animals received continuous infusion of saline (NaCl, DOX groups) or apelin (APE40 group). Apelin was administered as Apelin-13 TFA salt (Cat. No.: HY-P1944A, MedChemExpress, Sollentuna, Sweden) at a dose of 40 µg/kg body weight/day. Saline and apelin infusion were delivered via osmotic pumps (Alzet Corp., Cupertino,

CA, USA; model 2ML4), implanted subcutaneously on the back. All animals received intraperitoneal injections once a week on days 1, 8, 15, and 22. The NaCl group received injections of saline. The DOX and APE40 groups received doxorubicin (DOX) at a dose of 3.5 mg/kg body weight per week (Cat. No.: HY-15142, MedChemExpress, Sollentuna, Sweden).

Enzyme-linked immunosorbent assay (ELISA)

Levels of MDA and 8-OHdG in left ventricular (LV) homogenates were subjected to analysis using commercial kits, in accordance with the instructions provided by the manufacturer (Double Antibody Sandwich Rat Malondialdehyde (MDA) ELISA Kit, ca. no. EIA06027r; Wuhan Newqidi Biotech Co.,Ltd, Wuhan, China; Double Antibody Sandwich Rat 8-OHdG ELISA Kit, ca. no. EIA05009r; Wuhan Newqidi Biotech Co.,Ltd, Wuhan, China).

Compliance with ethical standards

All procedures applied in the experiment were approved by the II Local Animal Research Ethics Committee (Application No. WAW2/087/2021, release date: 2.06.2021).

Statistical analysis

Datasets passed the normality test (Shapiro-Wilk test), thus one-way ANOVA with Sidak's post hoc test was used for statistical analysis. Values in the Figures are presented as medians with interquartile ranges (IQRs).

RESULTS

DOX treatment significantly increased MDA levels compared to the NaCl group (**$p = 0.0064$). The APE40 group also showed significantly elevated MDA levels compared to NaCl group (***$p < 0.0001$). However, no statistically significant difference was observed between the DOX and APE40 groups ($p = 0.2267$). (Fig.1.)

The DOX showed a significant reduction in 8-OHdG levels compared to the NaCl group (*$p = 0.0445$). Administration of apelin-13 (APE40) restored 8-OHdG levels to values comparable to those observed in animals from NaCl group ($p=0.1797$). Moreover, 8-OHdG levels in the APE40 group were significantly higher than those in the DOX group (***$p = 0.0002$). (Fig.2.)

DISCUSSION

Previous studies have shown that apelin-13 can exert protective effects in cardiac models by reducing ROS, MDA and inducing antioxidant enzyme activity (superoxide dismutase, catalase, glutathione peroxidase), as well as by activating PI3K/Akt (phosphoinositide 3-kinase/protein kinase B) signaling and delaying MPTP (mitochondrial permeability transition pore) opening [7]. In contrast to those observations, apelin-13 did not reduce MDA in our experiment (Fig.1.), suggesting that DOX-induced oxidative mechanisms may be resistant to its effects. Nonetheless, the modulation of 8-OHdG levels (Fig.2.) indicates a potential effect of apelin-13 on nuclear or mitochondrial responses, effectively affecting markers of DNA damage despite the absence of an anti-lipid effect.

Our data suggests that the role of apelin-13 in oxidative protection against DOX is more complex than expected and may be dependent on the type of OS and the target tissue or type of damage. Similar observations were reported by Duan et al. (2019), who showed that apelin-13 reduced MDA levels and oxidative damage in a model of ischemic stroke [8] and by Xu et al. (2020), who demonstrated decreased OS markers following apelin-13 treatment in spinal cord ischemia–reperfusion injury [9]. In contrast, our results suggest that apelin-13 may exert more selective, context-dependent effects on nuclear rather than lipid oxidative damage. Possible explanations include differences in subcellular targets of apelin-13, with greater effects on nuclear rather than membrane lipid compartments. Apelin-13 may enhance DNA repair pathways or preferentially modulate nuclear ROS, without significantly affecting mitochondrial lipid peroxidation reflected by MDA levels. The findings may have translational relevance for patients undergoing long-term DOX therapy. Although apelin-13 did not attenuate lipid peroxidation, its ability to restore 8-OHdG levels suggests potential modulatory effects on nuclear OS and DNA integrity. Understanding these mechanisms could contribute to the development of adjunctive strategies aimed at minimizing DOX-induced cellular damage without compromising its antitumor efficacy.

