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Antioxidant effect of nicotinic acid on experimental doxorubicin-induced chronic heart failure

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ABSTRACT

The antioxidant effects of niacin (nicotinic acid) were investigated in doxorubicin (DXR)-induced chronic heart failure in rats. For the experiment 30 Wistar albino rats (180-220 g) were divided into three groups. The control group received normal saline for 5 weeks. Experimental chronic heart failure (CHF) was induced by administering DXR (5 mg/kg) intramuscularly once a week for 5 weeks; nicotinic acid was administered intraperitoneally daily (10 mg/kg) for 5 weeks. In this experiment we studied the changes in the concentration levels of superoxide anion radical (SOR), hydrogen peroxide (HP), lipid peroxidation (LPO) products (diene conjugates (DC), thiobarbituric acid active products (TBA-AP) and Schiff bases (SCHB), and changes in the superoxide dismutase (SOD) and catalase (CAT) activity in the myocardium of animals. The results indicate that niacin administration against the DXR background decreases the oxidant parameters (SOR, HR), increases the antioxidant parameters (SOD, CAT), and LPO reaches a normal level. The obtained results are indicative of free radical scavenging attenuation in cardiomyocytes in rats with chronic heart failure, which allows us to consider nicotinic acid as a product with cardioprotective activity.

KEYWORDS: reactive oxygen species, superoxide dismutase, catalase, lipid peroxidation, nicotinic acid, experimental chronic heart failure, doxorubicin

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ABBREVIATIONS

AOS	-	antioxidant system
CAT	-	catalase
CHF	-	chronic heart failure
CVDs	-	cardiovascular diseases
DC	-	diene conjugates
DXR	-	doxorubicin
HP	-	hydrogen peroxide
HR	-	hydroperoxyl radical
LPO	-	lipid peroxidation
NA	-	nicotinic acid
NAD	-	nicotinamide adenine dinucleotide
NADP	-	nicotinamide adenine dinucleotide phosphate
PUFA	-	polyunsaturated fatty acid
SCHB	-	Schiff bases
SOD	-	superoxide dismutase
SOR	-	superoxide anion radical
TBA-AP	-	thiobarbituric acid active products

INTRODUCTION

Cardiovascular diseases (CVDs) are the foremost health problem in the world today. More than 500 thousand Ukrainians annually, i.e. about 1370 people everyday on average, die from CVDs. According to the State Statistics Service of Ukraine 2013, about 50% deaths are caused by CVDs. The primary cause of heart diseases is disorders in the metabolic processes at the cellular and sub-cellular levels. It is mainly connected with membrane LPO, excess synthesis of reactive

oxygen species and free radical formation in cardiomyocytes [1, 2].

In this work we used an experimental model of CHF caused by toxic effects of DXR, an anthracycline antibiotic. There are currently several explanations for the cytotoxicity of DXR. Among them are the DXR-induced free radical formation [3], inhibition of DNA replication enzymes [4], cellular enzyme inhibition by doxorubicinol (a substance formed as a result of DXR biochemical modifications in the cell) [5], and the SOR generation in the cells through the redox-cycling activity of DXR that ultimately leads to intracellular oxidative stress [6].

An anthracycline antibiotic-induced myocardial injury clinically leads to serious complications with fatal outcomes [7]. Hence looking for ways to protect the myocardium during DXR administration is one of the most important aspects needing attention. We considered nicotinic acid as a drug with potential cardioprotective activity. Nicotinic acid (niacin) has long been used as a treatment for lipid metabolism disorders and various CVDs. The recently discovered nicotinic acid receptor GPR109A (HM74A or PUMA-G), associated with G-proteins, has allowed expanding the knowledge about molecular mechanisms that are the basis of its metabolic and vascular effects [8, 9, 10]. It offers opportunities to make broader use of drugs based on nicotinic acid for CVD treatment and prevention.

In recent years, a number of *in vitro* and *in vivo* studies of the mechanisms of action of nicotinic acid show that it has antioxidant effects [11-13]. The aim of this work was to verify the possibility to use nicotinic acid to protect the myocardium from oxidative stress related to DXR-induced cardiomyocyte death, taking into account the pathological role of oxidative stress in the heart disease development and data of antioxidant effects of nicotinic acid.

