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Activation of ATP-sensitive potassium channels prevents doxorubicin-induced mitochondrial dysfunction in the heart and impaired vascular responses in rats

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One of the side effects of the anticancer drug doxorubicin is its mitotoxicity. At the same time, a sufficient number of functioning mitochondria is required for normal energy supply to the heart. The system of ATP-sensitive potassium channels (K_{ATP}-channels) of cytoplasmic and mitochondrial membranes is considered to be the central metabolic sensor of energy supply, and their opening triggers mechanisms of protection against cell damage and death under the influence of pathological factors. The aim of our study was to investigate the effect of K_{ATP}-channel opener flocalin on doxorubicin-induced mitochondrial dysfunction in the heart, impaired vascular contraction-relaxation function, and oxidative stress. Acute cardiotoxicity was modelled by short-term intraperitoneal injection of doxorubicin in a total dose of 15 mg/kg. To prevent damage, animals were administered flocalin at a dose of 2.5 mg/kg for 5 consecutive days. It was found that the rate of formation of superoxide anion ($•O_2^-$) and hydroxyl radical (•OH) in the heart mitochondria significantly increased after administration of doxorubicin by 10.5 and 3.4 times, respectively, and the level of H_2O_2 increased by 5.3 times. When rats were administered flocalin against the background of doxorubicin, oxidative stress indicators were significantly reduced, namely, the rate of $•O_2^-$ and •ON generation was 4 and 1.6 times lower, respectively, and the H_2O_2 levels were 4.6 times lower. Under conditions of impaired redox status in the rat heart after doxorubicin administration, mitochondrial permeability transition pore opening was activated: the amplitude of spontaneous swelling doubled, and Ca^{2+}-induced swelling increased 1.5-fold. The use of flocalin reduced the amplitude of mitochondrial swelling in calcium-free medium by 84.6%, and under the conditions of action inducer of mPTP opening calcium, this index was restored to control values. Endothelium-dependent relaxation of aorta preparations of doxorubicin-treated animals to acetylcholine (0.1 μmol/l) was 47% less than in the control group. Contractions of aortic rings in these animals under the influence of norepinephrine (10 μmol/l) were reduced by 59% compared to control rats. When flocalin, a K_{ATP}-channel opener, was injected into rats, the contractile responses of isolated rat aortic rings were restored almost to the values of control animals, while the endothelium-dependent vasodilator effects of acetylcholine (0.1 μmol/l) under the influence of flocalin were restored by 69% compared with animals injected with doxorubicin. Thus, the opening of K_{ATP}-channels by flocalin prevents doxorubicin-induced mitochondrial dysfunction in the heart, reduces oxidative stress and prevents vascular contraction-relaxation disorders.

Key words: doxorubicin; K_{ATP}-channels; flocalin; mPTP; oxidative stress; heart; vascular responses.

INTRODUCTION

Anthracyclines are potent cytotoxic antibiotics with a wide range of clinical applications as anticancer agents [1]. The main anthracyclines approved by the FDA for clinical use are doxorubicin and its derivatives: daunorubicin and idarubicin [2]. It is known that doxorubicin, when it enters a cell, on the one hand, inhibits tumor progression by interfering with DNA replication and transcription. This occurs through intercalation into the DNA strand, interference with DNA unwinding, changes in helicase activity, displacement of histones from open and transcriptionally active chromatin regions and, as a result, changes in the epigenome [2]. On

