ORIGINAL ARTICLE

The effect of *Aronia melanocarpa* extract on the phospholipid composition of the rat myocardium during stress

Vliv extraktu *Aronia melanocarpa* na složení fosfolipidů myokardu potkana během stresu

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Received November 3, 2021 / Accepted April 27, 2022

Summary

The research was performed on 80 male rats of the Wistar line. Animals of two age groups were used: adults (10–12 months) and old (22–25 months). The obtained data show that the development of immobilization stress in adult and aged rats are accompanied by the formation of a characteristic complex of changes in the phospholipid composition of the myocardium. Intraperitoneal injection of the chokeberry extract (*Aronia melanocarpa*) at a dose of 0.2 g/kg 60 minutes before the immobilization has limited stress modulation of myocardial phospholipid composition in aged animals. Thus, the extract of *Aronia melanocarpa* increases the myocardial resistance to the injury effect of stress.

Key words: aging • immobilization • myocardium • *Aronia melanocarpa*

Souhrn

Výzkum byl proveden na 80 samcích potkanů linie Wistar. Byla použita zvířata dvou věkových skupin: dospělí (10–12 měsíců) a staří (22–25 měsíců). Získaná data ukazují, že rozvoj imobilizačního stresu u dospělých a starých potkanů je doprovázen tvorbou charakteristického komplexu změn ve fosfolipidovém složení myokardu. Intraperitoneální injekce extraktu z temnoplodce černoplodého (*Aronia melanocarpa*) v dávce 0,2 g/kg 60 minut před imobilizací má omezenou modulaci stresu složení fosfolipidů myokardu u starých zvířat. Extrakt z *Aronia melanocarpa* tak zvyšuje odolnost myokardu vůči negativnímu účinku stresu.

Klíčová slova: stárnutí • imobilizace • myokard • *Aronia melanocarpa*

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Introduction

Stress is an important etiological factor in the development of cardiovascular diseases[1–4]. It is associated with myocardial ischemia, arrhythmias, coronary, and cardiosclerosis, etc. Therefore, the search for drugs with marked antistress and cardioprotective effects is highly relevant. Herbal drugs are their promising representatives, including medications from *Aronia melanocarpa* extract. Extracts from this plant contain a wide range of biologically active substances, including bioflavonoids and their representatives – anthocyanidins[5–7].

The biological activity of bioflavonoids appears through their significant antioxidant, cardioprotective and anti-inflammatory effects[8–12]. At the same time, the mechanism of their cardiotropic effects is still far from a final understanding. It can be assumed that it is associated with their influence on the structure and

function of biological membranes and, in particular, on the state of the lipid bilayer of cardiomyocyte membranes. Considering the pronounced age-dependent nature of the stress damaging effect on the heart, the work aimed to study the impact of *Aronia melanocarpa* extract on the phospholipid composition of the different age rat myocardium during immobilization stress.

Experimental part

Eighty Wistar male rats were used in the study. Animals were kept in constant environmental conditions (20 °C, 12-h light/dark cycle) and were on a standard laboratory diet. Animals of two age groups were adults (10–12 months) and old (22–25 months). We divided both age groups of animals into four subgroups:

1 – intact rats
2 – control rats that were affected by immobilization stress by fixing them in a dorsal position for 30 minutes
3 – rats that were intraperitoneally administered with the *A. melanocarpa* extract, 0.2 g/kg, 60 minutes before the immobilization[9, 13]
4 – rats that were intraperitoneally administered the dimethyl sulfoxide (DMSO), 175 mg/kg, 60 minutes before the immobilization[14]

The effectiveness of stress was controlled pathomorphologically and by measuring the level of glucocorticoid hormones (11-hydroxycorticosteroids) in the blood by the fluorimetric method using a spectrofluorimeter Hitachi MPF-4 (Japan)[15].

The study was conducted in accordance with the requirements of the European Council Directive of November 24, 1986, for Care and Use of Laboratory Animals (86/609/EEC)[16], and according to the general ethical principles of experiments on animals adopted by the First National Congress of Ukraine on Bioethics (2001), as well as other international agreements and legislation of Ukraine in this area (Protocol No.10, approved 15.10. 2019 by Bioethics commission of Zaporizhzhia state medical university).

Immediately after immobilization, animals were decapitated by guillotine under ether anaesthesia. The heart was removed and washed from the blood. The left ventricular myocardium was isolated and homogenized with 0.1 M sodium phosphate buffer (pH 7.5). 10% of homogenates were used for lipid extraction[17]. Lipid extracts were fractionated by the thin-layer chromatography (TLC) technique using a CAMAG TLC Scanner 3 (Camag, Switzerland).

