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EXPERIMENTAL PAPERS

Effect of Empagliflozin on Reactivity of Mesenteric Arteries and Skin Microvessels in Rats Treated with Doxorubicin

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Abstract—The potential protective effect of empagliflozin (EMPA) on the functional state of various types of vessels was assessed in Wistar rats that received a single injection of the anthracycline antibiotic doxorubicin (DOX), an anticancer chemotherapeutic agent widely used in clinical practice. The rats were divided into 3 groups, by 15 animals per group. Rats in the DOX group were once injected intraperitoneally with DOX (4 mg/kg), while animals in the DOX+EMPA group, following a single DOX administration (4 mg/kg), received EMPA (1 mg/kg) daily over 5 weeks through a gastric tube. The control group consisted of intact animals. After 4 weeks of the experiment, the rats were examined for the indices of the initial cutaneous microcirculation and their changes after acetylcholine (ACh) and sodium nitroprusside (NP) iontophoresis using laser doppler flowmetry (LDF). A week after LDF, the mesenteric artery dilation was assessed by the changes in the vascular diameter before and after ACh or NP exposures, without blockers and under conditions of pre-incubation of vessels with the NO synthase (NOS) blocker L-NAME. In the control group of rats, ACh iontophoresis evoked an increase in perfusion intensity by 78.5%, while in the DOX group, the change was less pronounced (by 55.2%). EMPA prevented a decrease in the skin microvascular response to ACh; the perfusion index in rats of the DOX+EMPA group increased by 82.8%. After NP iontophoresis, the increase in the microcirculation index in the DOX+EMPA group did not differ from the control, while being significantly lower in the DOX group. ACh-induced dilation of the mesenteric arteries in the DOX group was 24.3% lower than in the control. In DOX-treated rats, EMPA administration improved arterial reactivity. Compared to the vascular reactivity without blockers, incubation of vessels with L-NAME reduced the amplitude of ACh-induced dilation in all groups, although to a lesser extent in the DOX group (45.6%). After EMPA administration, the differences in the relaxation amplitude before and after NOS blockade increased (54.4%), but did not reach the control values (64.1%). Thus, DOX treatment led to a decrease in the reactivity of various types of vessels to the effect of vasodilators ACh and NP. EMPA had a protective effect in DOX-treated animals, improving the dilation of mesenteric arteries and skin microvessels. Presumably, the effect of EMPA is associated with an improvement of the efficiency of NO-dependent vasorelaxant mechanisms disrupted by DOX treatment.

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Keywords: empagliflozin, doxorubicin, cutaneous microcirculation, endothelium-dependent vasodilation, mesenteric arteries

INTRODUCTION

Empagliflozin (EMPA), a sodium-glucose cotransporter 2 (SGLT2) inhibitor, is widely used in the therapy of diabetes mellitus as a hypoglycemic drug [1]. SGLT2 receptors, located in the renal proximal tubules, are responsible for the simultaneous reabsorption of glucose and sodium from primary urine [2]. Inhibiting SGLT2, EMPA promotes an enhancement of urinary glucose excretion [3], thereby reducing the glycemia level and increasing natriuresis [4, 5]. Clinical observations shows that the use of EMPA in diabetic patients not only lowers blood glucose levels but also contributes to the improvement of cardiac and vascular health, reducing the risk of cardiovascular catastrophes [6, 7]. Meanwhile, recent studies demonstrate the cardioprotective effect of EMPA and other glyflosins in various diseases, both diabetes-related [8] and diabetes-unrelated [9, 10]. For example, EMPA improved contractility and reduced the severity of fibrosis in the myocardium of rats with heart failure [11, 12], and prevented myocardial remodeling in rats with metabolic syndrome [13, 14]. Despite ample research, the mechanism of action of SGLT2 inhibitors has not been fully elucidated [15, 16]. Most of the studies focus on the effect of glyflosins on the myocardial contractile function and remodeling [11, 17], whereas its effect on the functional state of blood vessels is inadequately studied [15]. Several experimental studies have shown that glyflosins can exert a protective effect on blood vessels, reducing endothelial dysfunction [12, 14, 18, 19].

We hypothesized that EMPA may improve the functional state of vessels with endothelium-dependent relaxation impaired by the toxic effect of doxorubicin (DOX), a classical anticancer drug having a pronounced negative side effect on the heart [20] and vessels [21, 22].

This study was aimed to reveal a possible protective effect of EMPA on the functional state of different types of vessels in Wistar rats after a single administration of DOX.

MATERIALS AND METHODS

Male Wistar rats aged 4 months were obtained from the Biocollection CCU of Pavlov Institute of Physiology of the Russian Academy of Sciences (PIP RAS). The rats were divided into 3 groups ($n = 15$ per group). In the DOX group, the animals were once injected intraperitoneally (i.p.) with DOX (4 mg/kg), while in the DOX+EMPA group, following a single injection of DOX (4 mg/kg), the rats received EMPA (1 mg/kg) daily over 5 weeks through a gastric tube. The control group comprised intact animals.

The rats were housed in the vivarium of PIP RAS under standard conditions, with ad libitum access to drinking water and food. The exposure duration was 35 days after DOX administration. Systolic blood pressure (BP) was measured by the tail-cuff method (Systola, Russia).

Four weeks after DOX administration, the animals anesthetized with tiletamine/zolazepam (20 mg/kg, Zoletil 100, Virbac Sant Animale, France) were examined for the indices of cutaneous microcirculation (MCR) on the dorsolateral surface of the lumbar spine by laser doppler flowmetry (LDF) using a LAKK-OP analyzer (Lazma, Russia). Acetylcholine (ACh; Sigma-Aldrich, USA) and sodium nitroprusside (NP; ICN Biomedicals, USA) were used as vasodilators to assess the functional state of skin microvessels. ACh and NP (1% solutions) were injected into the skin for 2 min using an ELFOR-PROF device (Russia). The baseline MCR indices and their changes after vasodilator exposures were recorded and analyzed.

LDF measurements lasted for 8 min at each step of the experiment: the baseline indices were recorded first, followed by ACh or NP iontophoresis, and the MCR indices were finally documented after the application of each vasodilator.

The following indices were analyzed: MCR index, i.e. the mean value of MCR amplitude over the observation period, maximum values of perfusion fluctuations in the endothelial (Ae), neurogenic (An), and myogenic (Am) ranges (Wavelet analysis). The endothelial component of vascular tone (endothelial tone, ET) was calculated by the formula:

$$ET = (\sigma \times P) / (Ae \times M),$$

where ET—endothelial tone, σ—mean square deviation of the MCR index, P—mean systolic blood pressure, Ae—maximum value of the amplitude of perfusion fluctuations in the endothelial range, M—mean value of the MCR index.