the other hand, doxorubicin inhibits tumor cell topoisomerase IIα, forming a complex TOPIIα-Dox-DNA, which leads to double-stranded DNA breaks [3]. In contrast to tumor cells, cardiomyocytes do not express TOPIIα but instead express TOPIIβ [4], which can also form a TOPIIβ-Dox-DNA complex, causing double-stranded DNA breaks and leading to cardiomyocyte death. It is assumed that mitochondrial DNA, compared to nuclear DNA, may be more sensitive to such damage due to its low repair capacity and proximity to the respiratory chain. Given that mitochondrial DNA encodes about 37 genes required for the functioning of oxidative phosphorylation [1], it is clear that the activity of respiratory chain enzymes is reduced under the influence of doxorubicin, leading to mitochondrial dysfunction. According to previous studies, the effect of doxorubicin on mitochondria may also be the induction of the mitochondrial permeability transition pore (mPTP), the opening of which can trigger apoptosis and cell death [5]. Doxorubicin induces calcium homeostasis disorders, but an increase in Ca^{2+} content alone is relatively ineffective in triggering mPTP opening, howsoever sensitivity to this inducer can be significantly enhanced by ATP depletion and oxidative stress [6]. It is known that the doxorubicin molecule must be reduced to a semiquinone to become active. The reduction of the drug occurs in the respiratory chain, resulting in the generation of free radicals: superoxide anion (·O2-) and hydrogen peroxide (H_2O_2), which is subsequently metabolized in the Fenton and Haber-Weiss reactions to form hydroxyl radical (·OH) [7]. In addition, doxorubicin forms an oxygen radical by being directly reduced in the reductase domain of eNOS. When the concentration of doxorubicin increases, the uncoupled state of eNOS is observed, in which the enzyme produces $•O_2^-$ instead of NO. It has also been shown that doxorubicin is reduced in free unchelated iron to form Fe^{2+}, which is a catalyst for the Fenton reaction. These factors make the mitochondrial inner membrane highly permeable to all dissolved substances with a molecular weight of up to 1.5 kDa. The mPTP opening leads to a collapse of the mitochondrial inner membrane potential, uncoupling of the respiratory chain, the influx of calcium ions, stoppage of ATP synthesis, the release of pro-apoptotic proteins, and, ultimately, swelling, rupture and death of cell mitochondria. Consequently, doxorubicin has mitotoxic properties, while a sufficient number of functioning mitochondria is required for the normal energy supply of the heart function [8]. Calcium overload of organelles and their hyperproduction of reactive oxygen species (ROS) are crucial in cardiomyopathies andunderlie the development of cardiovascular pathology [9]. At the same time, there are endogenous protection mechanisms in response to these metabolic shifts. One of them isthe system of adenosine triphosphate-sensitive potassium channels (K_{ATP}-channels) of cytoplasmic and mitochondrial membranes. They are considered to be the central metabolic sensor of energy supply [10], and their opening triggers mechanisms of protection against cell damage and death under the influence of pathological factors [11, 12]. The aim of our study was to investigate the effect of K_{ATP}-channel opener flocalin on doxorubicin-induced mitochondrial dysfunction in the heart, impaired vascular contraction-relaxation function, and oxidative stress.

METHODS

Laboratory animals. Experiments were performed on 30 male Wistar rats weighing 250-350 g, aged 6 months. Animals were housed in the vivarium of Bogomoletz Institute of Physiology in a neutral temperature environment (22 ± 2°C) on a natural day-to-night cycle with free access to water and on a standard diet. All procedures were conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (22.09.2010). The study used doxorubicin “Ebewe” 50 mg/25 ml (Austria) and domestic fluorine-containing opener of K_{ATP} channels flocalin. The drug was

administered intraperitoneally. Animals were removed from the experiment on the 5th day. Rats were randomly divided into three groups of 10 animals each: in group I there were control rats that were injected with physiological solution (5 ml/kg); in the II group, rats were administered doxorubicin in a total cumulative dose of 15 mg/kg for 2 consecutive days; in group III, rats were administered doxorubicin in the same regimen and flocalin in a dose of 2.5 mg/kg for 5 consecutive days, on the first two days 30 min after the administration of doxorubicin. Flocalin was administered after previously dissolving according to the scheme: 0.032 ml of dimethylsulfoxide and 0.9 ml of physiological solution were added to 1 mg of flocalin.