CONCLUSION

Our preliminary study aimed to explore the potential role of apelin-13 under chronic DOX treatment. Although apelin-13 did not mitigate DOX-induced lipid oxidative damage, it may selectively influence nuclear OS markers. This suggests a complex

and context-dependent role of apelin-13 in modulating OS associated with DOX treatment. However, the use of a single dose, the measurement of only two biomarkers, and absence of functional or histopathological assessments limit the strength of these conclusions. Further studies are needed to confirm these findings and to clarify the mechanisms involved.

Conflicts of interest: Authors declare no conflicts of interest.

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Data availability: The corresponding author, Katarzyna Kamińska, will provide the data that supports the findings of this study upon request.

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FIGURES WITH LEGENDS

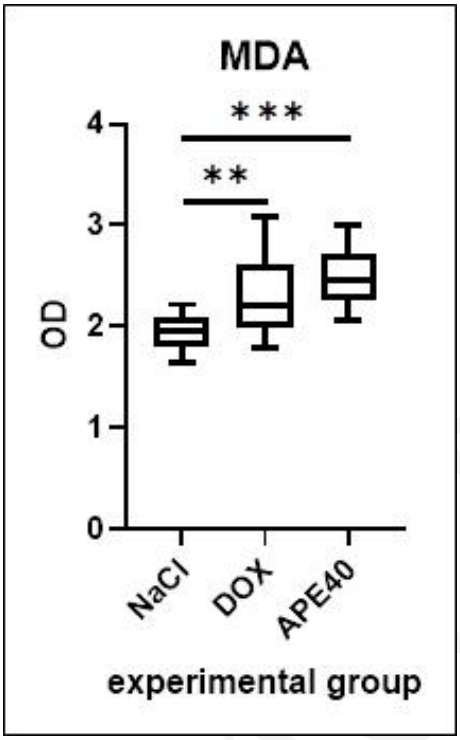

Figure 1. Levels of malondialdehyde (MDA) in the left ventricle (LV) of rats from the NaCl group, the doxorubicin-treated (DOX) group, and the doxorubicin- and apelin-13-treated (APE40) group, presented as optical density (OD) values. Data are presented as medians with interquartile ranges (IQR), derived from two technical replicates per animal. The number of technical replicates included in the analysis was as follows: NaCl: n = 14, DOX: n = 16, APE40: n = 15.

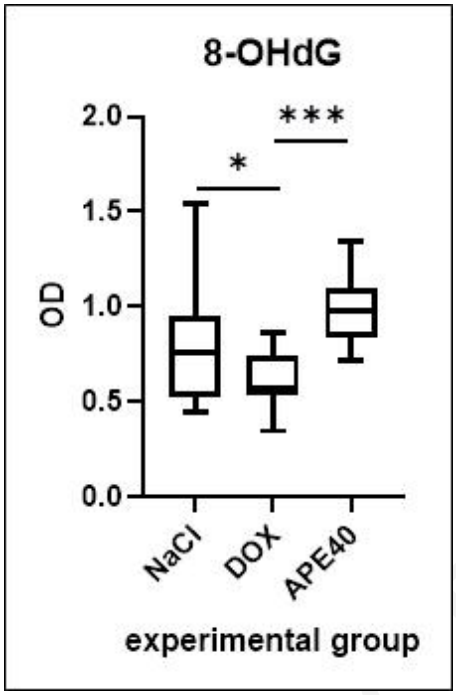

Figure 2. Levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the left ventricle (LV) of rats from the NaCl group, the doxorubicin-treated (DOX), and the doxorubicin- and apelin-13-treated (APE40) group, presented as optical density (OD) values. Data are presented as medians with interquartile ranges (IQR), derived from two technical replicates per animal. The number of technical replicates included in the analysis was as follows: NaCl: *n* =15, DOX: *n* = 15, APE40: *n* = 16.