Neurogenic and myogenic tones (NT and MT, respectively) were calculated in the similar manner.

At the end of the experiment, the in vivo reactivity of mesenteric arteries was studied in animals anesthetized as described above. A loop of the small intestine with mesenteric vessels was fixed in a chamber (at 37°C) through which a solution containing (mmol/L) NaCl 120.4, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2; NaH_2PO_4 1.2, $NaHCO_3$ 15.5, glucose 11.5, pH 7.4 was run. After a 30-min stabilization, the mesenteric arteries were examined according to the protocol.

Endothelium-dependent and endothelium-independent vasodilation was assessed by the response to ACh (1×10^{-5} mol/L) or NP (1×10^{-6} mol/L). Vasodilator exposures were performed after vascular pre-constriction with phenylephrine (PE, 1×10^{-5} mol/L) (Sigma-Aldrich, USA), with the dilation amplitude being expressed as a percentage of the amplitude of PE-induced vasoconstriction.

The NO synthase (NOS) blocker L-NAME (1×10^{-4} mol/L, N_ω-Nitro-L-arginine methyl ester hydrochloride, ICN Biomedicals) was used to assess the NO-dependent vasodilator mechanisms. The vessels were incubated with L-NAME for 30 min. The contribution of NO-dependent vasorelaxant mechanisms was calculated by the difference in the amplitude of ACh-induced arterial dilation before and after L-NAME application. In addition, the concentration-dependent impact of ACh on blood vessels was studied by assessing the cumulative effect of gradually increasing ACh concentrations (from 1×10^{-10} to 1×10^{-5} mol/L) on the vasorelaxation amplitude.

The mesenteric artery diameter was measured using a Biomed MS-1T-ZOOM microscope (Russia) and a BASLER acA4600-10uc camera (Germany); photo and video records were analyzed in the MultiMedia Catalog (MMC) software.

Statistical data processing was carried out using the Statistica for Windows v.12. Data were presented as $M \pm SEM$. The indices with normal distribution were analyzed using the one-way ANOVA followed by post-hoc pairwise comparisons using the Tukey's HSD test, with the normality of distribution being tested using the Shapiro–Wilk test. The Kruskal–Wallis test was used in the cases of data distribution other than normal. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Our studies demonstrated that after 5 weeks of the experiment, the rats of all the three groups under study did not differ significantly in their body weight (DOX+EMPA—432 ± 19 g, DOX—395 ± 17 g, and Control—387 ± 16 g, $p > 0.05$) and systolic BP level (DOX+EMPA—123 ± 8 mmHg, DOX control group—120 ± 7 mmHg, Control—125 ± 10 mmHg, $p > 0.05$). Meanwhile, left ventricular mass index (LVMI) and myocardial mass index (MMI) were significantly increased in the animals treated with DOX alone compared to the control, with this being not the case in the rats co-treated with DOX and EMPA (Fig. 1).

An LDF analysis of the skin blood flow showed that the rats of all these groups did not differ in the baseline skin MCR index (Fig. 2). A spectral analysis of MCR index fluctuations revealed that the endothelial and neurogenic spectral density did not differ significantly between the groups under study, while myogenic tone was significantly higher in the DOX+EMPA group compared to the control and DOX groups (Fig. 3).

However, the response of cutaneous microvasculature to the effect of vasodilators differed significantly between the experimental rat groups. DOX evoked a decrease in the skin microvascular reactivity, as the increase in the perfusion index after ACh iontophoresis in DOX rats was 55.2%, whereas in the control and DOX+EMPA rats, it amounted to 78.5 and 82.8%, respectively (Fig. 4). After NP iontophoresis, the skin MCR index in the DOX rats increased by as little as 51.9%, whereas in the control rats, by 81.5%. The increase in the MCR index in the DOX+EMPA animals amounted to 89%, being non-significantly different from the control.

Thus, DOX administration reduced ACh-induced vasodilation in the cutaneous microcirculatory bed, as well as NO sensitivity of smooth muscle cells (SMCs), whereas EMPA treatment prevented the development of these effects. DOX alone and in combination with EMPA had no significant effect on the baseline MCR. However, in the DOX rats, there was noted a worsening of the vasodilator response of skin microvessels, whereas EMPA application leveled the toxic effect of DOX on the skin microvasculature, preventing a decrease in vasoreactivity.

A study of the in vivo dynamics of the mesenteric

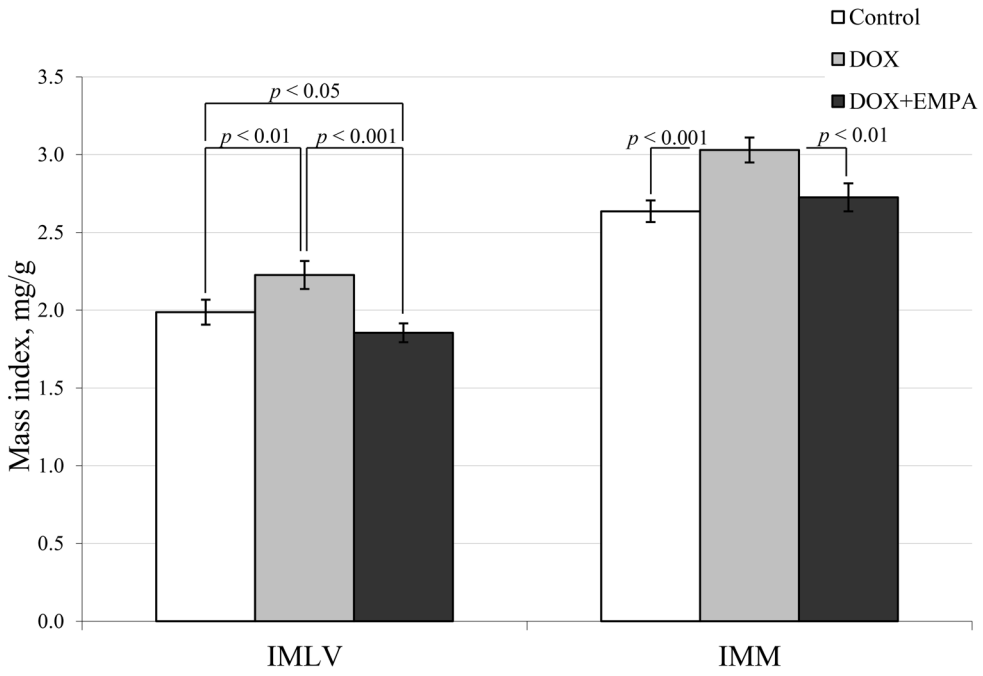

Fig. 1. Effect of empagliflozin (EMPA) on left ventricular mass index (IMLV) and myocardial mass index (IMM) in rats treated with doxorubicin (DOX). Experimental groups: DOX—animals treated with a single dose of DOX (4 mg/kg), DOX+EMPA—animals treated once with DOX (4 mg/kg) and daily with EMPA (1 mg/kg) over 5 weeks. $M \pm SEM$.