Registration of mitochondrial swelling in the heart of rats. Mitochondria were isolated by the method of differential centrifugation in our modification [13]. Hearts were washed with cooled 0.9% KCl solution (4ºC). The heart tissue was minced and homogenized in isolation medium (mmol/l): sucrose - 250, EDTA - 1, Tris-HCl - 25; pH 7.4. The homogenate was centrifuged at 700g for 8 min at 2°C to pellet nuclei and cell fragments. The resulting supernatant was subjected to centrifugation at a speed of 11,000g for 16 min and 2ºC to precipitate the mitochondrial fraction and washed again. The sediment was stored in a resuspension medium (mmol/l): sucrose - 250, Tris-HCl - 25; pH 7.2. After 30 min, the isolated organelles were used in the experiment. mPTP opening was investigated by spectrophotometric recording of mitochondrial swelling using a spectrophotometer UV 1900 Shimadzu (Japan). For this purpose, mitochondria were placed in the incubation medium of isotonic composition (mmol/l): KCl - 120, Tris-HCl - 25, KH_2PO_4 - 3, sodium succinate - 5, pH 7.4, and a decrease in the optical density in mitochondria suspension was recorded at λ = 520 nm for 15 min of the mitochondria swelling. The change in the amplitude (ΔD_{520}) of organelle swelling was determined as the difference between the mitochondrial swelling amplitude at 15 min and relative to the initial value at 1 min. As a control, mitochondria suspension was used in the incubation medium in the absence of an inducer, with the following registration of the optical density for 15 min. A decrease in the optical density of the solution with mitochondria indicated their swelling. Protein concentration was 0.4 mg/ml.

Study of biochemical indicators of oxidative stress in heart mitochondria of rats. The methods used to assess oxidative stress are described in detail in our previous work [14]. We measured the rate of superoxide ($\bullet O_2^-$) and hydroxyl radical (•OH) formation and the levels of hydrogen peroxide (H_2O_2) in the heart mitochondria of rat. The method for determining the content of superoxide anion is based on the ability of cytochrome c to oxidize $\bullet O_2^-$ to O_2. Changes in the extinction of the samples were recorded after 30 min of incubation (37°C) at λ = 550 nm. To calculate the concentration of superoxide, the molar absorption coefficient of 28,000 $mol^{-1} \cdot cm^{-1}$ was used [15]. The rate of hydroxyl radical generation was determined by the method of deoxyribose oxidation, was measured by the increase in absorbance at λ = 532 nm, and was expressed in conventional units $\Delta E \cdot 10^2$ in 60 min per 1 mg of protein [16]. H_2O_2 definition consisted of the indirect registration of its utilization during the oxidation of iodide (I-) to iodine (I_3-) under conditions of excess lactoperoxidase. The formation of I_3- was recorded spectrophotometrically at λ = 353 nm, and its amount was determined using the molar absorption coefficient of 26,000 $mol^{-1} \cdot cm^{-1}$ [17].

Registration of contraction-relaxation of isolated preparations of the aorta. The contraction-relaxation reactions of isolated vascular preparations were studied in experiments on isolated aortic rings of rats, perfused at the temperature of 37°C normal Krebs solution. The isolated vascular rings had a diameter of 2 mm and a width of 1.5 mm. Measurements of contraction-relaxation reactions were made in the isometric mode, at the initially set tension of the vascular preparation, when it developed maximum contractions in response to

the introduction of noradrenaline (10 μmol/l). The temperature of the working solution in the perfusion system and the experimental chamber was maintained using a KISS 208B automatic thermostat (Huber, Germany). The working solution was saturated with oxygen using a gas mixture containing 95% oxygen and 5% carbon dioxide. Before the experimental measurements, the aortic rings fixed and stretched as indicated above were kept in the working chamber until their stabilization. Krebs solution contained (in mmol/l): NaCl - 120.4; $NaHCO_3$ - 15.5; NaH_2PO_4 - 1.2; KCl - 5.9; glucose - 11.5; $CaCl_2$ - 2.5; $MgCl_2$ - 1.2. Research on the effects of contraction-relaxation of aorta preparations was carried out with the help of norepinephrine (10 μmol/l) and acetylcholine (0.1 μmol/l).

Statistical analysis. Excell (MS Office 2021) and Origin 8.0 ("Microcal Software Inc.", USA) programs were used for statistical analysis of the obtained results. Shapiro-Wilk test was used to evaluate the normality of distribution of data in each group. Comparison between groups was made using one-way analysis of variance (ANOVA) followed by post hoc Tukey HSD test or non-parametric Kruskal-Wallis test for multiple independent samples with post hoc test by the methods of Conover. $P < 0.05$ was assumed as statistically significant. The data were expressed as mean ± SEM.

