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BIOSYNTHETIC PROCESSES IN CARDIOMYOCYTES OF ALBINO RATS AFTER ADMINISTRATION OF DIHYDROQUERCETIN

DOI 10.25789/YMJ.2018.63.36

ABSTRACT

The aim of the research was to study the effect of dihydroquercetin on DNA synthesis and anabolic processes in the myocardium; to analyze the role of changes in biosynthetic processes in cardioprotective effects during oxidative stress. Materials and methods. Dihydroquercetin ("Ametis", Russia) was injected to newborn and adult albino rats Wistar intraperitoneally in a dose 50 mg/kg during 5 days daily. Oxidative stress was modeled by hypobaric hypoxia. Biosynthetic activity was evaluated by autoradiography with 3H-thymidine and morphometry of nucleo-nucleoli apparatus. Parameters of free-radical oxidation was investigated by chemiluminescence. Results. Administration of dihydroquercetin to intact newborn rats from 2 to 6 postnatal days depressed the DNA-synthetic activity of cardiomyocytes and increased the nucleoli amount in right ventricle cardiomyocytes. Administration of dihydroquercetin to newborn rats, exposed to intrauterine hypoxia, corrected negative cardiac consequences of antenatal hypoxia. In adult male rats injections of dihydroquercetin induce the decrease of cardiomyocytes' nuclei size and total area of nucleoli in cardiomyocytes' nuclei. These changes may reflect the inhibition of cardiomyocytes anabolic activity. Administration of dihydroquercetin to adult male rats preliminarily before hypobaric hypoxia corrected the post-hypoxic changes of heart weight and total area of nucleoli of right ventricle cardiomyocytes. Conclusion. Dihydroquercetin has a positive corrective effect on the biosynthetic processes in the myocardium of albino rats in the presence of oxidative stress. But in «healthy» heart dihydroquercetin induces adverse affects, because it inhibits DNA synthetic processes in the myocardium of newborn animals and significantly reduces the morphometric nucleo-nucleolar parameters of adult rats cardiomyocytes. The data obtained allow to recommend the using of antioxidants in cardiology only in the presence of pronounced oxidative stress.

Keywords: cardiomyocytes, antioxidants, DNA synthesis, nucleoli, free-radical oxidation.

Introduction

Antioxidants are recommended for the prevention of cardiac pathology [2]. Plant bioflavonoid dihydroquercetin (DHQ) is considered as a reference antioxidant [6] and is used for prevention and treatment cardiovascular disorders [8]. DHQ and it analog quercetin have a positive effects in ischemically-reperfusion [12], post-traumatic [11], diabetic [13] and other myocardial lesions. DHQ reduces the angiotensin II level in the myocardium, decreases the formation of reactive oxygen species (ROS) by inhibiting the NADPH-oxidase activity of cardiomyocytes (CMC) [13].

The aim of the research was to study the effect of dihydroquercetin on DNA synthesis and anabolic processes in the myocardium, to analyze the role of changes in biosynthetic processes in cardioprotection under oxidative stress.

Materials and methods

Newborn and adult albino Wistar rats were used in experiments. All experimental manipulation were performed according to all institutional and national guidelines for the care and use of laboratory animals. Rats were kept in a well-ventilated cage under standard laboratory conditions. They were fed pellet diet and water *ad libitum*. The permission of the Ethical Committee of the Far Eastern State Medical University was obtained for carrying out the experiments.

At the first stage of the study, newborn offspring of intact female rats and female rats exposed to hypobaric hypoxia during pregnancy were used. For modeling the hypobaric hypoxia, pregnant females from 14 to 19 gestation days were placed in a hypobaric chamber, in which a low pressure (224 mm Hg) was created. The partial pressure of oxygen was 42 mm Hg, accordingly. The duration of daily hypoxic exposure was 4 hours: decompression and pressure increasing continuance were one hour, the duration of the stationary hypobaric state was two hours. Other group of pregnant rats (control) did not incubate in hypoxic conditions. The offsprings were divided into four experimental groups randomly. The "Control group": animals were subjected to intraperitoneal injection of isotonic sodium chloride solution (0,1 ml) from 2 to 6 postnatal days daily. The "DHQ group": animals were subjected to intraperitoneal injection of dihydroquercetin (50 mg/kg; "Ametis", Russia) from 2 to 6 postnatal days daily. The "Antenatal hypoxia group": animals were exposed to intrauterine hypoxia and were subjected to intraperitoneal injection of isotonic sodium chloride solution (0,1 ml) from 2 to 6 postnatal days daily. The "Antenatal hypoxia+DHQ group": animals were exposed to intrauterine hypoxia and were subjected to intraperitoneal injection of of dihydroquercetin (50 mg/kg; "Ametis", Russia) from 2 to 6 postnatal days daily.

