



STUDY OF ANTIATHEROSCLEROTIC AND ENDOTHELIOPROTECTIVE ACTIVITY OF PEPTIDE AGONISTS OF EPOR/CD131 HETERORECEPTOR

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Introduction. The drugs affecting a mitochondrial dysfunction, oxidative stresses, apoptosis and inflammation of the vascular wall, have a high potential for the prevention and treatment of atherosclerotic lesions. In this regard, the use of EPOR/CD131 heteroreceptor agonists which have a similar spectrum of pharmacological effects, is one of the promising strategies in the treatment of cardiovascular diseases.

Materials and Methods. The study was carried out on 68 C57Bl/6J male mice. Atherosclerosis was simulated in transgenic animals with an endotheliospecific knockdown of the *Polg* gene by simulating a balloon injury and keeping on a Western diet. Then, the studied drugs were injected once every 3 days at the dose of 20 µg/kg for 27 days. On the 28-th day, the animals were euthanized and the area of atherosclerotic plaques was assessed. The gene expression associated with the processes of inflammation, antioxidant protection, apoptosis, and angiogenesis was also determined in the aortic tissues. In addition, the endothelium protective effect of peptides on primary cultures of endothelial cells of wild and transgenic *Polg-D257A* mice was studied.

Results. No statistically significant effect of drugs on the area of lipid infiltration have been found. However, the studied peptides have significantly reduced the expression of proinflammatory genes (*iNos*, *Icam1*, *Vcam1*, *Sele*, *Il6*, *Tnfa*), the genes associated with angiogenesis (*Vegfa*, *Kdr*, and *Hif1a*), the expression of proapoptotic factors; they decreased the Bax/Bcl-2 ratio by more than 1.5 times. In addition, when supplemented with H₂O₂ in vitro, peptides dose-dependently increased endothelial cell survival.

Conclusion. The erythropoietin-based peptides can be used to improve the functional state of the vascular wall against the background of atherosclerotic lesions and have a depressing effect on pathobiological processes associated with a mitochondrial dysfunction. In addition, the studied peptides have a significant endothelial protective effect in the induction of oxidative stress in vitro.

Keywords: atherosclerosis, erythropoietin derivatives, mitochondrial dysfunction, oxidative stress.

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ИЗУЧЕНИЕ АНТИАТЕРОСКЛЕРОТИЧЕСКОЙ И ЭНДОТЕЛИОПРОТЕКТИВНОЙ АКТИВНОСТИ ПЕПТИДНЫХ АГОНИСТОВ ГЕТЕРОРЕЦЕПТОРА EPOR/CD131

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Введение. Препараты, воздействующие на митохондриальную дисфункцию, оксидативный стресс, апоптоз и воспаление сосудистой стенки, обладают высоким потенциалом при профилактике и лечении атеросклеротических поражений. В этой связи применение агонистов гетерорецептора EPOR/CD131, которые обладают подобным спектром фармакологических эффектов, является одной из перспективных стратегий в лечении кардиоваскулярных заболеваний.

Материалы и методы. Исследование было проведено на 68 самцах мышей C57Bl/6J. Атеросклероз моделировали на трансгенных животных с эндотелиоспецифичным нокаутом гена *Polg* путем моделирования баллонной травмы и содержания на западной диете. Затем в течение 27 дней вводили изучаемые препараты 1 раз в 3 дня в дозе 20 мкг/кг. На 28-й день животных эвтаназировали и оценивали площадь атеросклеротических бляшек. Также в тканях аорты определяли экспрессию генов, связанных с процессами воспаления, антиоксидантной защиты, апоптоза, ангиогенеза. Кроме того, было изучено эндотелиопротективное действие пептидов на первичных культурах эндотелиоцитов диких и трансгенных мышей *Polg-D257A*.

Результаты. Мы не обнаружили статистически значимого влияния препаратов на площадь липидной инфильтрации. Однако исследуемые пептиды значительно уменьшили экспрессию провоспалительных генов *iNos*, *Icam1*, *Vcam1*, *Sele*, *Il6*, *Tnfa*, генов, связанных с ангиогенезом *Vegfa*, *Flt-1* и *Hif1a*, экспрессию проапоптотических факторов и более чем в 1,5 раза снизили соотношение *Bax/Bcl-2*. Кроме того, пептиды дозозависимо увеличили выживаемость эндотелиоцитов при добавлении H_2O_2 in vitro.