RESULTS AND DISCUSSION

In the experiments on heart mitochondria, it was shown that the change in the amplitude of organelle swelling ($\Delta D_{520} \cdot 10^2$) in the calcium-free medium was 4.6 and 9.9 units of extinction in control rats and rats after a course of doxorubicin administration, respectively (Fig. 1A). When loaded with calcium ions (0.1 mmol/l) in the incubation medium, a high-amplitude swelling of the heart mitochondria in the control and doxorubicin-treated rats was recorded, which differed significantly in amplitude: these values increased to 18.1 and 27.5 units of extinction, respectively (Fig. 1B). Thus, an increase in the amplitude of spontaneous and Ca^{2+}-induced mitochondrial swelling in the hearts of rats after a course of doxorubicin administration compared with control rats was due to the toxic effect of the drug on the heart mitochondria of experimental rats and indicates activation of pore formation in the heart after its administration.

In the experiments with activation of K_{ATP}-channels by flocalin, Ca^{2+} at a concentration of 0.1 mmol/l was used to induce mPTP opening. In our study, it was found that the use of flocalin in doxorubicin-treated animals reduced the amplitude of mitochondrial swelling in calcium-free medium by 84.6% (see Fig. 1A). Its value was 6.0 units of extinction. With the use of the inducer of mPTP opening calcium, the amplitude of swelling of rat heart mitochondria after flocalin treatment was practically the same as in control animals (see Fig. 1B). Thus, the administration of flocalin effectively prevented the mitotoxic effect of doxorubicin, which was manifested in the opening of the mPTP. Previously, we have shown that preincubation of mitochondria with flocalin reduced calcium-induced swelling of organelles in the heart of adult rats [18]. Thus, the preliminary opening of mitochondrial K_{ATP}-channels with a fluoride-containing opener prevented the calcium-induced opening of the mPTP in the heart of animals. In addition, in vivo experiments have shown that flocalin injection reduces the sensitivity of mPTP to calcium in rat hearts [18]. There are several explanations for this effect of drugs in this class. On the one hand, it is a decrease in calcium ion entry into mitochondria, which is a consequence of depolarization of the mitochondrial inner membrane potential after activation of mitochondrial K_{ATP}-channels. On the other hand, it is a moderate mitochondrial swelling that occurs as a result of potassium ions and the simultaneous passive intake of weak acid and water anions, which to some extent protects mitochondria by preserving the structure and function of their intermembrane space [11].

The antioxidant properties of K_{ATP}-channels and their activators, in particular flocalin, are

well known [19, 20]. It has also been previously shown that activation of K_{ATP}-channels reduced plasma uric acid pools, which may indicate inhibition of xanthine oxidase activity [19, 20], which acts as a catalyst in the reduction of doxorubicin to form $\bullet O_2^-$. Flocalin also inhibited the activity of the heme oxygenase reaction, reducing the pools of free unchelated iron [20]. However, how the doxorubicin-induced oxidative stress changes with the administration of flocalin has not yet been studied. Fig. 2 shows biochemical parameters characterizing oxidative stress in the heart mitochondria of control and

