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INFLUENCE OF THE COMPOSITION OF DIHYDROQUERCETIN AND ARABINOGALACTAN FOR CONTRACTION OF SMOOTH MUSCLE CELLS OF RAT PORTAL VEIN

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INFLUENCE OF THE DIHYDROQUERCETIN AND ARABINOGALACTAN COMPOSITION ON THE CONTRACTION OF RAT'S PORTAL VEIN SMOOTH MUSCLE CELLS

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The purpose of the work: to evaluate the effect of the composition of dihydroquercetin (DHA) and arabinogalactan (AG) on contraction smooth muscle cells (SMC) of the portal vein of rats. The contractile activity of the portal vein segments was studied by mechanography on an experimental setup with a precision force sensor FT10g. The contractile response of HMC to the addition of 40 mM KCl and 10–5 M phenylephrine was assessed after 20 min of preincubation with DHA.
(0.001%), AG (0.005%) and their compositions in a ratio of 1:5 (0.006%). Amplitude of KCl induced contraction after preincubation with DHA was 61.0±5.2% of the original. The use of a combination of DHA and AG reduced the inhibitory effect of the former on the KCl induced contraction. At the same time, all the studied substances suppressed contraction of MMC induced by phenylephrine.
Key words: dihydroquercetin, arabinogalactan, smooth muscle cells, rat portal vein.

The aim of the study was to evaluate the influence of the dihydroquercetin (DQ) and arabinogalactan (AG) composition on the contraction of murine portal vein smooth muscle cells (SMC). The contractile activity of the rat portal vein segments was by the mechanographic method by using a device with mechano electrical transducer (FT10g, World *Precision* Instruments Inc. USA). The SMC contractile responses to 40 mM KCl and 10 mM phenylephrine were evaluated after 20 min pre incubation with DQ (0.001%), AG (0.005%), and their composition with ratio of 1:5 (0.006%). The amplitude of KCl induced contraction after pre incubation with DQ was 61.0±5.2% of the initial contraction. treatment with the composition of DQ and AG reduced the inhibitory effect of DQ on the KCl induced contraction. Studied substances and their composition suppressed SMC contractions induced by phenylephrine.
Key words: dihydroquercetin, arabinogalactan, smooth muscle cells, portal vein.

Introduction

Dihydroquercetin (DHA) is a well-known flavonoid possessing anti-inflammatory, decongestant, angioprotective and other properties, making it promising for the development of a phleboprotective agent [1]. Previously it was shown that DHA is not has a direct effect on the tone of the isolated portal vein, but reduces the amplitude of its contractile response to KCl and norepinephrine [3]. On the other hand, the relaxing effect on blood vessels of DHA is less than that of most other flavonoids [11].

Recent experiments have shown the possibility of modulating the pharmacological effects of DHA, due to its use in composition with arabinogalactan (AG) [4]. The purpose of the work: to evaluate the effect of DHA, AG and their compositions on the contraction of SMC of the portal vein rats.

Material and methods

We used DHA (98.6%) and AG (98.7%) isolated from the wood of Dahurian larch (Larix dahurica Turcz.), as well as the composition of DHA and AG in a ratio of 1:5 (the content of DHA and AG is 14.9% and 80.1%, respectively). Substances for research provided CJSC "Ametis".

Experiments were performed on 24 outbred rats Wistar males weighing 300–350 g obtained from the vivarium of the FGBU “N.I. E.D. Goldberg” SO RAMN. Animals were kept in plastic cages standard conditions with 12/12 light cycles and free access to food and water. Rats were exposed euthanasia by CO2 inhalation . Then the abdominal cavity opened, isolated a fragment of the portal vein with a length 2-3 cm, cleaned it of fat and connective tissue and washed in Krebs solution (NaCl - 120.4 mM; KCl - 5.4 mm; CaCl2 - 2.5 mM; MgCl2 -1.2 mM; KH2 PO4 - 0.6 mM; tris hydroxyaminomethane - 15.5 mM; glucose - 5.5 mM; pH=7.44) at 25 o C. We used fragments veins in the form of rings 2 mm wide, which were placed between a rod fixed at the bottom of the chamber and a hook connected to a force sensor (FT10g AD Instrument, Ukraine). The myogram was recorded using a personal computer with a 14 bit ADC L791 (L Card, Moscow).