After 24 hours after the final injection, the 7-day-old animals were weighed and withdrawn from the experiment by rapid decapitation under anesthesia. One hour prior to euthanasia, 7-day-old animals were intraperitoneally injected with ^{3}H-thymidine at a dose of 1 μCiurie per gram to assess the DNA synthesizing activity of CMC.

At the second stage of the study adult (60-days-old) male rats were used. Four experimental groups was formed also. The "Control group": animals were subjected to intraperitoneal injection of isotonic sodium chloride solution (0,1 ml) 5 days daily. The "DHQ group": animals were subjected to intraperitoneal injection of dihydroquercetin (50 mg/kg; "Ametis", Russia) 5 days daily. The "Hypoxia group": animals were exposed to hypobaric hypoxia during 4 hours 5 days daily (partial pressure of oxygen was 42 mm Hg) with prior administration of isotonic sodium chloride solution (0,1 ml) 60 mins before hypoxic exposure. The "DHQ+hypoxia group": animals were exposed to hypobaric hypoxia during 4 hours 5 days daily (partial pressure of oxygen was 42 mm Hg) with prior administration of dihydroquercetin (50

mg/kg; "Ametis", Russia) 60 mins before hypoxic exposure. 24 hours after the final experimental procedure, the adult male rats were weighed and than rapid decapitation after 30-second raush anesthesia with chloroform vapors was carried out. After organ extraction the heart weight was evaluated.

Than the tissue fragment was placed in the Carnoy fixator with standard histological procedure. The autoradiographs of 7-day-old animals' heart was prepared according to the procedure adopted in the laboratory [1]. The index of labeled nuclei (ILN, %) was determined by viewing at least 2,000 CMC nuclei in each investigated area of the myocardium. The intensity index (II), which indirectly characterizes the rate of DNA synthesis, was estimated as the average number of tracks over the nucleus.

In order to analyse the protein-synthesizing activity of CMC, histological sections of heart stained with silver nitrate was prepared [4]. The average number of nucleoli was counted by viewing at least 100 nuclei in the subendocardial zones of the left and right heart ventricles. Computer morphocytometry was performed on the MEKOS-C image analyzer: the area of nuclei and nucleoli of cardiomyocytes, the average number of nucleoli in the nuclei of cardiomyocytes were determined.

Reactive oxygen species generation in heart homogenates were evaluated by chemiluminescence assay (CML). Registration of CML was carried out on a luminescent spectrometer LS 50B "PERKIN ELMER". The standardization of the CML signal was performed using special program «Finlab». The CML study included the determination of a number of parameters for the intensity of spontaneous and activated luminescence: Ssp. – parameter of 1 minute spontaneous CML, which directly correlates with the reactive oxygen species' production; H1 – maximum of amplitude of the Fe2+-induced luminescence quick flash, which indicates the content of lipid peroxidation products; Sind 1 – parameter of 2 minutes Fe2+-induced CML, reflecting the rate of peroxide radicals generation; H2 – maximum of amplitude of the H2O2-induced luminol-dependent CML, that inversely correlates with substrate' peroxide resistance; Sind 2 – parameter of 2 minutes H_2O_2-induced luminol-dependent CML, which inversely correlates with antioxidant capacity. CML parameters were calculated per 1 g tissue and expressed in relative units.

The statistical analysis was performed using the Statistica software version 6.0 by the t-Student test. Differences between groups were considered significant at $p<0,05$. The total number of animals used in the work was 126 rats.

Results and discussion

First of all, we studied the effect of DHQ in the myocardium of newborn rats. Five-fold administration of DHQ does not change the body and heart weight of 7-day-old rats (Table 1). When DNA-synthetic processes in the myocardium was studied, a decrease of the amount of CMC in the S-phase of the cell cycle was found in the left atrium myocardium (by 31,4%), in the right atrium myocardium (by 35,1%). Also a decrease of the CMC intensity index of the right ventricle myocardium was registered (by 12,6%) (Table 2). We registered the increase of nucleoli amount (by 9,5%) in the nuclei of the right ventricular CMC of the experimental rats [9]. Thus, DHQ induced inhibition of proliferative and activation of protein-synthetic activity of CMC. This effects can indicate on the stimulation of differentiation processes in the myocardium of newborn animals under the antioxidant influence. It is known, that ROS in developing CMC prevent cell differentiation, and pharmacological reduction of the ROS generation during cardiogenesis stimulates differentiation of CMC [10].

