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CORRECTION OF ENDOTHELIAL DYSFUNCTION WITH SULFAPORIN IN HYPERCHOLESTEROLEMIC RABBITS

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Abstract.

In animals with experimental hypercholesterolemia, depending on the time of survey, we observed activation of smooth muscle cells and macrophages, causing development of inflammation in the intima, as evidenced by high levels of C-reactive protein (CRP), endothelin-1, and homocysteine. The aim of the study was to investigate the molecular mechanisms of endothelial dysfunction in HCH and the possibility of their correction with sulfaporin. Material and methods of the study Experiments were conducted on 46 Chinchilla rabbits with an average weight of 2.5–3.0 kg, kept on a standard diet. The model of experimental HCH in animals was reproduced by orally administering cholesterol (CS) dissolved in sunflower oil in a ratio of 0.2 g per 1 kg of body weight daily for 3 months. The development of hypercholesterolemia was assessed by the increase in the level of totalcholesterol (TC), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), which were determined on a biochemical analyzer. Results of the study. In this case, a progressive increase in the homocysteine level was established: an increase of 1.72 ($P < 0.01$); 2.33 ($P < 0.001$) and 2.89 ($P < 0.001$) times, respectively, for the periods of cholesterol administration of 1, 2 and 3 months. Considering that hyperhomocysteinemia enhances the capture of LDL by endothelial cells, it was of interest to study the relationship between these indicators. The studies showed that with a cholesterol level in LDL of 2.38 ± 0.27 mmol / l, the homocysteine content is 3.46 ± 0.25 pg / ml. With a cholesterol level in LDL of 4.08 ± 0.10; 5.97 ± 0.09 and 6.48 ± 0.11 mmol/l, the homocysteine content increases to 5.96 ± 0.05; 8.07 ± 0.43 and 9.99 ± 0.17 pg/ml, respectively. Conclusions. Hypercholesterolemia is manifested by activation of smooth muscle cells and macrophages, causing the development of inflammation in the intima, which is confirmed by high levels of CRP, endothelin-1, homocysteine.

Key words: hypercholesterolemia, C-reactive protein, endothelin-1, homocysteine, treatment.

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In recent years, many new data have emerged, significantly expanding our understanding of the pathogenesis of atherosclerosis [1, 2]. According to a number of authors [3–5], it has been proven that dysfunction of the endothelium is one of the possible main stages in atherogenesis. Under physiological conditions, the vascular endothelium not only provides adequate vasodilation, but also inhibits the activation and adhesion of platelets, suppresses the coagulation activity of the blood, and prevents the inflammatory process, which is based on the activation of leukocyte adhesion [6, 7]. Dyslipidemia plays an important role in the pathogenesis of atherosclerosis by triggering the cascade mechanism of inflammation. [1, 7, 8]. Therefore, an important role in the development of therapeutic measures belongs to reducing the level of cholesterol and other saturated fats. Much attention is paid to natural biodegradable compounds, in particular chitosan and its derivatives. At the Institute of Polymer Chemistry and Physics of the Academy of Sciences of the Republic of Uzbekistan, various metal and nano derivatives of chitosan are being developed under the supervision of S.Sh. Rashidova [9]. However, the mechanism of action of the sulfated form of chitosan on endothelial function in hypercholesterolemia (HCH) is not fully understood.

The aim of the study was to investigate the molecular mechanisms of endothelial dysfunction in HCH and the possibility of their correction with sulfaporin.