Заключение. Используемые пептиды на основе эритропоэтина способны улучшать функциональное состояние сосудистой стенки на фоне атеросклеротического поражения и оказывают угнетающее влияние на патобиологические процессы, связанные с митохондриальной дисфункцией. Кроме того, исследуемые пептиды оказывают значимый эндотелиопротективный эффект при индукции оксидативного стресса in vitro.

Ключевые слова: атеросклероз, производные эритропоэтина, митохондриальная дисфункция, оксидативный стресс.

INTRODUCTION

Atherosclerosis is a chronic disease of the walls of vascular walls. It is characterized by aseptic inflammation, impaired perfusion of organs and tissues, a tendency to thrombosis and a progressive dysfunction of vascular walls in the process of aging. In 1912, at the meeting of the Society of Russian Physicians in St. Petersburg, a prominent Russian scientist N.N. Anitschkow together with S.S. Khalatov presented the first results of his revolutionary research concerning the identification of the relationships between alimentary factors, blood cholesterol levels and atherosclerosis. The prize for the

most outstanding research in the field of atherosclerosis is currently named after N.N. Anitschkow. Since the time of these works, atherosclerosis has been considered primarily as a disease caused by the accumulation of cholesterol in the vascular walls [1]. This concept is still the key to reducing a cardiovascular risk. However, it is now generally accepted that dyslipidemia is the only cause of atherosclerosis in familial hypercholesterolemia only. In other cases, atherosclerosis is the result of the combined effect of a number of pathogenetic factors; among them there is an endothelial dysfunction [2], hemodynamic overload [3–5], migration of smooth

muscle cells [6], chronic sterile vascular inflammation and a number of other processes.

In the pathobiology of atherosclerosis, a special place is given to the disruption of a mitochondrial function [7]. Mitochondria are the main generator of reactive oxygen species (ROS) in the cell. For example, it is known that passing along the redox gradient of the electron transport chain, 1–3% of electrons prematurely react with oxygen in complexes I and III to form superoxide and other types of ROS [8]. Under various pathological conditions, including hypoxia and inflammation, their number may increase. In addition, mitochondria play not only a metabolic role in vascular cells, but also an important regulatory and signaling one [8].

It is also important that a mitochondrial dysfunction in other cells, including neurons and cardiomyocytes, leads to a decrease in their resistance to ischemia, which is expressed in an increase in deaths against the background of cerebral strokes, coronary artery occlusions, infarctions of kidneys and other organs. In connection with the indicated information, the pathogenetic cascade combining a mitochondrial dysfunction and atherosclerosis, is becoming a relevant target for pharmacological effects.

Agonists of the heterodimeric erythropoietin receptor EPOR/CD131 can be considered as a promising therapeutic approach for influencing the mitochondrial link in case of damage to the vascular wall. The first drug from this group was the 11-amino acid peptide pHBSP (pyroglutamate helix B surface peptide), discovered by a research group guided by Michael Brines, in 2008 [9]. Previously, this peptide was demonstrated to have a pronounced endothelium protective effect in modeling an L-NAME-induced endothelial dysfunction in rats [10, 11]. However, in this study, a side effect in the form of a prothrombotic action has also been identified. In this regard, an attempt to modernize this molecule by adding tripeptide motifs with an antiplatelet effect was made. As a result, two fundamentally new compounds combining cytoprotective [12] and antiplatelet effects (the unpublished data of the authors' own), were obtained. Here, the reports on the results of studying the antiatherosclerotic activity of these compounds are presented.

THE AIM of the study was to evaluate the anti-atherosclerotic and endothelium protective properties of short peptide derivatives of erythropoietin.

MATERIALS AND METHODS

Animals and diet

C57Bl/6J mice were used as the main test system. The animals were donated to the Center for Collective Use of the Institute of Gene Biology, the Russian Academy of Sciences, and were kept in the preclinical research center of the Research Institute of Pharmacology of Living Systems. After passing a 14-day quarantine regime, the mice were stratified by weight and placed in separate conventional cages in accordance with their belonging

to the experimental group. Before and during the study, the animals were kept in the rooms with artificial lighting (12h/12h mode) at the temperature of 21–23° C, the humidity of 38–50%; they had a free access to food and water. The number of the conclusion of the independent ethical committee is 06-09/02-1 dated 12/16/2019.