artery diameter, as affected by different vasodilators, yielded the following results. In the DOX rats, the dilation amplitude of PE-preconstricted vessels under the effect of NP was by an average of 17.6% smaller than in control animals (Fig. 5). EMPA application prevented the suppression of the response to NP, characteristic of DOX, with the amplitude of NP-induced vasodilation in the DOX+EMPA rats being statistically indistinguishable from that in the control group.

The DOX rats showed a pronounced decrease in vasorelaxation compared to the control animals, with the differences increasing as ACh concentration increased (Fig. 6a). In the DOX+EMPA group, vasorelaxation was noted to increase compared to the DOX group, but its magnitude was lower than in control animals.

The area-under-the-curve-for-ACh analysis revealed a decrease in the efficiency of arterial dilation in the DOX group, which was by 18.9% lower than in the control. EMPA improved the overall dilation of the mesenteric vessels, which was by only 11.5% lower compared to the values in control animals (Fig. 6b).

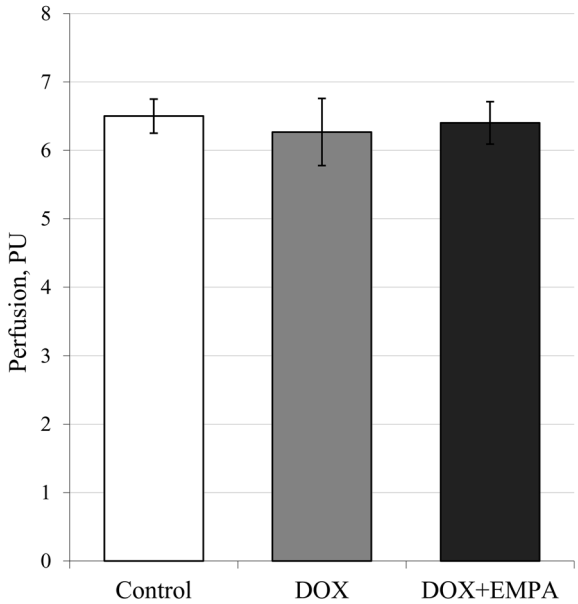

Fig. 2. Baseline microcirculatory index in the rat skin. *Y-axis*: perfusion (perfusion units, PU). Experimental groups: DOX—animals treated with a single dose of doxorubicin (DOX, 4 mg/kg), DOX+EMPA—animals treated once with DOX (4 mg/kg) and daily with empagliflozin (EMPA, 1 mg/kg) over 5 weeks. $M \pm SE$.

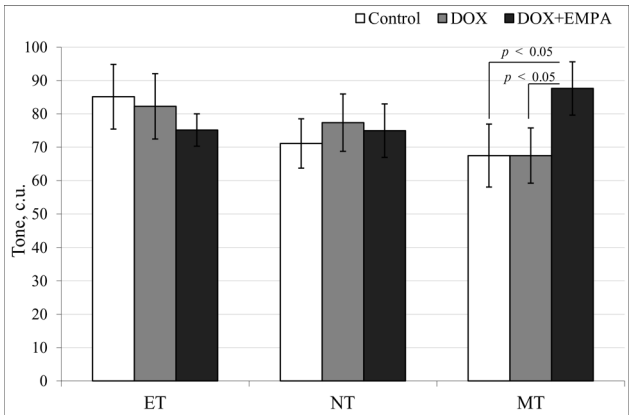

Fig. 3. Calculated value of endothelial (ET), neurogenic (NT) and myogenic (MT) microcirculatory vascular tone in the rat skin. *Y-axis*: tone values (conventional units, c.u.). Experimental groups: DOX—animals treated with a single dose of doxorubicin (DOX, 4 mg/kg), DOX+EMPA—animals treated once with DOX (4 mg/kg) and daily with empagliflozin (EMPA, 1 mg/kg) over 5 weeks. $M \pm SE$.

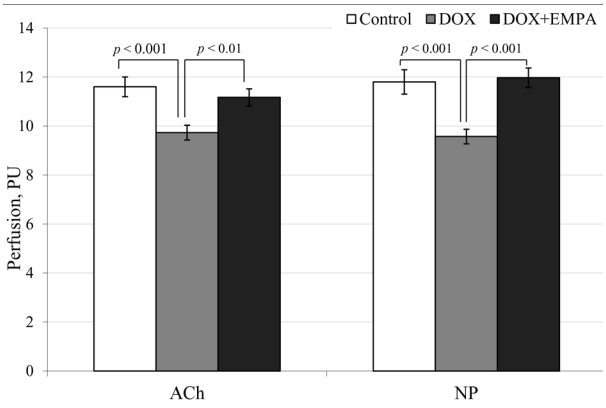

Fig. 4. Average microcirculatory index (perfusion) in the skin of rats after acetylcholine (ACh) and sodium nitroprusside (NP) iontophoresis. *Y-axis*: perfusion, PU. Experimental groups: DOX—animals treated with a single dose of doxorubicin (DOX, 4 mg/kg), DOX+EMPA—animals treated once with DOX (4 mg/kg) and daily with empagliflozin (EMPA, 1 mg/kg) over 5 weeks. $M \pm SE$.

To assess the NO-dependent vasodilator mechanisms, we measured the amplitude of ACh-induced (10^{-5} mol/L) mesenteric artery relaxation before and after L-NAME-induced NOS blockade. DOX administration led to a 24.3% decrease in the amplitude of ACh-induced dilation compared to the control. EMPA application in the DOX+EMPA group significantly improved the ACh-induced response of mesenteric arteries, but the dilation amplitude remained 8.1% lower than in the control (Fig. 7). After incubation with L-NAME, the relaxation amplitude significantly decreased in rats of all groups; however, a least pronounced decrease was observed in DOX rats (45.6%), while being the greatest in the control (64.1%). In animals treated with EMPA, the difference between the magnitude of dilation before and after NOS blockade was greater (54.4%) than in the DOX group, but lesser than in control rats.