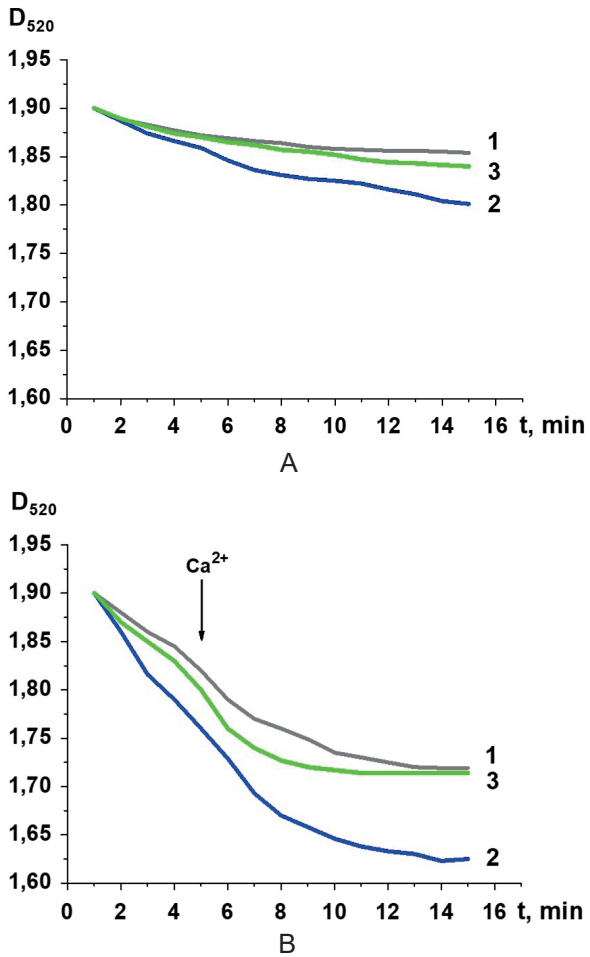

Fig. 1. Typical kinetic curves of mitochondrial swelling in adult rat hearts under the influence of doxorubicin at a dose of 15 mg/kg and flocalin at a dose of 2.5 mg/kg for 5 days in calcium-free (A) and calcium-containing (Ca^{2+}, 0.1 mmol/l) medium (B). 1 - control, 2 - exposure to doxorubicin, 3 - exposure to doxorubicin and focalin

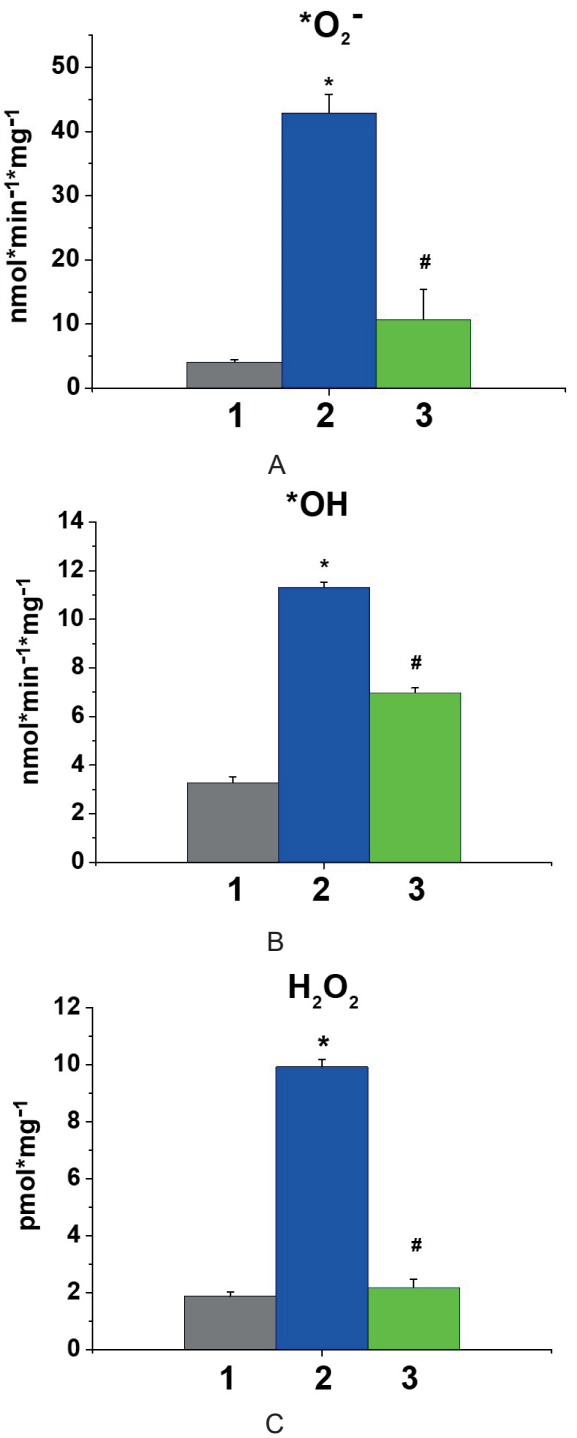

Fig. 2. The rate of superoxide anion ($\bullet O_2^-$) (A) and hydroxyl radical ($\bullet OH$) formation (B), and hydrogen peroxide (H_2O_2) levels (C) in the mitochondria of control animals (1), doxorubicin-treated rats (2) and rats after the combined effect of doxorubicin and flocalin (3). *P < 0.05 compared to the values of control animals; #P < 0.05 compared to the values of animals after doxorubicin administration

doxorubicin-treated rats, and in doxorubicin-treated animals after flocalin administration.