Material and methods of the study

Experiments were conducted on 46 Chinchilla rabbits with an average weight of 2.5–3.0 kg, kept on a standard diet. The model of experimental HCH in animals was reproduced by orally administering cholesterol (CS) dissolved in sunflower oil in a ratio of 0.2 g per 1 kg of body weight daily for 3 months. The development of hypercholesterolemia was assessed by the increase in the level of totalcholesterol (TC), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), which were determined on a biochemical

analyzer. Two months after the beginning of the experiment, the rabbits were divided into 5 groups: Group 1 - intact (6 rabbits), which were administered vegetable oil daily through the oral cavity at 1.0 ml / kg; Group 2 - hypercholesterolemia + H2O - control (8 rabbits); Group 3 - hypercholesterolemia + gemfibrazil at 100 mg / kg (8 rabbits); Group 4 - hypercholesterolemia + chitosan sulfate at 25 mcg / kg (8 rabbits); Group 5 - hypercholesterolemia + chitosan sulfate at 50 mcg / kg (8 rabbits). The effect of the drugs was studied dynamically: the initial 3-month state and one month after the administration of the drugs. The content of C-reactive protein (CRP) was determined on an automated biochemical analyzer «Human» (Germany) using special sets of reagents. The level of endothelin-1 and homocysteine was determined by the enzyme immunoassay using reagents from the company «ELIZA» [10]. The results obtained were compared with the indicators of the control and intact groups. The digital material was processed using the method of variation statistics.istration of the drugs. The content of C-reactive protein (CRP) was determined on an automated biochemical analyzer «Human» (Germany) using special sets of reagents. The level of endothelin-1 and homocysteine was determined by the enzyme immunoassay using reagents from the company «ELIZA» [10]. The results obtained were compared with the indicators of the control and intact groups. The digital material was processed using the method of variation statistics.

Results of the study and their discussion.

In recent years, the inflammatory theory of atherosclerosis has been considered. Attractants for inflammatory cells are deposits in the vessels of LDL themselves, which can regulate the expression of genes for the colony-stimulating factor of macrophages. This contributes to an increase in the inflammatory response in the vascular wall. In turn, specific inflammatory mediators increase the binding of LDL to the endothelium and smooth muscle cells, enhance the transcription of the LDL receptor gene. It turns out that the inflammatory cycle, modification of lipoproteins and further atherosclerotic inflammation in the vascular wall are «hostages» of modified LDL [8, 11, 12].

One of the indicators reflecting the presence of an inflammatory process in the body is CPb. Determination of this indicator in rabbits in the dynamics of GCS showed its increase by the end of the first month of CS administration to 13.50 ± 0.57 μg / ml ($P < 0.001$) with the value of this indicator in intact rabbits of 5.17 ± 0.40 μg / ml. The content of CPb in the subsequent periods continues to increase, amounting to 19.00 ± 0.96 ($P < 0.001$) and 21.88 ± 0.64 ($P < 0.001$) μg / l after 2 and 3 months from the beginning of the toxicant administration.

Local damage in the arterial wall triggers far from local processes, and this is natural. Local damage cannot be a local problem. One of the key links in “globalization” are chemotactic factors [8, 13]. The naturalness of immune disorders, one of the manifestations of which is the formation of LDL + IgG complexes, suggests changes in cellular and humoral immunity, which are necessarily observed. It turns out, as it should be, that with atherosclerosis, there are changes in markers characteristic of inflammation, including in the peripheral blood. These changes are clearly tied to the phase course of atherosclerosis, periods of its exacerbations and remissions, are determined by the total mass of vessels involved in the inflammatory process, and other signs [12, 14]. To clarify this issue, we analyzed the relationship between the indicators of atherogenic lipoproteins and the level of CRp. The studies showed that as the content of cholesterol in LDL increases, the level of CRp also increases, i.e. There is a direct dependence of the severity of inflammation on the content of cholesterol in LDL. Due to the activation of free radical oxidation of lipids and non-enzymatic glycosylation of proteins, the structures of lipids and apoB-100 in lipoproteins, as well as their receptors, change. Modified LDL are captured by macrophages through scavenger receptors, turn into foam cells, contributing to endothelial damage. When endothelial cells are damaged, platelets are activated into thromboxane A2 and platelet-derived growth factor, which stimulates the proliferation of smooth muscle cells and the development of inflammation. Recently, it has been believed that an increase in the level of CRP in the blood serum reflects the activity of inflammation, which is associated with the activity of atheromatosis even before the development of myocardial infarction or stroke [4, 13]. In this regard, an increase in the concentration of CRP should be considered as a sign of atherosclerosis. The strongest vasoconstrictor of endothelial origin is endothelin-1, the high activity of which plays a role in the aggravation of endothelial dysfunction [6,7]. The study of its content in the blood serum of rabbits with experimental hypercholesterolemia showed a progressive increase as the process worsened. The content of endothelin-1 in the blood serum increases by