Thus, DOX administration led to the suppression of mesenteric artery reactivity to ACh, while EMPA treatment exerted a protective effect, improving ACh-induced vasodilation in the animals that received DOX. Compared to the dilation without blockers, the dilation magnitude in DOX animals group changed to a significantly lesser extent after NOS blockade than in control animals. In rats of the DOX+EMPA group, this index had an intermediate value. It can be concluded that DOX significantly reduced the severity of mesenteric artery dilation in response to ACh application, and EMPA partially attenuated this effect. In this case, the most probable mechanism behind the implementation of the positive EMPA effect is NO-dependent vasoreactivity.

DISCUSSION

The widespread use of anthracycline drugs, which have considerable side effects, in the anticancer therapy of patients requires the development of a strategy for protection against their cardiotoxic effects. To this end, it is necessary to explore the pathophysiological mechanisms leading the reduction in myocardial and vascular functions during antitumor treatment. The strong cardiotoxic effect of DOX has been shown in multiple studies [20, 23], allowing DOX administration to animals to be regarded as a model of chronic heart failure [24].

Chronic heart failure is usually initiated by an intermittent DOX administration, when its cumulative concentration proves to be by far higher than that we used in this work. Our studies show that even a single administration of 4 mg/kg DOX to rats has a toxic effect on the myocardium and vessels. Specifically, we observed a myocardial remodeling in the DOX group of animals, as judged by the increased LVMI and IMM, indicative of a hypertrophy not only in the left ventricle but also in the right chambers of the heart against the background of retaining a normal level of BP.

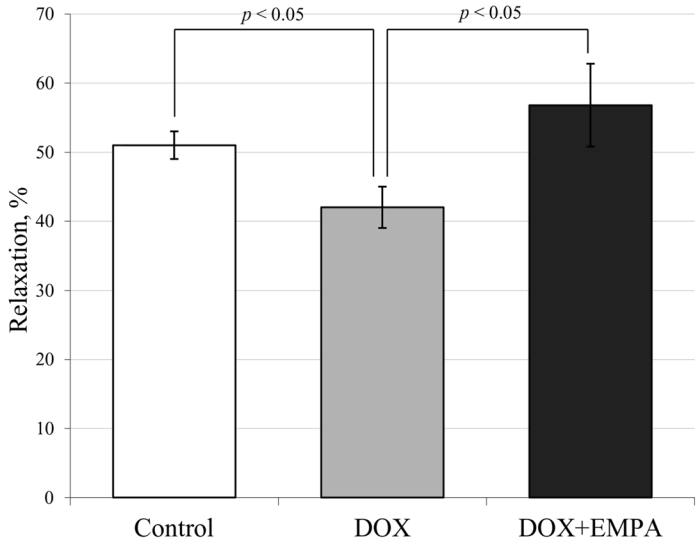

Fig. 5. Nitroprusside-induced vasorelaxation of the rat mesenteric arteries. Y-axis: vasorelaxation amplitude in the mesenteric arteries pre-constricted with phenylephrine (PE, 1×10^{-6} mol/L) under the action of sodium nitroprusside (NP, 1×10^{-6} mol/L), expressed as a percentage of the amplitude of PE-induced vasoconstriction. Experimental groups: DOX—animals treated with a single dose of doxorubicin (DOX, 4 mg/kg), DOX+EMPA—animals treated once with DOX (4 mg/kg) and daily with empagliflozin (EMPA, 1 mg/kg) over 5 weeks. $M \pm SE$.

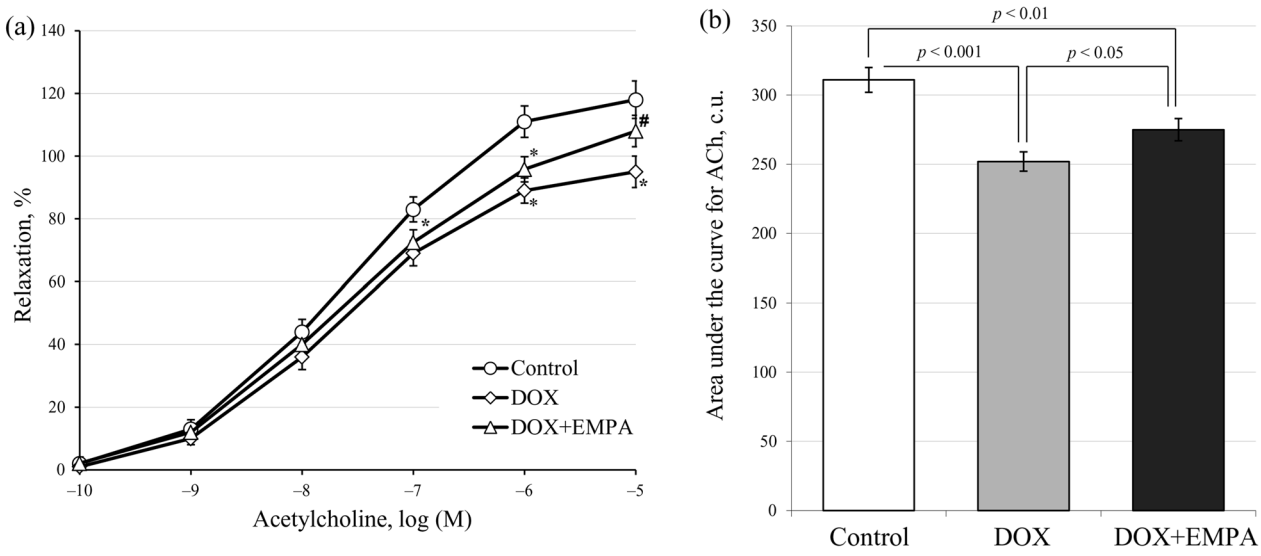

Fig. 6. Acetylcholine-induced dilation of phenylephrine-precontracted rat mesenteric arteries, cumulative effect. (a) Amplitude of acetylcholine (ACh)-induced dilation of the mesenteric arteries. *X-axis*: logarithm of ACh concentration of acetylcholine. *Y-axis*: vasorelaxation amplitude, expressed as a percentage of the amplitude of phenylephrine-induced preconstriction (1×10^{-6} mol/L). (b) AUC for ACh: ordinate—area under the curve for the ACh concentration-dependent dilation amplitude, conventional units (c.u.). Experimental groups: DOX—animals treated with a single dose of doxorubicin (DOX, 4 mg/kg), DOX+EMPA—animals treated once with DOX (4 mg/kg) and daily with empagliflozin (EMPA, 1 mg/kg) over 5 weeks. $M \pm SE$.