Separation of the lipid homogenate fractions was performed using one-dimensional thin-layer chromatography in a hexane : diethyl ether : acetic acid (79.2% : 19.8% : 1%) system on glass plates (10 × 15 cm) with a thin silica gel layer (Laane Kalur, Estonia). According to this division technique, the spot of the phospholipids states at the start.

Phospholipids (PLs) of the lipid extract were fractionated using two-dimensional TLC on "Kieselgel-60" plates (7 × 7 cm) (Merck). We used two different solvent systems for separation – chloroform : methanol : benzene : NH_4OH, 25% (58.5% : 27% : 9% : 5.5%) and chloroform : methanol : benzene : acetone : acetic acid – water (58.3% : 25% : 8.3% : 4,2% : 3.3% : 0.9%).

Identification of separated PLs was performed by R_f index that was calculated using CAMAG TLC Scanner 3 software and through using there various dying[17].

Isolated PLs were scraped off the plates and extracted with Folch reagent[17]. The extracts were evaporated and mineralized with $HClO_4$ for 20 min at 180 °C. Inorganic phosphorus in the samples was determined using the Vaskovsky reagent[18]. The protein concentration in homogenates was estimated by Lowry[19].

Statistical analyses were performed using Student's t-test.

Results and discussion

Figure 1 shows that immobilization does not significantly affect the total amount of phospholipids in the heart homogenates of adult rats.

However, the content of phosphatidylcholine (PC) reduces by 29%, and the contents of lysophosphatidylcholine (LPC) and phosphatidylserine + phosphatidylinositol (PS + PI) were increased by 158%, and 122%, respectively, compared to intact animals (Table 1).

The total PLs in the heart homogenates of old animals after immobilization were reduced by 15%, compared to initial values. PC and phosphatidylethanolamine (PE) content was decreased simultaneously by 36% and 49%, respectively, compared with unstressed rats. The content of PS and PI increased 2.8 times in the heart homogenates.

The study results showed that immobilization stress is accompanied by modifying the myocardial phospholipid structure. This modification appeared more in the old animal group.

While assessing a possible reason for the phospholipid composition modification of old rat hearts during stress, it is assumed that the inhibition of PE synthesis is closely linked in the process associated with the use of ATP and PC – by limiting the speed of PE methylation[20, 21]. The above-mentioned fact can be explained by the age-dependent decreasing of ATP-level in the myocardium of immobilized rats[4]. In this case, an alternative phospholipids biosynthesis pathway, associated with phosphatidylserine synthesis and the use of CDP-glycerol as the precursor, is activated[20, 21]. Another cause for the decrease in the level of PC in cardiomyocytes can be an increase in its hydrolysis in the reaction catalyzed by phospholipase A_2[22], resulting from an enhanced secretion of adrenaline in the adrenal glands during immobilization stress[23–25].

In adult immobilized animals, the occurred modification in the phospholipid composition manifested only by an increase in the hydrolysis of PC in the reaction catalyzed by phospholipase A_2 due to hypersecretion of catecholamines during stress. It can be proved by a prominent accumulation of LPC in the heart homogenates.

Preliminary administration of *Aronia melanocarpa* extract 60 minutes before the immobilization prevented the occurrence of all changes from the PL composition of the old rat heart homogenates and previsedа decrease in the total PLs amount. Administration of *A. melanocarpa* extract did not have a similar effect on the PL composition of the adult rat myocardium.

It can be assumed that the cardioprotective activity and positive effects on the myocardium phospholipids composition during stress may be associated with the antioxidant properties of *A. melanocarpa* extract[9, 13, 26–27].

To verify that assumption, we assay the effect of preliminary administration of DMSO on the PL composition of the rat myocardium during stress.

Studies pointed out that the myocardial phospholipid composition of both age groups of animals, which were administered DMSO 60 minutes before the immobilization, does not differ from the control group animals. It is an important argument in favor of the fact that changes in the myocardial phospholipid composition of both age group animals during stress were not associated with the stimulation of free radical processes in the myocardium. Therefore, the protective effect of *A. melanocarpa* extract during stress is not associated with its antioxidant properties[9, 13, 26–27].