1.34 (P < 0.05); 2.14 (P < 0.001) and 2.66 (P < 0.001) times, respectively, at 1, 2 and 3 months from the beginning of the experiment. At the same time, we established a direct dependence of the increase in its content on the level of hyperbetalipoproteinemia. In particular, with the LDL cholesterol content of 2.38 ± 0.27 mmol/l, the endothelin-1 level is 0.29 ± 0.01 μg/mg. With the LDL cholesterol level of 4.08 ± 0.10; 5.97 ± 0.09 and 6.48 ± 0.11 mmol/l, the endothelin-1 content progressively increases to 0.39 ± 0.01; 0.62 ± 0.03 and 0.77 ± 0.06 μg/ml, respectively, according to the terms. Recently, data have appeared on the leading role of homocysteine in the development of atherothrombosis [1, 5, 7]. This is associated with the formation of disulfide derivatives of proteins, which leads to sequencing of very low and low density lipoproteins by endothelial membranes, a decrease in the content of sulfo derivatives of glycosaminoglycans, causing a decrease in the elasticity of the vessel wall and activation of smooth muscle cell proliferation. High concentrations of homocysteine cause oxidative stress, increased production of nitric oxide radicals and activation of proinflammatory factors [15, 16]. To clarify the role of homocysteine in endothelial dysfunction, we determined its content in the blood serum of rabbits with HCS. In this case, a progressive increase in the homocysteine level was established: an increase of 1.72 (P < 0.01); 2.33 (P < 0.001) and 2.89 (P < 0.001) times, respectively, for the periods of cholesterol administration of 1, 2 and 3 months. Considering that hyperhomocysteinemia enhances the capture of LDL by endothelial cells, it was of interest to study the relationship between these indicators. The studies showed that with a cholesterol level in LDL of 2.38 ± 0.27 mmol / l, the homocysteine content is 3.46 ± 0.25 pg / ml. With a cholesterol level in LDL of 4.08 ± 0.10; 5.97 ± 0.09 and 6.48 ± 0.11 mmol/l, the homocysteine content increases to 5.96 ± 0.05; 8.07 ± 0.43 and 9.99 ± 0.17 pg/ml, respectively.

Thus, the reproduction of experimental GCS in experimental rabbits is accompanied by endothelial dysfunction due to the accumulation of very low-density and low-density lipoprotein cholesterol. Their mechanism involves interdependent changes in CRP, endothelin-1 and homocysteine, the severity of which depended on the duration of the experiment. At the same time, a clear relationship was revealed between the increase in the level of the above-mentioned compounds and the cholesterol content in LDL. These interdependent changes lead to atherogenesis, disruption of the integrity of the vascular endothelium and endothelial dysfunction. The conducted pharmacotherapy contributed to the reduction of inflammatory processes in the vascular endothelium (table). Thus, pharmacotherapy with gemfibrazilov contributed to the reduction of the CRP content in the blood serum of rabbits with GCS by 1.46 (P < 0.05) times relative to the values of the control group of animals. However, despite such a reduction, the content of this protein remained statistically significantly higher by 2.9 (P < 0.001) times than the values of intact rabbits, which indicated the preservation of inflammatory processes in the vascular endothelium. Contents of CRP, endothelin-1 and homocysteine in the blood serum of rabbits with experimental hypercholesterolemia during treatment