In our previous work, it has been shown that a single injection of 4 mg/kg DOX leads to a myocardial hypertrophy and deterioration of the functional state of mesenteric arteries already after 4 weeks [22]. There are studies demonstrating endothelial dysfunction involving a suppression of the vasodilator response during DOX administration [22, 25]. Given that vessels of different types and basins have specific features of the mechanisms for their tone regulation, we aimed to assess the functional state of the skin

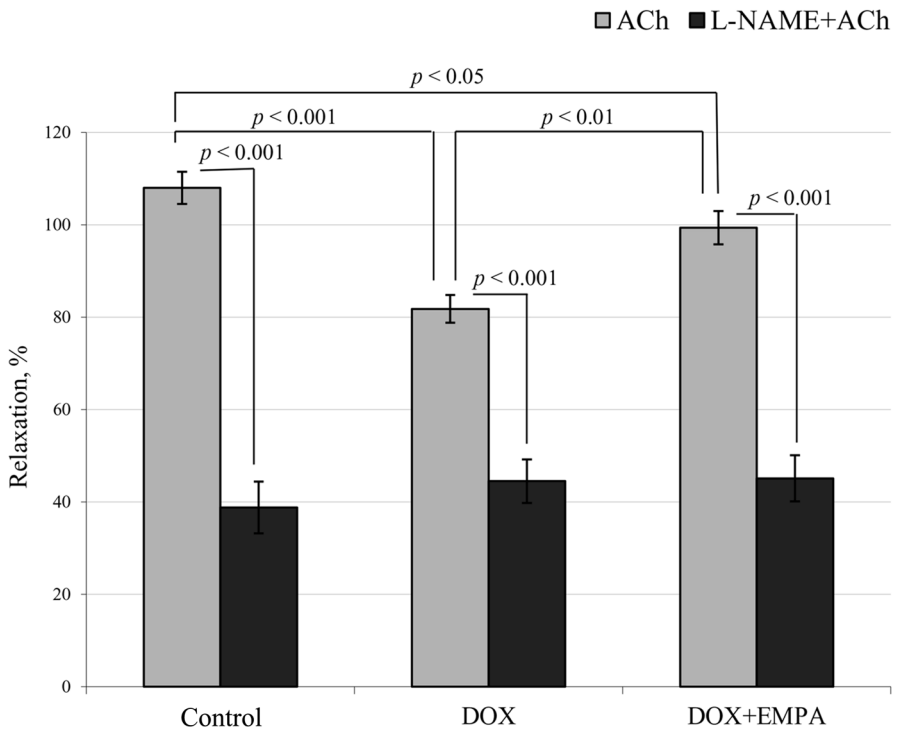

Fig. 7. Acetylcholine-induced dilation of phenylephrine (PE)-preconstricted rat mesenteric arteries in the absence of blockers and after the incubation of vessels with L-NAME (1×10^{-4} mol/L). *X-axis*: amplitude of ACh-induced (1×10^{-5} mol/L) dilation of mesenteric arteries preconstricted with PE (1×10^{-6} mol/L), expressed as a percentage of the amplitude of PE-induced preconstriction. Experimental groups: DOX—animals treated with a single dose of doxorubicin (DOX, 4 mg/kg), DOX+EMPA—animals treated once with DOX (4 mg/kg) and daily with empagliflozin (EMPA, 1 mg/kg) over 5 weeks. $M \pm SE$.

microvessels and larger-caliber resistance vessels in the same animals, for which we carried out both an LDF analysis of the vessels in the cutaneous microcirculatory bed and an in vivo examination of the mesenteric arteries. We also assessed vascular responses to the vasodilator action of ACh and NP and the role of NO-dependent mechanisms in the relaxation of mesenteric arteries.

A study of the baseline MCR index revealed no differences in either the mean values of the skin MCR index or the manifestation degree of endothelial and neurogenic tone, as calculated using a Wavelet analysis of MCR index fluctuations. This indicates that the resting intensity of skin blood flow in DOX-administered animals is retained at a level characteristic of control animals. EMPA application in DOX-administered rats also had no significant influence on MCR indices. However, functional tests with ACh and NP revealed the disturbances in the skin blood flow reactivity in DOX-administered animals. The reactivity to intracutaneous injection of ACh in rats of the DOX group was lower than in the control. Since the reactivity to ACh in the DOC+EMPA group proved to be similar to that in the control, EMPA exerts a protective effect on the cutaneous microcirculatory system. Given that one of the main mechanisms behind ACh-induced dilation is NO-dependent, we tested whether or not the fall in microvascular reactivity to ACh in rats of the DOC group is associated with a decrease in the NO sensitivity of vascular SMCs. It turned out that DOX administration led to a decrease in the response of the MCR index to NP iontophoresis, but EMPA prevented a decrease in the response to the exogenous source of NO, characteristic of DOX, and the NP iontophoresis-induced skin blood flow response corresponded to that in control animals.

Thus, the LDF skin blood flow assessment showed that EMPA administration to DOX-treated rats did not influence the resting baseline MCR index. At the same time, EMPA exerted a protective effect on the skin microvessels, preventing or slowing down a

decrease in their reactivity, characteristic of DOX. In addition, the decrease in the reactivity to NP in DOX-administered animals suggests the impaired sensitivity of skin microvascular SMCs to NO, whereas EMPA treatment after DOX administration corrects this impairment, promoting the retention of NO-mediated mechanisms in vascular SMCs of the cutaneous microcirculatory bed.

In order to assess the functional state of the mesenteric arteries, the vascular diameter dynamics was recorded under the action of vasodilator agonists. It was shown that DOX administration suppresses the vasodilator response to ACh, which is thought to be a hallmark of the incipient endothelial dysfunction. In animals administered with DOX, EMPA improves the arterial reactivity to ACh, but does not restore it completely.

