The improvement of the functional condition of micro-vascular endothelium among the aged people with metabolic syndrome under quercetin influence

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Abstract. The **Aim** of study is the impact of course application of quercetin on the functional state of the endothelium of the microvessels in elderly people with metabolic syndrome. **Materials and methods.** We examined 110 patients (55 primary and 55 control patients) with metabolic syndrome (MS). Patients in the main group (n = 55) received quercetin ("Kvertin", chewing tablets manufactured by PJSC "Borshchagovsky KFZ", 80 mg 3 times a day) during 3 months. The control group patients (n = 55) received placebo tablets. The volume velocity of skin blood flow in the region of the middle third of the inner surface of the forearm was determined using the laser doppler flowmetry (LDF) on the apparatus BLF-21D (Transonic Systems Inc, USA). The functional state of the endothelium of microvessels was examined in a sample with post-exclusive hyperemia by clamping shoulder vessels of with a cuff for 3 minutes, in which the pressure exceeded the systolic blood pressure of the subject by 50 mm Hg. Blood pressure was measured by the Erkameter 3000 (Germany) mercury sphygmomanometer on the shoulder artery in accordance with ESH recommendations. **Results.** In the group of patients receiving quercetin, when performing a test with post-occlusive hyperemia, there was a statistically significant increase in the maximum volumetric velocity of the skin blood flow and the duration of the recovery period, the volumetric velocity of the skin blood flow to the initial level, which indicates an improvement in the vasomotor function of the microvessel endothelium. Improvement of the functional state of the endothelium occurred in 38 of 55 patients (69%) examined and was accompanied by a significant decrease in the level of systolic and diastolic blood pressure. Patients in the control group showed no improvement in the indices of vasomotor function of the microvessel endothelium. **Conclusions.** Course application of quercetin improves the functional state of the endothelium of microvessels, which helps to lower blood pressure in patients with metabolic syndrome.

Key words: metabolic; syndrome; quercetin; functional state of the endothelium; arterial pressure.

In many studies of recent years, the involvement of the endothelial dysfunction in the occurrence and development of various diseases has been actively investigated.

It is proved that disturbance of the balance between synthesis by endothelium of biologically active substances with vasodilating and vasoconstrictor properties towards increase of production of the last indicated, influences the ability of endothelium to support vascular homeostasis [1]. The known endothelial vasoconstrictors and proaggregants are endothelin (I, II, III), angiotensin II, prostaglandin F_{2α}, thromboxane A₂, leukotrienes C and D. Nitrogen oxide (NO), bradykinin, endothelial hyperpolarization factor, prostacyclin, prostaglandin E₂ belong to vasodilators. The main neurohumoral mediators that increase NO

production are adrenaline, norepinephrine, acetylcholine, histamine, bradykinin, ADP, serotonin, thrombin, endothelin, melatonin. Endothelium-dependent vasodilation is an important indicator of vasomotor endothelial function among the patients with diabetes mellitus and vascular diseases. Endothelium-dependent vasodilation is disturbed among the patients with ischemic heart disease [2], arterial hypertension [3, 4], among the patients with various risk factors, in particular, insulin resistance [5]. For today, insulin resistance (IR) and hyperinsulinemia, overweight, visceral obesity impaired glucose tolerance, dyslipidemia are related to cardiovascular risk factors and very often accompany the development and clinical course of arterial hypertension and other cardiovascular diseases [6].

As well, in the development of insulin resistance, the particular attention is paid to the suppressive role of oxidative stress. It should be noted that under the influence of free radicals there is a violation of NO synthesis and inhibition of the production of the secondary messenger of the action of insulin - cGMP [7]; impaired insulin signal transduction due to inhibition of protein kinase B activation and intracellular translocation of the glucose transporter in peripheral tissues. As well, activation of the nuclear factor NF- κ B by free radicals influences on different pathways of signal transduction, which causes the development of endothelial dysfunction, pro-inflammatory and procoagulative state, that are the major risk factors for cardiovascular pathology [8].

The important role of the oxidative stress in the development of metabolic syndrome, justifies the expediency of using antioxidant drugs for its correction. Among the latest, quercetin is attributed, the most common representative of flavonoids. The effects of quercetin are related to its antioxidant properties, the influence on various enzyme systems and biological pathways that determine carcinogenesis, inflammation, and cardiovascular diseases [9,10]. Quercetin has an angioprotective, vasodilating, anti-inflammatory effects [11]. It is able to normalize the blood pressure due to its vasoprotective properties, in particular, it inhibits the activity of 5-lipoxygenase, synthesis of leukotrienes and the activity of the inflammatory process in the vascular endothelium, reduces the production of adhesive molecules by endothelium, inhibits the processes of thrombogenesis, raises the level of nitrogen oxide in the endothelial cells, dependently on dose [12, 13, 14].

The aim of our study was to investigate the impact of the course taking of quercetin on the functional state of endothelium of microvessels among the aged patients with metabolic syndrome.