Groups, terms (months)	Indicators		
	CRb, μg/ml	Endothelin-1, pg/ml	Homocysteine, pg/ml
Intact	5,17 ± 0,40	0,291 ± 0,010	3,46 ± 0,25
HCS + H2O	21,88 ± 0,64a	0,772 ± 0,060 a	9,99 ± 0,17 a
HCS + gemfibrazil	15,00 ± 1,87 a, б	0,328 ± 0,016 a, б	6,96 ± 0,32 a, б
HCS + sulfaporin 25 μg/kg	5,88 ± 0,44 б, в	0,311 ± 0,018 б	5,77 ± 0,28 a б, в
GHS + sulfaporin 50 mcg/kg	4,75 ± 0,45 б, в	0,253 ± 0,016 б, в	4,58 ± 0,23 б, в

Notes: a – significant in relation to the indicators of intact rabbits; b – significant in relation to the indicators of the control group; c – significant in relation to the indicators of gcs + gemfibrazil rabbits (p < 0.05).

Pharmacotherapy of GCS with sulfaporine at a dose of 25 μg/kg statistically significantly reduced the high level of CRP by 3.72 (P < 0.001) times relative to the values of untreated animals. However, its values remained above the norm. Increasing the dose of sulfaporine had a more pronounced effect, statistically significantly reducing the high values of CRP in the blood serum by 4.16 times (P < 0.001). This indicator did not differ significantly from the values of intact rabbits, and was slightly lower than the values of the group of animals receiving sulfaporine at a dose of 25 μg/kg. In animals with GCS, when using sulfaporine at doses of 25 and 50 μg/kg, the level of CRP decreased by 2.55

and 3.16 times, respectively, compared to the values of the group of animals receiving gemfibrazil. Comparison of the CRP level with the LDL cholesterol indices showed the following results. Thus, as the LDL cholesterol level decreased, the CRP content also decreased, which was apparently due to a decrease in the phagocytosis of mlDL by smooth muscle cells. In this case, gemfibrazil had the least effect on these processes, while chitosan sulfo derivatives, significantly reducing the sequencing capture of mlDL, contributed to a decrease in the inflammatory process in the vascular endothelium. At the same time, analysis of the relationship between changes in the CRP level and the HDL cholesterol content showed a decrease in the severity of the inflammatory process as the HDL cholesterol content increased. If in untreated animals the level of cholesterol in HDL statistically significantly decreased against the background of a sharp increase in CRP, then with the use of sulfaporine, the restoration of the level of cholesterol in HDL led to a decrease in the content of CRP in the blood serum. Apparently, this was due to the increased intake of cholesterol in HDL due to the activation of LCAT and transfer to the liver. The capture of excess cholesterol leads to an improvement in the functional activity of the endothelium and the restoration of the production of prostacyclins, a noticeable decrease in proinflammatory cytokines and thromboxane, which increase inflammatory reactions in cells. Pharmacotherapy suppressed the production of a powerful chemoattractant and vasodilator - endothelin-1 (see table). Thus, pharmacotherapy with gemfibrazilov reduced the high level of endothelin-1 by 2.35 ($P < 0.001$) times relative to the values of the untreated group of animals. Despite such a decrease, this indicator remained above the values of intact rabbits. Unlike gemfibrazil, sulfaporin at doses of 25 and 50 μg/kg reduced the level of endothelin-1 by 2.48 ($P < 0.001$) and 3.05 ($P < 0.001$) times relative to the values of the untreated group of animals and did not differ significantly from the indicators of intact rabbits. Compared with gemfibrazil, sulfaporin at a dose of 50 μg/kg reduced the level of endothelin-1 by 1.3 ($P < 0.05$) times, while at a dose of 25 μg/kg only a tendency towards a decrease was noted.