The basic mechanism of endothelium-dependent vasodilation is commonly believed to be associated with endothelial NO (eNO) production. To assess the involvement of this mechanism in vasorelaxation, the vessels were preincubated in a solution containing the NO synthase blocker L-NAME, and the dilation amplitudes were compared before and after blocker application. The magnitude of ACh-induced relaxation was significantly decreased after L-NAME application; however, this decrease was significantly smaller in the DOX group than in control animals. This fact indicates the impairment of the NO-dependent vasodilator mechanisms in rats administered with DOX. Meanwhile, in the DOC+EMPA group, the contribution of the NO-mediated mechanism to vasodilation was greater than in the DOX group, although it did not reach the level observed in control animals. The disruption of the NO-dependent vasorelaxation mechanism is typically associated with a decrease in NO bioavailability, which can be mediated by both a decrease in endothelial NO production and impaired SMC sensitivity to NO. The assessment of the magnitude of arterial responses to NP, which was significantly lower in DOX-administered rats, suggests that the decrease in the amplitude of vasorelaxation may be partially mediated by the impaired efficiency of the NO/soluble guanylate cyclase (sGC)/cyclic guanosine monophosphate (cGMP) cascade in SMCs. It is possible that EMPA, while preventing a decrease in SMC sensitivity to NO, characteristic of DOX, exerts a protective effect on ACh-induced vascular reactivity, including due to preserving the sGC-associated pathway. This effect of EMPA appears to be characteristic of both mesenteric arteries and the vessels of the cutaneous microcirculatory bed. On the other hand, it cannot be ruled out that the disruption of NO-dependent vasorelaxation mechanisms, revealed in our experiments in DOX-administered animals, may also be partially related to a decrease in eNO production due to the lack of L-arginine (the substrate for NO synthesis), an increase in the level of asymmetric dimethylarginine (a competitive eNOS inhibitor), a decrease in eNOS expression, or NO inactivation because of its interaction with reactive oxygen species (ROS) [26]. The protective effect of EMPA on NO-dependent vasorelaxation mechanisms in the mesenteric arteries of DOX-administered rats, demonstrated in our studies, may also be mediated by the improvement of one or more of the above-mentioned pathways of vascular reactivity reduction. EMPA is believed to have vasodilator properties to some extent. For example, the literature data on the effect of EMPA on the vascular function in animals lacking diabetes-associated pathology, obtained mainly on isolated vascular segments in vitro, show that the EMPA vasodilator mechanism may relate to the activity of voltage-dependent potassium channels (K_v) [18, 27]. Hasan et al. [18] noted that EMPA introduction to a perfusion chamber evokes a dose-dependent vasodilation, which is not abolished by the eNOS blocker (L-NNA), sGC and protein kinase KT5823 inhibitors, or indomethacin-induced cyclooxygenase blockade. Moreover, these authors showed that denuded vessels also respond to EMPA, as do the vessels with the preserved endothelium, suggesting an endothelium-independent vasodilator effect of EMPA directly on SMCs. Interestingly, voltage-dependent potassium channels expressed in SMCs [28] were found to be involved in EMPA-induced vasorelaxation, since their 4-aminopyridine-induced blockade significantly diminished the amplitude of the response to EMPA [18]. On the other hand, the effect of EMPA was not mediated by the large-conductance calcium-activated potassium (BK_{Ca}) channels or ATP-dependent potassium channels, because the preincubation with paxillin (a BK_{Ca} channel blocker) or glibenclamide (K^+_{ATP} inhibitor) did not alter the magnitude of arterial dilation during EMPA action [18]. The possible effect of EMPA on

Ca^{2+} homeostasis in SMCs should also be considered, as there are reports on the ability of EMPA to prevent excessive Ca^{2+} accumulation in cardiomyocytes, characteristic of DOX [30, 31].

Apparently, EMPA may have both a direct effect on blood vessels, causing vasorelaxation, and an indirect influence on the state of the entire vascular system, modulating the state of energy metabolism and reducing inflammation. Since the model of DOX-induced disorders, used in our study, includes multiple factors, such as inflammation, mitochondrial dysfunction, and ROS production [32, 33], it cannot be ruled out that EMPA has an effect on these processes as well [30, 34]. Specifically, Soares et al. [35] demonstrated that EMPA application decreases DOX-induced oxidative stress and energy metabolism disorders by reducing the level of malonic dialdehyde, a marker of lipid peroxidation, and suppressing the pathways associated with ROS generation and hydrogen peroxide metabolism in mouse aortas. The antioxidant and anti-inflammatory effects of EMPA have also been shown in a number of studies [31, 36, 37]. One cannot also exclude a possible protective effect of EMPA on the endothelial glycocalyx, which is specifically associated with a shear stress-mediated NO production [38]. For example, it has been shown that damage to the glycocalyx often accompanies endothelial dysfunction, while EMPA treatment promotes the restoration of its structure and integrity [39]. So, further studies are required to elucidate the mechanisms underlying the EMPA vascular effects in DOX-administered rats.

Thus, DOX leads to a decrease in the reactivity of different types of vessels to the effects of vasodilators, specifically, ACh and NP. The use of EMPA exerts a protective effect in animals after DOX administration, improving the dilation of mesenteric arteries and skin microvessels. The effect of EMPA is probably due to the improvement of the efficiency of NO-dependent vasorelaxation pathways disrupted by DOX administration.

AUTHORS' CONTRIBUTION

Conceptualization (A.N.K., S.V.O.), experimental design, data collection and processing (G.T.I., O.N.B.), writing and editing the manuscript (G.T.I., O.N.B., A.N.K., S.V.O.).

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ETHICS APPROVAL

Animal-related experiments were conducted in compliance with the NIH Guidelines for the care and use of laboratory animals (http://oacu.od.nih.gov/regs/index.htm) and approved by the Ethics Committee of Pavlov Institute of Physiology of the Russian Academy of Sciences (Minutes No. 04/03 of April 3, 2023).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflict of interest.