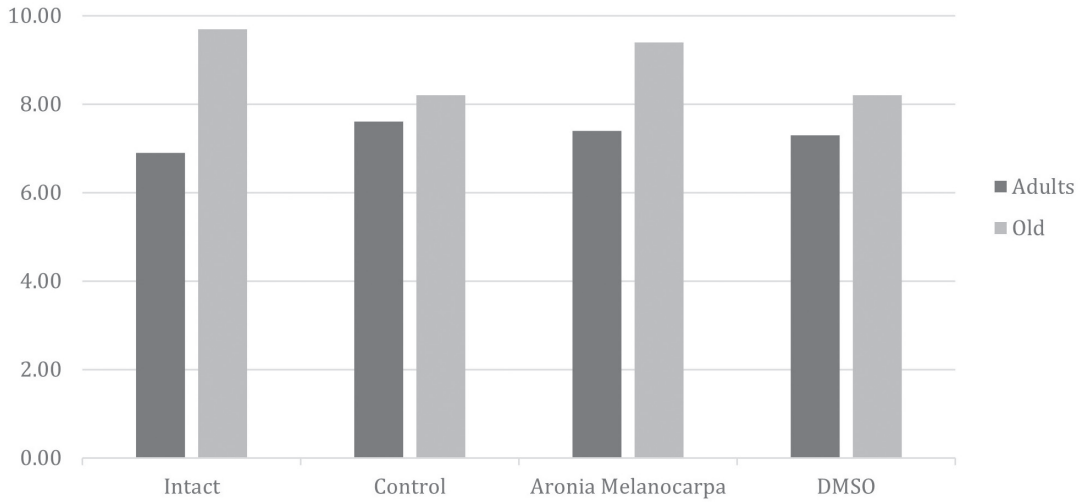

Fig. 1. The effect of Aronia melanocarpa extract on the total amount of PLs in myocardial homogenates of different age group rats during immobilization stress, μg P_{inorg}/mg protein (M ± m)

Table 1. Individual phospholipids in the heart homogenates of different age group rats, mcg P_{inorg}/mg protein (M ± m)

Animal group	PC	PE	PS + PI	LPC
Adult animals				
Intact	2.4 ± 0.2	2.2 ± 0.2	0.9 ± 0.1	0.19 ± 0.03
Stress	1.7 ± 0.2*	2.2 ± 0.1	2.0 ± 0.4*	0.49 ± 0.11*
Stress + DMSO	1.8 ± 0.1*	2.1 ± 0.2	1.8 ± 0.2*	0.48 ± 0.08*
Stress + *A. melanocarpa*	1.9 ± 0.3*	2.2 ± 0.2	1.7 ± 0.2*	0.44 ± 0.04*
Old animals				
Intact	2.8 ± 0.3	3.9 ± 0.3#	1.0 ± 0.1	0.32 ± 0.03#
Stress	1.8 ± 0.2*	2.0 ± 0.1*	2.8 ± 0.6*	0.42 ± 0.04
Stress + DMSO	1.9 ± 0.3*	2.1 ± 0.2*	2.5 ± 0.3*	0.39 ± 0.05
Stress + *A. melanocarpa*	2.6 ± 0.2**	3.7 ± 0.3**	1.2 ± 0.1**	0.34 ± 0.04

*significantly different relative to the intact group ($P < 0.05$)
**significantly different relative to the control group ($P < 0.05$)
#significantly different relative to the adult animals ($P < 0.05$)

Thus, the cardioprotective[13, 16] effect of *A. melanocarpa* extract is age-dependent and associated with its action on the phospholipid structure of cardiomyocyte membranes.

One of the possible mechanisms of that effect can be associated with the inhibition of phospholipids hydrolysis in the reaction catalyzed by phospholipase A_2. It may also be based on the effect of *A. melanocarpa* extract components on the secretion of catecholamines and intracellular calcium balance. Moreover, we propose the possibility of a direct effect of *A. melanocarpa* extract on the synthesis of cardiomyocyte phospholipids. We assume that the direct effect may be associated with the impact of extract components on the biosynthesis of cardiomyocyte phospholipids or with the increase in the efficiency of its energy supplement. However, the detailed mechanism of the protective effect of *A. melanocarpa* extract on the heart during stress is still not clear. We will focus on their explorations in the future.

Conclusions

The development of immobilization stress in rats is accompanied by appears of age specific changes in the phospholipid composition of the myocardium. Intraperitoneal injection of the chokeberry extract (*Aronia melanocarpa*) 60 minutes before immobilization at a dose of 0.2 g/kg restricts the stress modulation of myocardial phospholipid composition in old animals.

Conflict of interest: none.

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