After fixing a fragment of the portal vein in a chamber with a thermostatically controlled Krebs solution (35.0 ± 1.0 °C) stretched it with a load of 250–300 mg and left for 45 min for complete stabilization. The solution was changed every 15 min. Before testing the test substances, the reaction of the vessel to the introduction of 40 mM KCl was checked three times. For rate influence of the investigated substances on the reduction of MMC 20 min preincubation was carried out with substances in the following concentrations: DHA - 0.001%, AG - 0.005%, composition of DHA and AG in a ratio of 1:5 - 0.006%. After preincubation period induced a contraction of SMC by adding KCl (40 mM) or phenylephrine (PE, 10–5 M). The initial contraction amplitudes were taken as 100%: in in the first case, the reaction of the vessel to KCl; in the second case, the reaction of the vessel to PE.

Statistical processing was carried out using of the STATISTICA 6.0 software package, the reliability of the results obtained was assessed by nonparametric the Mann-Whitney test. The data are presented in the form M±m, where M is the mean value, m is the standard error of the mean value.

results

The studied substances at the concentrations used did not by themselves induce contraction of the SMC in the isolated segment of the rat portal vein.

At a concentration of 0.001% DHA had an inhibitory effect on SMC contraction induced by 40 mM KCl (Table 1). The contraction amplitude decreased by 39% relative to the control. The addition of 0.005% AG, on the contrary, led to a significant increase in the amplitude of the hyperkalotic contraction by 24%. When using the composition of DHA and AG, the amplitude of contractions was significantly more by 31% compared to the values obtained by incubation with DHA alone.

When studying the effect of DHA, AG and their composition on contraction of SMC of the portal vein of rats, induced PE, it was found that all the studied substances had a pronounced inhibitory effect (Table 2).

Amplitude of contraction after incubation period with DHA decreased by 70% compared with the baseline, with hypertension - by 66%, with a composition of DHA and AG - by 90%.

Discussion

It is known that many flavonoids (quercetin, campferol, naringenin) inhibit the contractile response vascular smooth muscle for norepinephrine, PE, high KCl concentration and other stimuli [5, 11].

Depolarization of the cell caused by the addition of excess KCl initiates the opening of voltage-dependent Ca2+ L type channels and development of hyperkalemia contracture. It has been shown that a number of flavonoids (quercetin, myricetin) activate L-type Ca2+ channels [9]. Wherein it is known that quercetin, despite the activation of the input Ca2+ inside the cells has an inhibitory effect on the contraction of vascular smooth muscles [5, 8]. Therefore, the observed decrease in the amplitude of KCl induced contraction after preincubation with DHA may be due to other mechanisms associated, for example, with the inhibition of intracellular enzymes. Watch my increase in hyperkalemia contraction amplitude on the background of hypertension, possibly due to a decrease in potassium membrane conductivity. It is interesting to note that the use of DHA in combination with AG makes it possible to reduce

Table 1

Effect of dihydroquercetin (0.001%), arabinogalactan (0.005%) and their compositions (0.006%) on the amplitude of contraction induced by KCl (40 mM)

Groups	Amplitude of contraction, %
Dihydroquercetin, n=8	61.0±5.2
Arabinogalactan, n=8	124.5±6.6*
Composition of dihydroquercetin and arabinogalactan, n=8	92.0±6.5*+

Note: * – p<0.01 in comparison with dihydroquercetin, + – p<0.05 in comparison with dihydroquercetin compared to arabinogalactan.

table 2

Effect of dihydroquercetin (0.001%), arabinogalact on (0.005%) and their composition (0.006%) on the amplitude contraction caused by FE (10-5M)

Groups, n=8	Amplitude of contraction, %
Dihydroquercetin	29.6±7.1
Arabinogalactan	34.0±7.2
Composition of dihydroquercetin and arabinogalactan	9.4±3.8+

Note: + – p<0.05 compared to arabinogalactan.

inhibitory effect of DHA on downregulation of SMC gate veins of rats.