MATERIALS AND METHODS

Studies were conducted in adult male Wistar rats (weight: 180-220 g). The animals were maintained (including euthanasia) pursuant to the *European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes*, as well as the provisions of the *General Ethical Principles of Animal Experiments* approved by the 1st National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine No. 3447-IV *On the Protection of Animals from Cruelty*. The animals were fed a normal, balanced diet and had free access to water in the animal house (vivarium) of the Bogomolets National Medical University (Kyiv City, Ukraine).

DXR-KMP in the form of a 0.01 g lyophilized powder for the solution for injection (manufactured by Kyivmedpreparat, OJSC, Ukraine) and nicotinic acid (niacin) BP, crystalline powder (substance) (manufactured by Aarti Drugs Ltd, India) were used in the work.

The animals were randomly divided into 3 groups (10 animals in each group):

1st group – animals that received weekly intramuscular injections of normal saline for 5 weeks (control);

2nd group – an experimental model of CHF: animals that received weekly intramuscular injections of DXR at 5 mg/kg of body weight for 5 weeks (experimental CHF);

3rd group - animals that received weekly intramuscular injections of DXR at 5 mg/kg of body weight for 5 weeks together with a daily intraperitoneal injection of nicotinic acid at 10 mg/kg of body weight (experimental CHF + nicotinic acid).

In the myocardial homogenates, we determined the content of reactive oxygen species (ROS) - SOR by the XTT formazan accumulation [14, 15] and concentration of HP by photocolorimetric method [16, 17]. We measured antioxidant enzyme activity in the myocardium, that is, SOD (EC 1.15.1.1), by the S. Chevari *et al*. method [18] and CAT (EC 1.11.1.6) activity by measuring the reaction of CAT with HP [19].

LPO rate in cardiac tissues was determined by examining the accumulation of polyunsaturated fatty acid (PUFA) peroxidation products, that is, DC, TBA-AP and SCHB. DC in the homogenates was measured spectrophotometrically [20], TBA-AP was determined by the I. D. Stalnaya *et al.* method [21] and SCHB by fluorescence intensity of lipid solutions in chloroform using the Shimadzu RF-510 fluorometer (Japan) [22].

Protein concentration in homogenate was determined by the Lowry method [23]. Significance of differences between experimental and control groups was evaluated by Student's t-test. We regarded the differences as statistically significant at $p < 0.05$.

RESULTS

DXR belongs to the category of anthracycline antibiotics and is one of the most commonly used chemotherapy drugs for the treatment of human cancers. At the same time it is well known that DXR is characterised by a number of adverse effects, primarily due to a significant cardiotoxicity. A great number of scientific research papers have been published in which the authors describe various molecular mechanisms that explain DXR-induced cardiotoxicity [24].

In our work, intensive SOR and HP formation were observed in myocardium of rats with experimental CHF as compared to rats in the control group ($p < 0.05$) (Table 1). Unfortunately, the increase in ROS is associated with a low antioxidant activity of the myocardial tissue. SOD activity in cardiomyocytes of animals with experimental CHF decreased by 1.4 times from 2.15 ± 0.18 conv. units. × min^{-1} × mg $protein^{-1}$ (control) to 1.51 ± 0.11 conv. units. × min^{-1} × mg $protein^{-1}$ ($p < 0.05$), while CAT activity decreased by 1.6 times from 4.17 ± 0.35 nm × min^{-1} × mg $protein^{-1}$ to 2.54 ± 0.23 nm × min^{-1} × mg $protein^{-1}$ (control) ($p < 0.05$) (Table 2).

High rates of LPO products namely DC, TBA-active products and SCHB in the myocardium of rats with experimental CHF as compared to control group ($p < 0.05$) (Tables 3, 4) indicate the development of oxidative stress in animals. These results are consistent with the data from literature related to activation of free radical reactions as well as LPO reactions in the myocardium of animals that received DXR injections.

Hence the model established in rats with experimental CHF was further regarded as an oxidative stress model. The main aim of our research was to search for ways to rectify violations found, as well as to protect myocardium from DXR toxic effects.

Table 1. The nicotinic acid effect on the reactive oxygen species content in myocardium in rats with experimental chronic heart failure (M ± m), n = 10.