A comparative analysis of the level of endothelin-1 with the content of LDL-C showed a decrease in the former as LDL-C decreased. In these studies, gemfibrazilov et al. showed that if the LDL-C level decreased by an average of 2 times, then the endothelin-1 content decreased by 2.35 times. In the other groups, a more pronounced decrease in endothelin-1 was also noted compared to LDL-C. We found the opposite changes when comparing the HDL-C level with the endothelin-1 content. If the HDL-C content increased approximately 1.3 times relative to the untreated group when treated with gemfibrazilov, then the endothelin-1 level decreased by more than 2 times. When sulfaporine was used at a dose of 25 μg/kg, the HDL-C content increased by 1.8 times, and the endothelin-1 level decreased by 2.48 times. As noted earlier, hypercholesterolemia is accompanied by a sharp increase in the homocysteine level, which triggers a cascade of free-radical processes in endothelial cells. Gemfibrasil GCS pharmacotherapy contributed to a statistically significant decrease in hyperhomocysteinemia by 1.43 ($P < 0.01$) times relative to the values of the control group of rabbits (see table). However, this indicator still remained 2 ($P < 0.001$) times higher than the values of intact rabbits. In animals with GCS treated with sulfaporine at doses of 25 and 50 μg/kg, the homocysteine level decreased by 1.73 ($P < 0.001$) and 2.18 ($P < 0.001$) times, respectively, relative to the values of the control group of animals. At the same time, its values remained higher than the standard values by 1.67 ($P < 0.01$) and 1.32 ($P < 0.05$) times, respectively, depending on the dose. It should be noted that, compared with gemfibrasil, sulfoporin reduced the homocysteine level in the blood serum of rabbits with GCS by 2.21 ($P < 0.001$) and 1.52 ($P < 0.01$) times, respectively, depending on the dose.

Analysis of the homocysteine level with the LDL-C content showed the unidirectional changes in the studied parameters. Despite the unidirectional changes in the studied parameters, their severity was different. Thus, gemfibrazil reduced the LDL-C level by an average of 2 times, while the homocysteine level decreased by an average of 1.4 times. The same changes were characteristic of other drugs. That is, the drugs reduced the LDL-C content to a greater extent than the homocysteine level, which was apparently due to the specificity of their action on the lipid spectrum. Analysis of the homocysteine level with the HDL-C content showed their opposite direction. In terms of the degree of action and severity of action, these indicators did not differ significantly, equally increasing the HDL-C content and reducing the homocysteine level. Sulfaporin was the most effective, while gemfibrazilin had a weak effect.

It is known that sulfated derivatives of chitosan are used as affinity ligands in the

creation of sorbents, for the prevention and treatment of atherosclerosis, coronary heart disease, hypercholesterolemia, etc. [17, 18]. The substitution reaction of sulfo groups (SO3H) in chitosan allows for the production of its sulfated derivative, which has highly effective antibacterial, anticoagulant, antitumor properties [19], as well as an increased ability to specifically bind blood LDL [20].

Conclusions

1. Hypercholesterolemia is manifested by activation of smooth muscle cells and macrophages, causing the development of inflammation in the intima, which is confirmed by high levels of CRP, endothelin-1, homocysteine. The level of their increase coincides with high values of cholesterol in low-density lipoproteins and low values of cholesterol in HDL.

2. Sulfated chitosan derivative reduces high levels of CRP, endothelin-1 and homocysteine. This coincides with its pronounced hypolipidemic properties. The effect of the studied drugs is superior to the classic hypolipidemic drug gemfibrazil.

LIST OF REFERENCES

[1] Klimov A.N., Shlyakhto E.V. Atherosclerosis. Problems of pathogenesis and therapy. - St. Petersburg: Medical literature, 2006. - 248 p.

[2] Titov V.N. Atherosclerosis as a pathology of polyene fatty acids (biological basis of pathogenesis, diagnosis, prevention and treatment of atherosclerosis). - Moscow: Publishing house «Altus», 2002.

[3] Kawashima S., Yokoyama M. Dysfuncthion of endothelial nitric synthase and atherosclerosis // Atheroscler Thromb Vasc Biol. - 2004. - Vol. 24. - P. 998-1015.

[4] Clinical significance of endothelial dysfunction, oxidative stress and hemostasis indices in patients with myocardial infarction / O.L. Barbarash, V.V. Kashtalap, V.N. Karetnikova et al. // Blood circulation pathology and cardiac surgery. - 2007. - No. 2. - P. 28-33.