REFERENCES

1. Ma J, Lu J, Shen P, Zhao X, Zhu H (2023) Comparative efficacy and safety of sodium-glucose cotransporter 2 inhibitors for renal outcomes in patients with type 2 diabetes mellitus: a systematic review and network meta-analysis. Ren Fail 45(2): 2222847. https://doi.org/10.1080/0886022X.2023.2222847
2. Vrhovac I, Balen Eror D, Klessen D, Burger C, Breljak D, Kraus O, Radović N, Jadrijević S, Aleksic I, Walles T, Sauvant C, Sabolić I, Koepsell H (2015) Localizations of Na+-d-glucose cotransporters SGLT1 and SGLT2 in human kidney and of SGLT1 in human small intestine, liver, lung, and heart. Pflügers Arch-Eur J Physiol 467: 1881–1898. https://doi.org/10.1007/s00424-014-1619-7
3. Salvatore T, Carbonara O, Cozzolino D, Torella R, Nasti R, Lascar N, Sasso FC (2011) Kidney in diabetes: From organ damage target to therapeutic target. Curr Drug Metab 12: 658–666. https://doi.org/10.2174/138920011796504509
4. Chao EC, Henry RR (2010) SGLT2 inhibition—A novel strategy for diabetes treatment. Nat Rev Drug Discov 9: 551–559. https://doi.org/10.1038/nrd3180
5. Rieg T, Masuda T, Gerasimova M, Mayoux E,

Platt K, Powell DR, Thomson SC, Koepsell H, Vallon (2014) Increase in SGLT1-mediated transport explains renal glucose reabsorption during genetic and pharmacological SGLT2 inhibition in euglycemia. Am J Physiol 306: F188–F193. https://doi.org/10.1152/ajprenal.00518.2013

6. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE (2015) Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 373(22): 2117–2128. https://doi.org/10.1056/NEJMoa1504720
7. Younis F, Leor J, Abassi Z, Landa N, Rath L, Hollander K, Naftali-Shani N, Rosenthal T (2018) Beneficial effect of the SGLT2 inhibitor empagliflozin on glucose homeostasis and cardiovascular parameters in the cohen rosenthal diabetic hypertensive (CRDH) rat. J Cardiovasc Pharmacol Ther 23(4): 358–371. https://doi.org/10.1177/1074248418763808
8. Li C, Zhang J, Xue M, Li X, Han F, Liu X, Xu L, Lu Y, Cheng Y, Li T, Yu X, Sun B, Chen L (2019) SGLT2 inhibition with empagliflozin attenuates myocardial oxidative stress and fibrosis in diabetic mice heart. Cardiovasc Diabetol 18(1): 15. https://doi.org/10.1186/s12933-019-0816-2
9. Inzucchi SE, Kosiborod M, Fitchett D, Wanner C, Hehnke U, Kaspers S, George JT, Zinman B (2018) Improvement in Cardiovascular Outcomes With Empagliflozin Is Independent of Glycemic Control Circulation. Circulation 138(17): 1904–1907. https://doi.org/10.1161/CIRCULATIONAHA.118.035759
10. Prikhodko VA, Okovity SV, Kulikov AN (2023) Gliflozins in non-alcoholic fatty liver disease: Perspectives of use outside diabetes, cardiac and nephroprotection. Therapy 9(7): 130–141. (In Russ). https://dx.doi.org/10.18565/therapy.2023.7.130-141
11. Lee HC, Shiou YL, Jhuo SJ, Chang CY, Liu PL, Jhuang WJ, Dai ZK, Chen WY, Chen YF, Lee AS (2019) The sodium–glucose co-transporter 2 inhibitor empagliflozin attenuates cardiac fibrosis and improves ventricular hemodynamics in hypertensive heart failure rats. Cardiovasc Diabetol 18(1): 45. https://doi.org/10.1186/s12933-019-0849-6
12. Kulikov AN, Krasnova MV, Ivkin DYu, Karpov AA, Kaschina E, Okovityy SV, Demakova NV (2021) Efficacy of empagliflozine in experimental chronic heart failure in normoglycemiaobucheniye. Cardiology: news, opinions, training 9(1): 9–16. (In Russ). https://doi.org/10.33029/2309-1908-2021-9-1-9-16
13. Kusaka H, Koibuchi N, Hasegawa Y, Ogawa H, Kim-Mitsuyama S (2016) Empagliflozin lessened cardiac injury and reduced visceral adipocyte hypertrophy in prediabetic rats with metabolic syndrome. Cardiovasc Diabetol 15(1): 157. https://doi.org/10.1186/s12933-016-0473-7
14. Park SH, Farooq MA, Gaertner S, Bruckert C, Qureshi AW, Lee HH, Benrahla D, Pollet B, Stephan D, Ohlmann P, Lessinger JM, Mayoux E, Auger C, Morel O, Schini-Kerth VB (2020) Empagliflozin improved systolic blood pressure, endothelial dysfunction and heart remodeling in the metabolic syndrome ZSF1 rat. Cardiovasc Diabetol 19(1): 19. https://doi.org/10.1186/s12933-020-00997-7
15. Salvatore T, Caturano A, Galiero R, Di Martino A, Albanese G, Vetrano E, Sardu C, Marfella R, Rinaldi L, Sasso FC (2021) Cardiovascular Benefits from Gliflozins: Effects on Endothelial Function. Biomedicines 9(10): 1356. https://doi.org/10.3390/biomedicines9101356
16. Salvatore T, Galiero R, Caturano A, Rinaldi L, Di Martino A, Albanese G, Di Salvo J, Epifani R, Marfella R, Docimo G, Lettieri M, Sardu C, Sasso FC (2022) An Overview of the Cardiorenal Protective Mechanisms of SGLT2 Inhibitors. Int J Mol Sci 23(7): 3651. https://doi.org/10.3390/ijms23073651
17. Sabatino J, De Rosa S, Tamm L, Iaconetti C, Sorrentino S, Polimeni A, Mignogna C, Amorosi A, Spaccarotella C, Yasuda M, Indolfi C (2020) Empagliflozin prevents doxorubicin-induced myocardial dysfunction. Cardiovasc Diabetol 19(1): 66. https://doi.org/10.1186/s12933-020-01040-5
18. Hasan A, Hasan R (2021) Empagliflozin Relaxes Resistance Mesenteric Arteries by Stimulating Multiple Smooth Muscle Cell Voltage-Gated K^+ (KV) Channels. Int J Mol Sci 22(19): 10842. https://doi.org/10.3390/ijms221910842
19. Adingupu DD, Göpel SO, Grönros J, Behrendt M, Sotak M, Miliotis T, Dahlqvist U, Gan LM, Jönsson-Rylander AC (2019) SGLT2 inhibition with empagliflozin improves coronary microvascular function and cardiac contractility in prediabetic ob/ob(-/-) mice. Cardiovasc Diabetol 18(1): 16. https://doi.org/10.1186/s12933-019-0820-6
20. Tan C, Zeng J, Wu G, Zheng L, Huang M, Huang X (2021) Xinshuitong Capsule extract attenuates doxorubicin-induced myocardial edema via regulation of cardiac aquaporins in the chronic heart failure rats. Biomed Pharmacother 144: 112261. https://doi.org/10.1016/j.biopha.2021.112261