It was shown that the rate of formation of $\bullet O_2^-$ and •OH radicals in heart mitochondria significantly increased after doxorubicin administration by 10.5 and 3.4 times, respectively (see Fig. 2A, B). The levels of H_2O_2 in doxorubicin-treated rats significantly increased by 5.3-fold compared to intact animals (see Fig. 2C). This indicates a significant increase in free radical processes in the heart organelles. The administration of flocalin to doxorubicin-treated rats significantly reduced the oxidative stress (see Fig. 2). In particular, the rate of generation of $\bullet O_2^-$ decreased 4-fold, the rate of formation of the most active oxygen radical •OH after activation of K_{ATP}-channels decreased 1.6-fold, and the levels of H_2O_2 were 4.6-fold lower, compared to the values in rats damaged by doxorubicin. Thus, our studies indicate that K_{ATP}-channels opening by flocalin inhibits free radical processes in heart mitochondria activated by the toxic effects of doxorubicin.

Along with the theory of tumor cell genome damage, there is an assumption that toxic damage to endothelial cells with the development of severe endothelial dysfunction is the main mechanism of doxorubicin's antitumor effect [21]. It is known that the vascular endothelium plays a crucial role in forming a separation barrier between blood and tissues, as well as regulating vascular tone. Endothelial dysfunction is a common phenomenon in many diseases, including toxic lesions of various etiologies. Such imbalance in the circulatory system as blood pressure instability in the form of a significant increase is often observed in patients during treatment with doxorubicin, which is an additional risk factor for cardiovascular complications and requires the introduction of cardiological drugs into the anti-cancertreatment regimens. It is believed that the formation of superoxide radical anions as a result of redox cycles of doxorubicin is crucial in mediating endotheliotoxicity, whichleads to loss of endothelial barrier function, increased permeability, impaired vasculartone regulation and increased arterial stiffness [21].

Pharmacological opening of K_{ATP}-channels of smooth muscle and endothelial cells leads to dilation of blood vessels, which contributes to increased supply of oxygen and energy resources to tissues. It should be noted that the vasodilator effects of activators of these channels, in particular fluoride-containing ones, are quite powerful and dose-dependent even in pathological conditions accompanied by a significant violation of systemic blood pressure [22, 23]. For example, in experiments on isolated rings of aorta from spontaneously hypertensive rats, the effects of the fluorine-containing potassium channel opener flocalin were virtually indistinguishable in amplitude from those in animals with normal blood pressure [22]. In vivo experiments have shown that intravenous and oral administration of flocalin dose-dependently reduces systemic blood pressure and dilates coronary arteries. In accordance with the reduction in blood pressure, peripheral vascular resistance decreased in a dose-dependent manner [24, 25].

In experiments on isolated aortic vascular rings, we demonstrated doxorubicin-induced disruption of both vascular relaxation and contraction mechanisms (Fig. 3).

In particular, the effects of aortic rings contraction in doxorubicin-treated rats in response to norepinephrine (10 μmol/l) in our experiments were 2.4-fold lower ($P < 0.002$) compared with control rats (see Fig. 3A). A similar weakening of norepinephrine-induced contraction in the aorta of doxorubicin-treated animals was also shown in other studies [26, 27]. Moreover, in other vessels, the changes were statistically insignificant and the authors explained this by regional differences in the density of alpha-adrenoceptors in animal vessels and a decrease in the number of corresponding receptors due to the effect of doxorubicin. However, the mechanism of selective downregulation of alpha-adrenoceptors in these experiments was not elucidated [26, 27].

At the same time, vascular relaxation in doxorubicin-modified rats at a dose of acetylcholine of 0.1 μmol/l was 47% lower ($P < 0.03$) than in control animals (see Fig. 3B), that confirms the general idea of a decrease in endothelium-dependent vascular relaxation in doxorubicin-treated animals [27, 28]. A decrease in the endothelium-dependent vasodilator effects of acetylcholine may indicate a disturbance in the nitric oxide system, which is a powerful mechanism of vascular relaxation, including through signalling pathways such as cGMP activation and K_{ATP}-channels [29].