[5] Shlyakhto E.V., Berkovich O.A., Maiseyeva O.M. Cellular and molecular genetic aspects of endothelial dysfunction // Bulletin of the Russian Academy of Medical Sciences. - 2004. - No. 6. - P. 50-52.

[6] Brevetti G., Schiano V., Chiariello M. Endothelial dysfunction: a key to the pathophysiology and natural history of peripheral arterial disease? // Atherosclerosis. – 2008. – Vol. 197. – P. 1–11.

[7] Endothelial dysfunction. Causes, mechanisms, pharmacological correction / edited by N. N. Petrishchev. — SPb.: Publishing house of St. Petersburg State Medical University. 2003. – 184 p.

[8] Nagornev V. A., Maltseva S. V. Autoimmune and inflammatory mechanisms of atherosclerosis development // Archives of pathology. – 2005. – Issue 5. – P. 6–15.

[9] Rashidova S. Sh., Milusheva R. Yu. Chitin and chitosan Bombyx mori. Synthesis, properties and application. – Tashkent: Fan, 2009. – 246 p.

[10] Levina A.A., Rakhnyanskaya A.A., Melik-Nubarov N.S., Mamukova Yu.I., Tsvetaeva N.V., Romanova E.A., Makarik T.V., Khoroshko N.D. Enzyme immunoassay for homocysteine determination // Pathogenesis. - 2004. - No. 1. - P. 92-96.

[11] Markov H.M. Nitric oxide and atherosclerosis. Nitric oxide, vascular endothelial dysfunction and pathogenesis of atherosclerosis // Cardiology. - 2009. - No. 11. - P. 64-74.

[12] Mallat Z., Taleb S., Ait-Oufella H., Tedgui A. The role of adaptive T cell immunity in atherosclerosis // J Lipid Res. – 2009. – Vol. 50 (Suppl). – S. 364–369.

[13] Abesadze I.T. Functional state of the endothelium and oxidative activity of leukocytes in patients with ischemic heart disease after coronary artery bypass grafting: Abstract of a PhD thesis. – St. Petersburg, 2008. – 21 p.

[14] Aukrust P., Otterdal K., Yndestad A., Sandberg W.J., Smith C., Ueland T., Øie E., Damås J.K., Gullestad L., Halvorsen B. The complex role of T-cell-based immunity in atherosclerosis. // Curr Atheroscler Rep. – 2008. – Vol. 10. – P. 236–243.

[15] Barkagan Z.S., Kostyuchenko G.I., Kotovshchikova E.F. Hyperhomocysteinemia as an independent risk factor for damage and thrombosis of blood vessels // Pathology of blood circulation and cardiac surgery. - 2002. - No. 1. - P. 65-71.

[16] Bairamukova A.A., Pavlenko M.A., Nabiev M.P., Mirrakhimov E.M. Homocysteineemia and the risk of developing atherosclerosis. // Central Asian Medical Journal. - 2006. - No. 2/3. - P. 137-144.

[17] Perova N.V., Metelskaya V.A. Plant sterols and stanols as food factors that reduce hypercholesterolemia by inhibiting intestinal cholesterol absorption // Cardiology.

- 2008. - No. 5. - P. 62-67.

[18] Treskunov K.A., Pogorelskaya L.V., Komarov B.A., Albulov A.I. Biologically active food supplements based on chitosan and dry extracts of medicinal plant mixtures // Practical Phytotherapy. - 2000. - No. 3. - P. 58-59.

[19] Vikhoreva T.L. Synthesis of polysaccharide sulfates and study of their biological activity // Cellbolose Chem. Technol. - 1981. - Vol. 15. - P. 487-504.

[20] Some mechanisms of hypocholesterolemic action of sulfaporin / F.Kh. Inoyatova, G.M. Kutlikova, R.Yu. Milusheva et al. // Vestnik TMA. - 2011. - No. 3. - P. 19-22.