Animal group	SOR, µmol of XTT formazan × mg $protein^{-1}$	HP, µmol × mg $protein^{-1}$
1st (Control)	8.39 ± 0.68	6.72 ± 0.56
2nd (experimental CHF)	15.17 ± 1.02*	10.03 ± 0.82*
3rd (experimental CHF + nicotinic acid)	12.03 ± 1.04*/#	8.36 ± 0.76*/#

*$p < 0.05$ compared to the 1st group (control group).
#$p < 0.05$ compared to the 2nd group.

Table 2. The effect of nicotinic acid on the superoxide dismutase and catalase activity in myocardium in rats with experimental chronic heart failure (M ± m), n = 10.

Animal group	SOD activity, conv. units. × min^{-1} × mg $protein^{-1}$	CAT activity, nm × min^{-1} × mg $protein^{-1}$
1st (Control)	2.15 ± 0.18	4.17 ± 0.35
2nd (experimental CHF)	1.51 ± 0.11*	2.54 ± 0.23*
3rd (experimental CHF + nicotinic acid)	1.77 ± 0.14	2.96 ± 0.27*

*$p < 0.05$ compared to the 1st group (control group).

Table 3. The effect of nicotinic acid on the LPO (content of diene conjugates) in myocardium in rats with experimental chronic heart failure (M ± m), n = 10.

Animal group	DC, nm × mg protein^{-1}
1st (Control)	237.61 ± 23.09
2nd (experimental CHF)	352.59 ± 29.48*
3rd (experimental CHF + nicotinic acid)	298.81 ± 26.92*$^{/\#}$

*$p < 0.05$ compared to the 1st group (control group).
$^{\#}p < 0.05$ compared to the 2nd group.

Table 4. The effect of nicotinic acid on the lipid peroxidation in myocardium in rats with experimental chronic heart failure (M ± m), n = 10.

Animal group	TBA-reactive products nm × mg protein^{-1}	SCHB conv. units. × mg protein^{-1}
1st (Control)	81.84 ± 7.43	7.54 ± 0.52
2nd (experimental CHF)	105.16 ± 9.75*	9.49 ± 0.88*
3rd (experimental CHF + nicotinic acid)	90.11 ± 7.79$^{\#}$	8.32 ± 0.71$^{\#}$

*$p < 0.05$ compared to the 1st group (control group).
$^{\#}p < 0.05$ compared to the 2nd group.

The fact that DXR toxic effects are associated with superoxide radical formation [25, 26] is an important argument in favour of justification for using antioxidants as agents preventing DXR side effects.

As a cardioprotective drug, we chose nicotinic acid (niacin, vitamin PP) - vitamin involved in many oxidative reactions of living cells [27]. Nicotinic acid and its amide play a significant role in the body; they are the part of NAD^+ and $NADP^+$ acting as coenzymes of various dehydrogenases that are hydrogen carriers and perform redox processes in cells [28].

In animals of the 3rd group receiving DXR together with nicotinic acid, the ROS concentration in myocardium was lower as compared to the group with experimental CHF (Table 1). Moreover, HP concentration in cardiomyocytes of the 3rd animal group (8.36 ± 0.76 μmol × mg protein^{-1}) decreased as compared to the group with experimental CHF (10.03 ± 0.82 μmol × mg protein^{-1}).

It should be noted that in the myocardium of animals with experimental CHF the SOR concentration increased by 1.8 times, from 8.39 ± 0.68 μmol of XTT formazan × mg protein^{-1} (control) to 15.17 ± 1.02 μmol of XTT formazan × mg protein^{-1} ($p < 0.05$), while in the group of animals receiving nicotinic acid this index increased only by 1.4 times, from 8.39 ± 0.68 μmol of XTT formazan × mg protein^{-1} (control) to 12.03 ± 1.04 μmol of XTT formazan × mg protein^{-1} ($p < 0.05$) (Table 1). Thus, nicotinic acid reduces the DXR ability to generate the SOR in cardiomyocytes that results in DXR cytotoxicity decrease.

The dynamics of antioxidant enzyme activity shows the opposite results both in the 3rd group of animals and in the group with experimental CHF. SOD activity in the myocardium of 3rd animal group increased (from 1.51 ± 0.11 conv. units × min^{-1} × mg protein^{-1} to 1.77 ± 0.14 conv. units × min^{-1} × mg protein^{-1}) ($p < 0.05$), the CAT activity increased from 2.54 ± 0.23 nm × min^{-1} × mg protein^{-1}

(2nd group) to 2.96 ± 0.27 nm × min^{-1} × mg $protein^{-1}$ (3rd group), but was 1.4 times lower than in the control group (4.17 ± 0.35 nm × min^{-1} × mg $protein^{-1}$) (Table 2).