Materials and methods

The clinical research was conducted in accordance with the laws of Ukraine and the principles of the Helsinki Declaration of Human Rights. The study design, information about study patient, and the information agreement form were agreed by the ethics committee of the Clinical Department of "D.F. Chebotaryov Institute of Gerontology NAMS of Ukraine" (Protocol N 11, date by June 17, 2016). There were selected men and women in the age of 60-74 years with the criteria for MS.

Metabolic syndrome was diagnosed in accordance with ATP III recommendations (2001) if three or more criteria were detected: 1) the waist circumference for the men is more than 102 cm and for the women is more than 88 cm; 2) the level of triglycerides is above 1.7 mmol / l; 3) high density lipoprotein cholesterol (HDL cholesterol) for the men is less than 1.03 mmol / l and for the women is less than 1.29 mmol / l; 4) the systolic blood pressure is above 130 mm Hg. and (or) diastolic blood pressure is more than 85 mm Hg.; 5) fasting blood plasma glucose level is exceeding 6.1 mmol / l [15]. All patients had a history of an increase of blood pressure, on average, in the range of $136.0 \pm 1.6 / 77.8 \pm 1.4$ mm Hg. (within 10.07 ± 4.4 years). Selected subjects had no serious pathology such as uncontrolled arterial hypertension, diabetes mellitus, stroke, neurocardial infarction, heart failure III-IV functional class, moderate and severe renal failure, diffuse diseases of connective tissue cancer. All subjects were distributed equally into the main and control groups. ACE inhibitors, statins, acetylsalicylic acid (75-100 mg per day) were taken as the basic therapy by the patients of both groups, the doses of the drugs were stable one month before the inclusion and throughout the study. The patients of the main group for three months received quercetin (drug "Quertin", chewable tablets manufactured by PJSC SPC "Borschagovsky chemical pharmaceutical plant", in dose 80 mg (2 tables.) 3 times per day). The patients of the control group received a placebo for 3 months (2 tables 3 times per day).

To determine the skin blood flow (SBF) rate, the Laser Doppler Flowmetry (LDF) methodology was used, which allows to register the changes in blood flow in the microcirculatory channel, both at state of rest and during functional tests, that are used to determine the functional state of vascular endothelium [16, 17].

The basis of the LDF method is the measurement of the Doppler component in the spectrum of the reflected laser signal, which is scattered on the shaped elements of blood (erythrocytes) in microvessels. The LDF signal gives a quantitative characteristic of blood flow in microvessels (arterioles, capillaries, venules). The skin blood flow (SBF) velocity was determined using a dual-channel laser Doppler flowmeter BLF-21D (Producer "Transonic Systems Inc", USA). The state of vasomotor function of the microvascular endothelium was determined in accordance with the methodology proposed by O.V. Korkushko and V.Y. Lishnevskaya [18]. At the beginning, the skin blood flow rate at rest (SBF_{initial}) was measured. Then a functional test with reactive hyperemia was carried out, to create which the vessels of the shoulder were pressed with a cuff within 3 minutes, in which the pressure exceeded the systolic blood pressure by 50 mm Hg. After the resumption of blood flow (cessation of clamping), there is an increase of blood flow in tissue due to vasodilation, that is conditioned by the release of nitric oxide by the endothelium of microvessels. In this period, the indices of maximum skin blood flow rate (SBF_{max}) and the length of restoration of blood flow to the initial level were measured (relative). The higher both indices are, the better is the functional state of the endothelium of microvessels.

Arterial blood pressure measurements were made using a mercury sphygmomanometer Erkameter 3000 (Germany) on the brachial artery in accordance with ESH recommendations. Considering that the indices had a distribution close to normal, the data are presented in $M \pm m$ format. Statistical processing of the obtained data was performed using the program "Statistica 6.0 for Windows" (StatSoft, USA). The probability of the difference between groups was estimated using the t-test (by Student). The differences were considered significant at $p < 0.05$. Pearson correlation analysis was performed (the results were considered to be reliable at $p < 0.05$).

Results and discussion

Many previous studies indicated that endothelial dysfunction is connected with such factors as insulin resistance, hyperglycemia, arterial hypertension, dyslipidemia, oxidative stress [19].

Endothelial dysfunction is one of the main pathogenetic links in the formation of arterial hypertension, in patients with MS. So, the correction of this disorder is very important.

In the group of patients receiving quercetin, there was observed an improvement of SBF. Thus, a statistically significant increase of maximum volumetric velocity of skin blood flow (SBF_{max}) and duration of the period of restoration of SBF to the initial level was observed during the post-occlusive hyperemia test (Tab. 1).