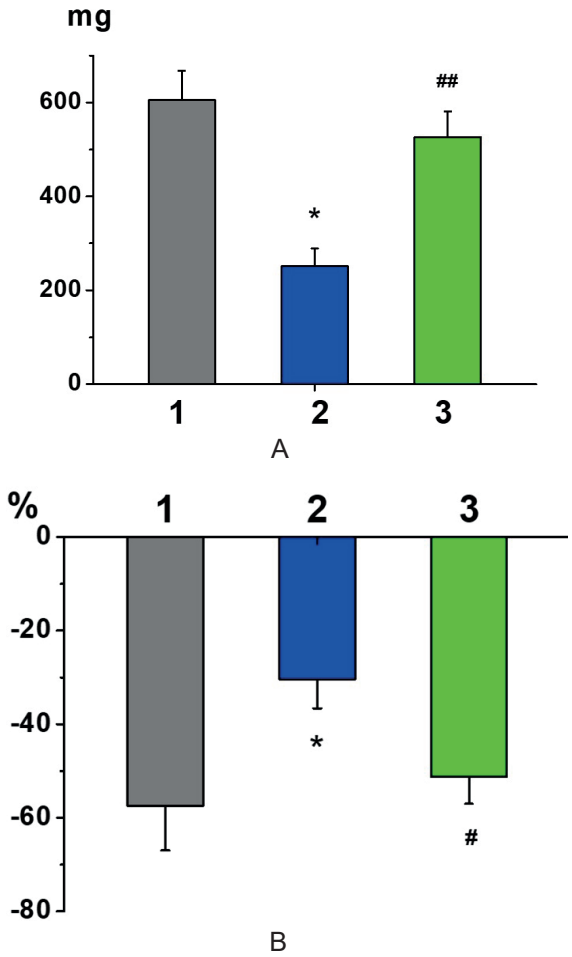

Fig. 3. Contractile responses of aortic rings to norepinephrine (10 μmol/l) (A) and endothelium-dependent relaxation of aortic rings to acetylcholine (0.1 μmol/l) (B). 1 - control group, 2 - effect of doxorubicin, 3 - effect of doxorubicin and flocalin. *$P < 0.05$ relative to the values of the control group, #$P < 0.05$ relative to the values of the group of rats treated with doxorubicin alone, ##$P < 0.002$ relative to the values of the group of rats treated with doxorubicin alone

Thus, the severe endothelial dysfunction demonstrated in our experiments on isolated aortic vascular rings of doxorubicin-modified rats indicates the vascular toxic effect of the drug caused by free radicals, which are products of anthracycline reduction. These substances adversely affect endothelial cell membranes and initiate apoptosis [30].

The administration of K_{ATP}-channels opener flocalin significantly reduced the disturbance of both contraction and relaxation of vascular preparations. In particular, after administration of flocalin, the contractile responses of isolated rat aortic rings were restored almost to the level of control animals, namely, increased by 2.1 times ($P < 0.002$) compared with doxorubicin-modified animals (see Fig. 3A). At the same time, the endothelium-dependent vasodilator effects of acetylcholine (0.1 μmol/l) under the influence of flocalin were restored by 69% ($P < 0.05$) compared with doxorubicin-modified animals (see Fig. 3B).

Thus, the activation of K_{ATP}-channels in smooth muscle and endothelial cells of rat arteries under the damaging effect of doxorubicin prevents significant impairment of vascular contraction-relaxation function. The mechanism underlying the vasodilator effects of $K_{ATФ}$-channel opening is hyperpolarization of the sarcolemmal membrane, reduction of Ca^{2+} influx into the smooth muscle cell, and decrease in vascular tone. Activation of K_{ATP}-channels in endothelial cells causes the release of nitric oxide molecules. The development of vasodilator responses may also involve the K_{ATP}-channels of mitochondrial membranes, the opening of which, through several signalling pathways, including protein kinases and ROS, can lead to the opening of K_{ATP}-channels of the sarcolemmal membrane [11, 31, 32]. However, in our case, administration of flocalin to animals prevents the weakening of endothelium-dependent relaxation to acetylcholine.