The reduction of MMC in response to the addition of catecholamines is caused by the activation of adrenergic receptors, leading to a decrease in cAMP levels and an increase in concentration of Ca2+ in the cytoplasm of SMCs [6]. Our data of the previous experiment indicate a decrease in the contractile response of SMC in the portal vein of rats on the addition of norepinephrine after preincubation with DHA [3]. As a result of this study, it was found that DHA inhibits the contractile response of smooth muscles veins for the addition of FE, which is an ÿ1 agonist adrenoreceptors. AG also reduced the amplitude of SMC contractions, and when using a combination of two substances this effect is cumulative.

As is known, the enzyme that degrades cAMP is cAMP dependent phosphodiesterase (PDE). In vitro studies have shown that quercetin and several other flavonoids inhibit the activity

PDE, while DHA, on the contrary, increases its activity [7]. Considering this fact, the data on the ability of DHA to increase the level of cyclic nucleotides and decrease the Ca2+ concentration in the platelet cytoplasm seem to be contradictory [2]. Unfortunately, there are no data in the literature on the effect of DHA on the content of cAMP and Ca2+ in SMA. FROM on the other hand, phosphodiesterase activity may not be of decisive importance in vasorelaxant the action of flavonoids, since it was shown that an increase in the level of cAMP does not change the quercetin-induced relaxation of MMC [5].

In addition to phosphodiesterase, an important role in the process contractions are played by protein kinases that phosphorylate proteins of the contractile apparatus and changing, such Thus, their sensitivity to Ca2+ ions [6]. Flavonoids, including DHA, inhibit the activity of protein kinase C and thus can prevent an increase in the Ca2+ sensitivity of contractile proteins [8, 10].

Conclusion

Thus, the data obtained indicate on the inhibitory effect of dihydroquercetin on the contractile response of portal vein smooth muscle cells rats to the action of various stimuli (KCl, norepinephrine, phenylephrine). The use of DHA in combination with AG reduces the inhibitory effect of DHA on KCl-induced contraction of smooth muscle cells of the portal vein of rats. All test substances inhibit the contraction of MMC induced by phenylephrine.

Literature

1. Kolkhir V.K., Tyukavkina N.A., Bykov V.A. etc. Dikvertin - new antioxidant and capillary protective agent // Chem. farm. magazine - 1995. - No. 9. - S. 61–64.
2. Kubatiev A.A., Tyukavkina N.A., Bykov V.A. etc. Suppression diquertin ADP and thrombin induced accumulation of cytoplasmic calcium in human platelets // Khim. farm. magazine - 1999. - No. 12. - P. 3–4.
3. Ivanov I.S., Sidekhmenova A.V., Nosarev A.V. dihydroquercetin on the tone of isolated veins of rats // Bull. experimental biol. and honey. - 2013. - T. 155, No. 1. - S. 71–72.
4. Composition with increased pharmacological activity based on dihydroquercetin and plant polysaccharides (options): Pat. 2421215 Russian Federation, IPC ÿ61ÿ31/15, ÿ61ÿ9/14 / A.V. Dushkin, E.S. Meteleva, V.P. Tikhonov and others; applicants and patent holders Open Joint Stock Company Plant of Ecological Equipment and Ecological Nutrition “DIOD” (RU). – No. 2421215: Appl. 04/15/2010; publ. 06/20/2011.
5. Duarte J., Perez Vizcaino F., Zarzuelo A. et al. Vasodilator effects of quercetin in isolated rat vascular smooth muscle // Eur. J. Pharmacol. - 1993. - Vol. 239, Iss. 1–3. – P. 1–7.
6. Khalil RA, van Breemen C. Sustained contraction of vascular smooth muscle: calcium influx or C kinase activation? // J. Pharmacol. Exp. Ther. - 1988. - Vol. 244, no. 2. – P. 537–542.
7. Kuppusamy UR, Das NP Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes // Biochem. Pharmacol. - 1992. - Vol. 44, no. 7. - P. 1307-1315.
8. Perez Vizcaino F., Duarte J., Andriantsitohaina R. Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols // Free Radic. Res. - 2006. - Vol. 40, no. ten. - P. 1054-1065.
9. Saponara S., Sgaragli G., Fusi F. Quercetin antagonism of Bay K 8644 effects on rat tail artery L type Ca(2+) channels // Eur. J. Pharmacol. - 2008. - Vol. 598, Iss. 1–3. – P. 75–80.
10. Wang YH, Wang WY, Liao JF et al. Prevention of macrophage adhesion molecule 1 (Mac 1) dependent neutrophil firm adhesion by taxifolin through impairment of protein kinase dependent NADPH oxidase activation and antagonism of G protein mediated calcium influx // Biochem. Pharmacol. - 2004. - Vol. 67, no. 12. – P. 2251–2262.
11. Xu YC, Leung SW, Yeung DK et al. Structure activity relationships of flavonoids for vascular relaxation in porcine coronary artery // Phytochemistry. - 2007. - Vol. 67, no. eight. - P. 1179–1188.