Table 1

Indices of volumetric velocity of skin blood flow at rest and in the test with post-occlusive hyperemia before and after the course treatment with quercetin ($M \pm m$)

Indices	Control group (n = 55)		Main group (n = 55)	
	Before treatment	After treatment	Before treatment	After treatment
SBF at rest, ml/(min. · 100g tissues)	1.08 ± 0.03	1.04 ± 0.02	1.03 ± 0.02	1.05 ± 0.02
Max. SBF at the peak of reactive hyperemia, ml/(min. · 100g tissues)	5.65 ± 0.23	5.25 ± 0.27	5.69 ± 0.25	6.67 ± 0.24*
Recovery time SBF to the initial level, sec	110.78 ± 5.35	101.36 ± 4.20	102.18 ± 5.15	123.04 ± 4.78*
Endothelial function, %	74.57 ± 4.66	66.00 ± 4.51	77.88 ± 4.89	93.68 ± 5.11*

Notes: validity of change in the indices under the influence of treatment: * - $p < 0.05$.

Such changes of these indices evidence a significant improvement of vasomotor function of the microvascular endothelium, which is also confirmed by an increase of the endothelial function index by $(15.80 \pm 5.34)\%$, $p < 0.05$.

Patients from the control group, who were on placebo, did not show any improvement in vasomotor function of microvascular endothelium after 3 months of treatment.

The analysis of individual data, indicate that 38 of 55 patients (69%) who received quercetin had an improvement in the functional state of the microvascular endothelium.

Before intake of quercetin these patients had the maximum volumetric velocity of SBF during the sample with the post-occlusive hyperemia was less than 5 ml / (min. * 100 g of tissue), that indicates about impaired vasomotor function of the endothelium (endothelial dysfunction) [18]. After course treatment with quercetin, a statistically significant increase of the maximum volumetric and an increase in the duration of recovery of the SBF to the baseline level (Tab. 2), also took place in these patients that is indicating a significant improvement of the vasomotor function of the endothelium.

Table 2

Indices of volumetric velocity of skin blood flow in the test with post-occlusive hyperemia before and after the course treatment with quercetin in subgroups of patients with different maximum SBF before treatment ($M \pm m$)

Indices	Max. SBF more than 5 ml/(min. -100g tissues) (n = 17)		Max. SBF less than 5 ml/(min. - 100g tissues) (n = 38)	
	Before treatment	After treatment	Before treatment	After treatment
SBF at rest, ml/(min. - 100g tissues)	1.11 ± 0.03	1.07 ± 0.03	0.93 ± 0.03	$1.04 \pm 0.04^*$
Максимальна ОШШК на піку реактивної гіперемії, ml/(min. - 100g tissues)	6.99 ± 0.26	6.94 ± 0.29	4.17 ± 0.14	$6.28 \pm 0.39^{**}$
Recovery time SBF to the initial level, sec	112.50 ± 7.32	119.07 ± 6.59	89.27 ± 6.26	$128.65 \pm 6.76^{**}$
Endothelial function, %	93.74 ± 7.29	95.02 ± 7.72	59.0 ± 3.58	$89.68 \pm 6.62^{**}$

Notes: validity of change in the indices under the influence of treatment: * - $p < 0.05$, ** - $p < 0.001$.

Thus, after long-term treatment with quercetin, an improvement of the functional state of the endothelium of microvessels among the aged people with endothelial dysfunction was obtained.

Due to its vasoprotective properties, the decrease of inflammation in the endothelium and the increase of the level of nitric oxide in the endothelial cells, quercetin is potentially has abilities to reduce a blood pressure [13].

Our data indicate that no significant changes in blood pressure occurred among the patients of the control group who were on placebo (tab. 3). At the same time, in main group of patients, who took quercetin, a statistically significant decrease in systolic and diastolic blood pressure was observed.

Such favourable changes in systolic blood pressure were observed in 43 of 55 (78%) patients and in diastolic BP - in 39 of 55 (71%) subjects investigated. One of the mechanisms of the reducing of blood pressure is the improvement of the vasomotor function of the endothelium. Confirmation of this is the direct

correlation between SBFmax and systolic blood pressure in patients who took quercetin ($r = 0.35$; $p < 0.05$; $n = 55$).

Thus, the results obtained by us correspond with the data of some other experimental and clinical researches, which point out that the use of quercetin leads to a decrease of high blood pressure, heart rate, improvement of diastolic function of the left ventricle [20, 13].

Vasoprotective effects of quercetin are predicted to be due to its ability to reduce the activity of inflammation in the vascular endothelium, increase the activity of endothelial NO-synthase (eNOS), which increases the level of nitrogen oxide in endothelial cells and improves endothelial function as well [21].

Conclusions

1. After course treatment with quercetin, the maximum volumetric velocity of skin blood flow at post-occlusive hyperemia test is increased, as well as the duration of the period of skin blood flow to initial level. These changes of both indices indicate about the improvement of the functional state of the endothelium of microvessels.
2. Quercetin shows the ability to improve the functional state of the endothelium of microvessels among the patients with endothelial dysfunction, whereas among the persons without endothelial dysfunction, the effect is absent.
3. In elderly patients with metabolic syndrome, who took quercetin for a long time, an improvement in endothelial function led to statistically significant decrease in a systolic blood pressure and a tendency to decrease a diastolic blood pressure.

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