The study included 16 wild-type males and 52 males (25–30 g) with the Polg-D257A/Cdh5-CRE genotype against the background of C57Bl/6J. The genotype is associated with an endothelial-specific knockdown of the Polg gene encoding the polymerase gamma enzyme. Disturbances in the work of this enzyme lead to the development of a mitochondrial dysfunction [13, 14]. This line was created at the Genome editing center of the IGB RAS to study the effects of the oxidative stress and mitochondrial dysfunction. The line is characterized by the presence of a mutant form of the Polg gene under the control of the CAG promoter and a stop cassette flanked by LoxP sites (the unpublished data); the basic structure is described in the article [15]. After cross-breeding with Cdh5-CRE mice, endothelial-specific removal of the stop cassette and overexpression of the mutant Polg form in the endothelium occur. 2 weeks before the operation, the animals were put on a Western diet with 2% cholesterol.

The carried out work met the requirements of the Law of the Russian Federation "On the Protection of Animals from Cruelty" dated June 24, 1998, the rules of laboratory practice when conducting preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), European Community directives (86/609 EU), the rules of the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental research (1997) and the Rules of laboratory practice adopted in the Russian Federation (order of the Ministry of Health of the Russian Federation No. 708 dated 29.08.2010).

Surgical procedures

The operation was performed on a heated table under a preparative microscope with anesthesia (Zolazepam (Virbac (Russia)) 2.5 mg/100 g + Xylazine (Biogel (Belarus)) 2 mg/100 g intraperitoneally); after performing a median laparotomy, the animals were isolated at the level between the bifurcation and the discharge of the renal arteries.

Two clips were placed on the exposed vessel, the arterial wall was incised between them, and the initial section of the balloon catheter was inserted (Fig. 1). Then the proximal clip was removed and the catheter was advanced cranially by 10 mm.

After that, water was pumped into the catheter balloon for 40 seconds, inflating it to a diameter of 1.5 mm at the pressure of 10 bar. Finally, the catheter was removed, three stitches were applied to the incision in the aorta, and the wound was sutured. After the operation, the animals were placed in individual cages with sterilized paper bedding and watched until their awakening.

To alleviate the postoperative pain syndrome, within 3 days from the moment of the operation, the animals received Metamizole sodium (Pharmstandard-Leksredstva (Russia)) with drinking water ad libitum at the concentration of 50 mg of the substance per 100 ml of water [16, 17].

Experiment design and drug administration

The animals with the *Polg-D257ACdh5-CRE* genotype were divided into 5 equal groups:

1) Intact – the animals without pathology modeling and without drug administration (n=12);

2) Control – the animals with pathology modeling (balloon injury + Western diet with a high cholesterol content), which, starting from the 1st day, were injected with water subcutaneously in the volume of 0.1 ml/10 g (n=12);

3) P-αB – the animals with pathology modeling, which, starting from the 1st day, were injected with the P-αB peptide (Pharmapark LLC) subcutaneously at the dose of 20 mcg/kg one time per 3 days for 27 days (total dose 180 mcg/kg) (n=12);

4) P-αB1 – the animals with pathology modeling, which, starting from the 1st day, were injected with the P-αB1 peptide (Pharmapark LLC) subcutaneously at the dose of 20 mcg/kg once every 3 days for 27 days (total dose 180 mcg/kg) (n=12);

5) P-αB3 – the animals with pathology modeling, which, starting from the 1st day, were injected with the P-αB3 peptide (Pharmapark LLC) subcutaneously at the dose of 20 mcg/kg once every 3 days for 27 days (total dose 180 mcg/kg) (n=12) (Table 1).

Measuring the atherosclerotic plaque area

Macroscopic examination of atherosclerotic aortic plaques was performed in four animals from each group. Briefly, on day 28 after the balloon trauma simulation, the animals were euthanized with an anesthesia overdose (Zolazepam (Virbac (Russia)) 10 mg/100 g intraperitoneally) and the abdominal aorta was carefully removed from the bifurcation to the diaphragm level.