21. He H, Wang L, Qiao Y, Zhou Q, Li H, Chen S, Yin D, Huang Q, He M (2020) Doxorubicin Induces Endotheliotoxicity and Mitochondrial Dysfunction via ROS/eNOS/NO Pathway. Front Pharmacol 10: 1531. https://doi.org/10.3389/fphar.2019.01531
22. Ivanova GT (2022) Effect of Doxorubicin on the Reactivity of Rat Mesenteric Arteries. J Evol Biochem Phys 58(6): 1914–1925. https://doi.org/10.1134/S0022093022060205
23. Wen J, Zhang L, Liu H, Wang J, Li J, Yang Y, Wang Y, Cai H, Li R, Zhao Y (2019) Salsolinol Attenuates Doxorubicin-Induced Chronic Heart Failure in Rats and Improves Mitochondrial Function in H9c2 Cardiomyocytes. Front Pharmacol 10: 1135. https://doi.org/10.3389/fphar.2019.01135
24. Huang C, Qiu S, Fan X, Jiao G, Zhou X, Sun M, Weng N, Gao S, Tao X, Zhang F, Chen W (2021) Evaluation of the effect of Shengxian Decoction on doxorubicin-induced chronic heart failure model rats and a multicomponent comparative pharmacokinetic study after oral administration in normal and model rats. Biomed Pharmacother 144: 112354. https://doi.org/10.1016/j.biopha.2021.112354
25. Luu AZ, Chowdhury B, Al-Omran M, Teoh H, Hess DA, Verma S (2018) Role of Endothelium in Doxorubicin-Induced Cardiomyopathy. JACC Basic Transl Sci 3(6): 861–870. https://doi.org/10.1016/j.jacbts.2018.06.005
26. Zhang Q, Malik P, Pandey D, Gupta S, Jagnandan D, Belin de Chantemele E, Banfi B, Marrero MB, Rudic RD, Stepp DW, Fulton DJ (2008) Paradoxical activation of endothelial nitric oxide synthase by NADPH oxidase. Arterioscler Thromb Vasc Biol 28(9): 1627–1633. https://doi.org/0.1161/ATVBAHA.108.168278
27. Seo MS, Jung HS, An JR, Kang M, Heo R, Li H, Han ET, Yang SR, Cho EH, Bae YM, Park WS (2020)Empagliflozin dilates the rabbit aorta by activating PKG and voltage-dependent K^+ channels. Toxicol Appl Pharmacol 403: 115153. https://doi.org/10.1016/j.taap.2020.115153
28. Jackson WF (2018) K(V) channels and the regulation of vascular smooth muscle tone. Microcirculation 25: e12421. https://doi.org/10.1111/micc.12421
29. Tamargo J, Caballero R, Gómez R, Valenzuela C, Delpón E (2004) Pharmacology of cardiac potassium channels. Cardiovasc Res 62(1): 9–33. https://doi.org/10.1016/j.cardiores.2003.12.026
30. Quagliariello V, De Laurentiis M, Rea D, Barbieri A, Monti MG, Carbone A, Paccone A, Altucci L, Conte M, Canale ML, Botti G, Maurea N (2021) The SGLT-2 inhibitor empagliflozin improves myocardial strain, reduces cardiac fibrosis and pro-inflammatory cytokines in non-diabetic mice treated with doxorubicin. Cardiovasc Diabetol20(1): 150. https://doi.org/10.1186/s12933-021-01346-y
31. Lin R, Peng X, Li Y, Wang X, Liu X, Jia X, Zhang C, Liu N, Dong J (2024) Empagliflozin attenuates doxorubicin-impaired cardiac contractility by suppressing reactive oxygen species in isolated myocytes. Mol Cell Biochem 479(8): 2105–2118. https://doi.org/10.1007/s11010-023-04830-z
32. Mele D, Tocchetti CG, Pagliaro P, Madonna R, Novo G, Pepe A, Zito C, Maurea N, Spallarossa P (2016) Pathophysiology of anthracycline cardiotoxicity. J Cardiovasc Med 17(Suppl 1): e3–e11. https://doi.org/10.2459/JCM.0000000000000378
33. Rea D, Coppola C, Barbieri A, Monti MG, Misso G, Palma G, Bimonte S, Zarone MR, Luciano A, Liccardo D, Maiolino P, Cittadini A, Ciliberto G, Arra C, Maurea N (2016) Strain analysis in the assessment of a mouse model of cardiotoxicity due to chemotherapy: sample for preclinical research. In Vivo 30(3): 279–290.
34. Zou R, Shi W, Qiu J, Zhou N, Du N, Zhou H, Chen X, Ma L (2022) Empagliflozin attenuates cardiac microvascular ischemia/reperfusion injury through improving mitochondrial homeostasis. Cardiovasc Diabetol 21(1): 106. https://doi.org/10.1186/s12933-022-01532-6
35. Soares RN, Ramirez-Perez FI, Cabral-Amador FJ, Morales-Quinones M, Foote CA, Ghiarone T, Sharma N, Power G, Smith JA, Rector RS, Martinez-Lemus LA, Padilla J, Manrique-Acevedo C (2022) SGLT2 inhibition attenuates arterial dysfunction and decreases vascular F-actin content and expression of proteins associated with oxidative stress in aged mice. Geroscience 44(3): 1657–1675. https://doi.org/10.1007/s11357-022-00563-x
36. Uthman L, Li X, Baartscheer A, Schumacher CA, Baumgart P, Hermanides J, Preckel B, Hollmann MW, Coronel R, Zuurbier CJ, Weber NC (2022) Empagliflozin reduces oxidative stress through inhibition of the novel inflammation/NHE/[Na(+)](c)/ROS-pathway in human endothelial cells. Biomed Pharmacother 146: 112515. https://doi.org/10.1016/j.biopha.2021.112515
37. Wang J, Huang X, Liu H, Chen Y, Li P, Liu L, Li J, Ren Y, Huang J, Xiong E, Tian Z, Dai X (2022)

Empagliflozin ameliorates diabetic cardiomyopathy via attenuating oxidative stress and improving mitochondrial function. Oxid Med Cell Longev 2022: 1122494. https://doi.org/10.1155/2022/1122494

38. Tarbell JM, Cancel LM (2016) The glycocalyx and its significance in human medicine. J Intern Med 280(1): 97–113. https://doi.org/10.1111/joim.12465
39. Cooper S, Teoh H, Campeau MA, Verma S, Leask RL (2019) Empagliflozin restores the integrity of the endothelial glycocalyx in vitro. Mol Cell Biochem 459(1–2): 121–130. https://doi.org/10.1007/s11010-019-03555-2

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