CONCLUSIONS

Thus, the activation of K_{ATP}-channels by flocalin prevents doxorubicin-induced dysfunction of rat heart mitochondria by inhibiting mPTP opening and significantly reducing oxidative stress indicators: the rate of superoxide anion and hydroxyl radical generation and the levels of hydrogen peroxide. Likely, these beneficial changes caused by the opening of K_{ATP}-channels by flocalin contribute to the prevention of vascular contraction-relaxation disorders, namely endothelium-dependent relaxation in response to acetylcholine and contraction in response to norepinephrine.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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АКТИВАЦІЯ АТФ-ЧУТЛИВИХ КАЛІЄВИХ КАНАЛІВ ПОПЕРЕДЖУЄ ДОКСОРУБІЦИНІНДУКОВАНУ МІТОХОНДРІАЛЬНУ ДИСФУНКЦІЮ В СЕРЦІ ТА ПОРУШЕННЯ СУДИННИХ РЕАКЦІЙ У ЩУРІВ

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Одним із побічних ефектів протипухлинного препарату доксорубіцину є його мітотоксичність. Водночас для нормального енергозабезпечення функції серця необхідна достатня кількість функціонуючих мітохондрій. Систему АТФ-чутливих калієвих каналів ($K_{АТФ}$-каналів) цитоплазматичних і мітохондріальних мембран вважають центральним метаболічним сенсором щодо енергозабезпечення і їх відкривання запускає механізми захисту від пошкодження і загибелі клітин при дії патологічних чинників. Метою нашої роботи було вивчення впливу відкривача $K_{АТФ}$-каналів флокаліну на доксорубіциніндуковані дисфункцію мітохондрій серця, порушення функції скорочення-розслаблення судин та оксидативний стрес. Гостру кардіотоксичність моделювали короткостроковим введенням щурам внутрішньоочеревинно доксорубіцину у загальній дозі 15 мг/кг. Для попередження пошкодження тваринам вводили флокалін у дозі 2,5 мг/кг 5 днів поспіль. Було виявлено, що швидкість утворення супероксид-аніона ($•O_2^-$) і гідроксильного радикала (•OH) у мітохондріях серця достовірно збільшувалася після введення доксорубіцину у 10,5 та 3,4 раза відповідно, а вміст H_2O_2 підвищилися у 5,3 раза. При введенні щурам флокаліну на тлі дії доксорубіцину значно зменшувалися показники оксидативного стресу: швидкість генерації $•O_2^-$ та •OH у 4 та 1,6 раза відповідно, а вміст H_2O_2 у 4,6 раза. В умовах порушення редокс-статусу у серці щурів після введення доксорубіцину активувалося відкривання мітохондріальної пори: амплітуда спонтанного набухання збільшувалася вдвічі, Ca^{2+}-індукованого набухання – у 1,5 раза. Застосування флокаліну зменшувало амплітуду набухання мітохондрій у безкальцієвому середовищі на 84,6%, в умовах дії індуктора цей показник відновлювався до контрольних значень. Ендотелійзалежна релаксація препаратів аорти тварин після введення доксорубіцину на дію ацетилхоліну (0,1 мкмоль/л) була меншою на 47%, ніж у контрольній групі. Водночас скорочення кілець аорти у цих щурів при дії норадреналіну (10 мкмоль/л) були меншими на 59%. При введенні щурам флокаліну скорочувальні відповіді ізольованих кілець аорти щурів відновлювалися практично до значень контрольних тварин, а ендотелійзалежні вазодилататорні ефекти ацетилхоліну (0,1 мкмоль/л) за дії флокаліну відновлювалися на 69% порівняно з тваринами, яким вводили доксорубіцин. Таким чином, відкривання $K_{АТФ}$-каналів флокаліном запобігає доксорубіциніндукованій дисфункції мітохондрій серця, зменшує оксидативний стрес та порушення функції скорочення-розслаблення судин.

Ключові слова: доксорубіцин; $K_{АТФ}$-канали; флокалін; окисний стрес; серце; судинні реакції.

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