Then the preparations were longitudinally dissected, spread out on a foam substrate, washed with a 50% ethanol solution and immersed in an Oil Red O solution for 15 minutes. After that, the preparations were washed with distilled water, and digital photographs were taken. The ratio of the area of the atherosclerotic plaque (colored red) to the intact tissue was calculated using the imageJ program.

Quantitative polymerase chain reaction

After euthanasia, the aortic tissue in the area of the balloon injury was sampled from the other animals, homogenized and incubated for 10 minutes at 37°C in the "Extract RNA" solution. After lysing the sample in the reagent, it was subjected to chloroform cleansing, the supernatant sample was collected and washed with isopropyl alcohol and 70% ethyl alcohol. The concentration

of the obtained RNA was measured using an IMPLEN NanoPhotometer spectrophotometer and adjusted to the concentration of 300 ng/μl.

A reverse transcription was performed using the MMLVRTSK021 set according to the manufacturer's protocol (Evrogen). The mixture was carefully mixed and heated for 2 minutes at 70°C to melt the secondary structures of RNA and then anneal the OligoDT primer. Then the samples were transferred to ice. The entire reaction mixture was incubated for 60 min at 40°C in a T100™ ThermalCycler (Bio-Rad).

To stop the reaction, the mixture was heated at 70°C for 10 minutes. The resulting DNA was diluted to the concentration of 1 ng/μl. The level of the gene expression was assessed relative to the values of the reference Gapdh gene. The calculation of the expression at the specific point was made according to the following formula: Gene expression = [(Ct (Gapdh)/Ct (Gene of interest))] (Table 2).

In vitro study of cytoprotective activity

In 8 intact mice (4 animals with the *Polg-D257A/Cdh5-CRE* genotype and 4 wild-type animals) after euthanasia under sterile conditions, the inferior vena cava was isolated and washed with a DPBS solution (Thermo FS) until the blood was completely removed. Then the vein was dissected to expose the inner surface and placed in a 0.2% collagenase solution in DPBS with the addition of 0.9 mM CaCl₂, 0.493 mM MgCl₂, 5.56 mM glucose, 0.327 mM sodium pyruvate, penicillin and streptomycin (Lonza), exposing the intima to enzymatic dissociation. To increase the efficiency of the endothelial cell separation, the inner layer was scraped off with sterile forceps. The collagenase solution containing the cells was collected in a 5 ml tube, the resulting endothelial cells were cultured in DMEM-F12 medium (Lonza) supplemented with 20 mM HEPES buffer (Lonza), 5 U/ml heparin, 200 μg/ml ECGF (Sigma-Aldrich), 10% fetal calf serum (ThermoFisher) at 37°C in a humid atmosphere containing 5% CO₂ [18].

Cell viability was measured by a quantitative colorimetric MTT analysis, which provides sensitive measurements of cellular metabolic statuses, in particular a mitochondrial status, which can reflect early redox changes. Briefly, exponentially growing cells were seeded in a 96-well plate at the density of 4×10⁴ cells per well. Then the cells were treated with the studied peptides (P-αB, P-αB1, P-αB3 (LLC "Pharmapark")) in 3 concentrations – 5, 30, 50 μg/ml for 2 hours. After pretreatment, 200 μM H₂O₂ was added for 24 hours to the culture medium. The negative control cells were treated with H₂O₂ only, and the positive control cells were not treated with anything at all. After the incubation for 24 hours, 10 μl of MTT assay set reagent was added to each well and the cells were incubated for an additional hour. The absorption of each reaction product was measured using a microplate reader at the wavelength of 450 nm. The results are expressed as a percentage of MTT uptake in control cells, which was assumed to be 100% (Fig. 2).