Analysis of CAT activity and HP concentration in cardiomyocytes of 3rd animal group shows that 1.2 time increase of CAT activity as compared to the 2nd group (Table 2) had influence on HP concentration, which decreased by 1.2 times from 10.03 ± 0.82 μmol × mg $protein^{-1}$ to 8.36 ± 0.76 μmol × mg $protein^{-1}$ as compared to the group with experimental CHF (Table 1).

In the presence of nicotinic acid, LPO activation was observed only during a stage of synthesis of the primary products - DC. The DC concentration in the 3rd group was 1.3 times higher than in the control group (298.81 ± 26.92 nm x mg $protein^{-1}$ and 237.61 ± 23.09 nm x mg $protein^{-1}$, respectively) ($p < 0.05$), but lower than 1.2 times as compared to the group of experimental CHF (352.59 ± 29.48 nm x mg $protein^{-1}$) (Table 3). At the same time, the levels of secondary products of LPO, TBA-APs, and end products of LPO, SCHBs, had no statistically significant differences compared to the control group (Table 4), but as in the case of DC, were 1.2 times lower compared to the group with experimental CHF.

DISCUSSION

Oxidative stress is caused by an imbalance between excessive ROS formation and decrease in the body's antioxidant defense. ROS play a dual role in a variety of physiologically normal and pathological conditions. In physiological concentrations, ROS transmit signals of external and internal environment of the body through regulatory metabolic cascades, act as mediators and redox messengers in various cellular processes and intracellular signalling systems [29]. At the same time, in certain pathological conditions, excessive ROS accumulation promotes cell death through induction of oxidative damage to cellular macromolecules, such as lipids, proteins and DNA [30]. Increased damage from ROS determines the cell fate through induction of cell cycle arrest and apoptosis [31]. Normal tissues, balancing between synthesis and elimination of ROS, maintain intracellular redox homeostasis [32].

As opposed to free-radical processes in the body, there is an antioxidant system consisting of a complex network of protective mechanisms for cells, tissues and organs that preserve and maintain body homeostasis. Balance between these two opposing components, in a state of physiological optimum, keeps peroxidation at a certain low level, preventing chain oxidative process, and describes the antioxidant status in the body [33]. One of the ways to prevent oxidative stress is antioxidant system activation, the components of which, in small concentrations, can inhibit the excessive free radical generation.

The obtained results are consistent with the results of Ganji *et al.* indicating that nicotinic acid inhibits endotheliitis by reducing ROS generation and subsequent oxidation of low density lipoproteins, as well as the production of cytokines, the key stages of atherogenesis [34]. Studies in both animal and cell culture recently made by Wu *et al.* show that nicotinic acid inhibits vascular inflammation through induction of Nrf2-regulated heme oxygenase activity (HO-1) [35].

Despite the fact that the basic mechanisms involved in antidyslipidemic effects of nicotinic acid are described in sufficient detail, the nicotinic acid participation in cell viability and oxidative stress development is not fully comprehensible. However, the reduction of free fatty acid concentration through activation of Gi-protein-mediated inhibition of adenylate cyclase and subsequent cAMP formation [36] that may affect the activity of cAMP-dependent protein kinase A and phosphorylation of hormone-sensitive lipase, may play an important role [37] in the mechanism for the antioxidant effects of nicotinic acid.

CONCLUSION

In this work we studied the influence of nicotinic acid on the development of anthracycline antibiotic-induced myocardial injury and its main mechanisms on the basis of a comprehensive examination of antioxidant system (AOS) and LPO in the myocardium. In the conditions of experimental CHF, we established that the presence of nicotinic acid reduces the free radical presence and DXR-caused deterioration of the metabolism. The antioxidant effects of nicotinic acid are

manifested by the normalization of redox process in the myocardium through inhibition of reactive oxygen species namely, SOR and HP, as well as oxidation of PUFA. This result prompts us to consider the potential for the use of nicotinic acid as a drug reducing DXR cardiotoxicity.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest with respect to this article.

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