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COMPARATIVE STUDY OF THE PROTECTOR EFFECT OF MODIFIED NANODIAMONDS OF EXPLOSIVE SYNTHESIS UNDER SYSTEMATIC EXPOSURE TO NICKEL AND CHROMIUM IONS ON GUINE PIG SKIN

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PROTECTIVE EFFECT OF MODIFIED DETONATION]SYNTHESIZED NANODIAMONDS ON NICKEL AND CHROMIUM ION]INDUCED ALLERGIC CONTACT DERMATITIS IN GUINEA PIGS

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The work is devoted to a comparative study of the protective effect of modified nanodiamonds (MND) explosive synthesis during the systematic exposure of the skin of guinea pigs to nickel and chromium ions, inducing the development of allergic contact dermatitis (AKD). The data obtained indicate that MND have a protective effect when exposed to the skin of animals with nickel ions. It has been shown that there is no protective effect of MND upon systematic exposure of animal skin to chromium ions. Revealed the differences are associated with different adsorption properties of MNDs for the studied ions. It follows from the results of atomic absorption spectroscopy of skin samples that MNDs effectively bind nickel ions and almost do not adsorb chromium ions. The prospects for the use of MND as a new adsorbent for the prevention of ACD caused by nickel ions are discussed.

Keywords: allergic contact dermatitis, modified explosive nanodiamonds, ions nickel and chromium, clinical morphological study.

The article presents the comparative study of the protective effects of modified nanodiamonds (MND), obtained by detonation synthesis, on nickel and chromium ion induced allergic contact dermatitis (ACD) in guinea pigs. treatment with MND exerted protective effects on guinea pig skin exposed to systematic application of nickel ions. No MND mediated protective effects were found in case of systematic application of chromium ions on the skin of experimental animals. The differences in the effects were caused by the differential adsorption properties of MND for nickel and chromium ions. Results of atomic adsorption spectroscopy of the skin samples showed that MND effectively bound nickel ions and did not adsorb chromium ions. The authors discuss prospects of the use of MHA as the new adsorbent agent for prevention ACD caused by nickel ions.
Key words: allergic contact dermatitis, modified detonation synthesized nanodiamonds, nickel and chromium ions, clinical morphological study.

Introduction

Allergic contact dermatitis is one of the most common diseases in dermatological practice, which occurs in 2.0–2.5% of the world's population. ball [5, 12, 17]. In recent years, there has been a steady increase in the incidence of ACD, which is associated with a significant increase in the number of chemicals used in everyday life and at work [12]. The most common cause of the onset and development of this disease is skin contact with chemical allergens, for example, divalent metal ions such as cobalt, nickel, and chromium [4, 6, 12]. important role in prevention of ACD belongs to the means capable of bind metal ions on the surface of the skin and thereby neutralize their toxic effect. new adsorbents for binding and neutralizing allergens of a chemical nature on the surface of the skin can be modified nanodiamonds (MND) deton