We analyzed the effect of DHQ on the biosynthetic activity of CMC of newborn rats, exposed to antenatal hypoxia. Antenatal hypoxia induced the decreasing of the body weight (by 24,8%) and the heart weight (by 25,6%) of 7-day-old rats (Table 1), and caused inhibition (by 30,8-34,0%) of DNA-synthetic activity of CMC of the examined heart chambers (Table 2). This results agree with the our early data about the cardiac consequences of antenatal hypoxia [1]. The administration of DHQ to animals, exposed to antenatal hypoxia, corrected the heart weight (Table 1), normalized of DNA synthesis in the myocardium (Table 2). According to data of Petruk N.S. et al. (2014), in newborn rats exposed to antenatal hypoxia, acute myocardial ischemia occurs on the 3rd postnatal day as a result of oxidative stress [7]. Influence of DHQ is able to reduce the intensity of oxidative stress and normalize the proliferative processes in the myocardium. In addition, in 7-day-old animals of this experimental group, we registered an increase of the nucleoli amount in the CMC nucleus of left (by 35.6%) and right (by 35.2%) ventricle (Table 2).

At the next stage of the investigation, we studied the effects of five-fold administration of DHQ in adult male rats. In adult animals, the administration of

Table 1

Gravimetric parameters of 7-days-old albino rats, exposed to antenatal hypoxia and administration of dihydroquercetin (DHQ)

Parameter	Control	DHQ	Antenatal hypoxia	Antenatal hypoxia + DHQ
Index of labeled nuclei of left atria CMC	14,40±0,36	14,26±0,47	10,83±0,41*	11,08±0,45*
Index of labeled nuclei of left ventricle CMC	95,20 ± 6,43	89,67 ± 8,23	70,86 ± 5,50*	78,64 ± 6,29

* In tables 1-5 - $p<0,05$ compared with "Control".

Table 2

Proliferative and anabolic activity of cardiomyocytes (CMC) of 7-days-old-rats, exposed to antenatal hypoxia and administration of dihydroquercetin (DHQ)

Parameter	Control	DHQ	Antenatal hypoxia	Antenatal hypoxia + DHQ
Index of labeled nuclei of left atria CMC	5,76±0,67	3,95±0,25*	3,87±0,29*	4,65±0,17
Index of labeled nuclei of left ventricle CMC	8,33 ± 0,67	7,78±0,35	5,60 ± 0,21*	7,60 ± 0,28
Intensity index of left ventricle CMC	21,90 ±0,93	19,95±0,62	17,44 ± 1,70*	19,74 ± 0,47
Nucleoli amount in left ventricle CMC	2,19±0,05	2,41±0,11	2,24±0,07	2,97±0,07*
Index of labeled nuclei of right atria CMC	5,49±0,67	3,56±0,21*	3,80±0,29*	4,58±0,33
Index of labeled nuclei of right ventricle CMC	6,56 ± 0,64	5,97±0,26	4,33 ± 0,17*	6,10 ± 0,21
Intensity index of right ventricle CMC	21,54 ± 0,71	18,83±0,20*	16,09 ± 1,57*	19,65 ± 0,53
Nucleoli amount in right ventricle CMC	2,10±0,05	2,30±0,07*	2,25±0,05	2,84±0,06*

DHQ did not change the body weight, the heart weight (Table 3), and the nucleoli amount in CMC nuclei (Table 4). Morphometric studies revealed a decrease of the CMC nuclei size (by 14% and by 33,3%) and the total area of the nucleoli in the CMC nuclei (by 31,2% and by 13,4%) of the left and right ventricles, respectively (Table 4). DHQ inhibits hypertrophy of CMC and protein-synthetic processes in CMC induced by angiotensin II. Moreover, it has been shown that the excess of ROS directly induced the CMC hypertrophy [14]. The role of ROS in maintaining of the structural and functional parameters of CMC is described in the literature [3]. Injection of DHQ significantly reduced the activity of free radical generation and increased antioxidant protection of myocardial tissue (Table 5). Accordingly, the antioxidant effect of DHQ can causes a reduction of the nucleo-nucleolar apparatus parameters of CMC and decrease the activity of protein-synthetic processes in the myocardium.

Further, we analyzed the effect of the preliminary administration of DHQ on the parameters of the CMC of adult male rats, exposed to hypobaric hypoxia. Five-fold hypobaric hypoxia induced a increase of adult rats heart weight (by 15.2%) (Table 3), a decrease of the CMC nuclei size in left and right ventricles (by 9.9% and by 20.8%, respectively) and a decrease of the total area of nucleoli in CMC nuclei in left and right ventricular (by 13.8% and by 26.5%, respectively) (Table 4). Also, in the heart homogenate of this experimental group animals, pronounced stimulation of free radical generation and a decrease of antioxidant activity were observed (Table 5). Preliminary (prior to hypoxic exposure) administration of DHQ corrected the changes of the heart weight (Table 3) and parameter of total area of the nucleoli in the CMC of the right ventricle (Table 4). Also a pronounced antioxidant effect was revealed according to the chemiluminescence data (Table 5).

Thus, the DHQ injections induce a significant decrease of the CMC morphometric parameters in adult animals. This effect shows the inhibition of biosynthetic processes in the heart cells under the antioxidant influence. At the same time, the preliminary administration of DHQ before hypoxic action partially correct the negative effect of hypoxia.

The effect of DHQ on the albino rats' heart has some ontogenetic features: the DHQ induced more pronounced deviations of the CMC parameters in intact adult animals and less correction

Table 3

Gravimetric parameters of adult albino rats, exposed to hypoxia and administration of dihydroquercetin (DHQ)

Parameter	Control	DHQ	Hypoxia	DHQ + hypoxia
Body weight (g)	236,25±25,6	241,88±20,34	242,5±8,86	232,22±13,02
Heart weight (g)	0,894±0,097	0,919±0,084	1,03±0,075*	0,932±0,068

Table 4

Nucleo-nucleolar parameters of cardiomyocytes (CMC) of adult albino rats, exposed to hypoxia and administration of dihydroquercetin (DHQ)

Parameter	Control	DHQ	Hypoxia	DHQ + hypoxia
Size of nuclei of left ventricle CMC	48,80±1,16	41,99±1,44*	43,97±1,23*	36,67±0,92*
Total area of nucleoli of left ventricle CMC	3,69±0,14	2,54±0,12*	3,18±0,12*	3,00±0,11*
Amount of nucleoli of left ventricle CMC	1,97±0,07	1,91±0,05	2,04±0,14	1,88±0,06
Size of nuclei of right ventricle CMC	50,44±1,53	33,67±1,29*	39,95±1,21*	28,8±1,01*
Total area of nucleoli of right ventricle CMC	2,75±0,10	2,38±0,094*	2,02±0,10*	2,94±0,12
Amount of nucleoli of right ventricle CMC	1,93±0,03	2,01±0,07	2,00±0,05	1,89±0,03

Table 5

Chemiluminescence parameters of homogenate of adult male rats' heart

	Control	DHQ	Hypoxia	DHQ + hypoxia
Ssp	0,10±0,009	0,071±0,007*	0,301±0,024*	0,138±0,012
Sind-1	0,699±0,053	0,527±0,047*	1,177±0,091*	0,857±0,078
H1	0,465±0,038	0,350±0,024*	1,314±0,063*	0,731±0,066*
Sind-2	4,288±0,277	3,377±0,250*	10,222±0,828*	6,115±0,436*
H2	3,248±0,248	2,017±0,131*	8,538±0,689*	4,467±0,488

effects during oxidative stress, compared with newborn rats. The reason of this difference may be the low structural maturity (low differentiation) of CMC and a large proportion of anaerobic processes in the metabolic profile of the newborn animals myocardium [5].

Conclusion

Dihydroquercetin has a positive corrective effect on the biosynthetic processes in the myocardium of albino rats in the presence of oxidative stress. But in «healthy» heart dihydroquercetin induces adverse effects, because it inhibits DNA synthetic processes in the myocardium of newborn animals and significantly reduces the morphometric nucleo-nucleolar parameters of adult rats cardiomyocytes. The data obtained allow recommending the using of antioxidants in cardiology only in the presence of pronounced oxidative stress.

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