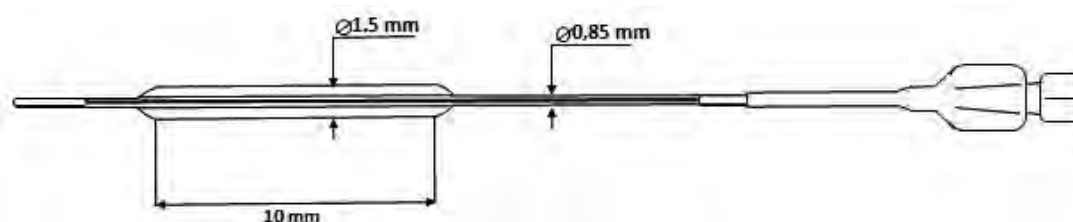


Figure 1 – Schematic image and dimensions of the balloon catheter

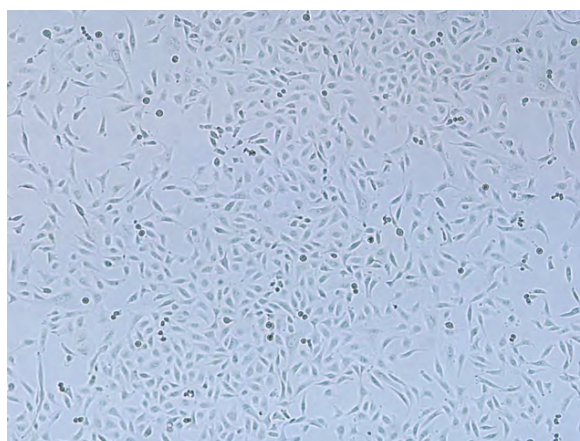


Figure 2 – Primary monolayer of mouse endothelial cells (a 40× magnification)

Table 1 – Amino acid sequence of the tested compounds

Laboratory cipher	Amino acid sequence
P-αB	QEQLERALNSS
P-αB1	RGDQEQLERALNSS
P-αB3	KGDQEQLERALNSS

Table 2 – Primers used for quantitative PCR

Gene	F-primer	R- primer	Product length (b.p.)	GenBank
Trp53 (p53)	CGACTACAGTTAGGGGGCAC	CCATGGCAGTCATCCAGTCT	95	NM_001127233.1
Bcl2	TCACCCCTGGTGGACAACAT	TTCCACAAAGGCATCCCAGC	102	NM_009741.5
Bax	CCCAGCTGATCAGAACCAT	GAGGCTTCCCAGCCAC	96	NM_007527.3
Pon2	CTTCCACACTGCCACCTGAT	TCCTGGGAATTTTAGACCCACA	105	NM_000305.3
Sod2	GGCTGGCTTGGCTTCAATAAG	AGCGGAATAAGGCTGTTGTT	95	NM_013671.3
Vegfa (VEGF-A)	GGGCCTCCGAAACCATGAA	TGCAGCCTGGGACCACTTG	95	NM_001025250.3
Kdr(VEGFR)	TGCAGGAAACTACACGGTCA	CGATCTGGGGTGGGACATTC	95	NM_001363216.1
Nos2 (iNOS)	GCTCTAGTGAAGCAAAGCCCA	GGGATTCTGGAACATTCTGTGC	103	NM_001313921.1
Icam1	CTCCGGACTTTCGATCTTCCA	CCTTCCAGGGAGCAAAACAAC	98	NM_010493.3
Vcam1	TACTGTTTGCAGTCTCTCAAGC	CGTAGTGCTGCAAGTGAGGG	101	NM_011693.3
Sele (E-selectin)	GGGAAGAAGACTGTCTAGCC	AGGGGAGCTGGCTTCTTAAG	96	XM_006496715.3
Hif1a	AGAACAACCTTGAGCTGGCGT	TGGAGGTGAACTAGGCTCTGT	103	NM_001092957.1
Casp1 (Caspase-1)	TGTATTCACGCCCTGTTGGA	CCCTCAGGATCTTGTCAGCC	100	NM_009807.2
Casp3 (Caspase-3)	GCTTGAACGGTACGCTAAG	CTTGCTCCCATGTATGGTCTT	105	NM_001284409.1
Il6	GACTGGGGATGTCTGTAGCTC	TGGATGGAAGTCTCTTGCAAG	103	NM_001314054.1
Tnfa (TNFα)	ACTGAACCTCGGGGTGATCG	ACTTGGTGGTTTGTGAGTGTG	105	NM_001278601.1
Gapdh	GGGTCCCAGCTTAGGTTTCATC	CCCAATACGGCCAAATCCGT	100	NM_001289726.1

Statistical processing

The data obtained were checked for a normal distribution using the Shapiro-Wilk test. The data with the normal distribution were compared with each other using One-way ANOVA with Tukey's HSD post hoc test. The data with the abnormal distribution were compared using the Kruskal-Wallis test and the post-hoc analysis according to Dunn's method.

RESULTS

Macroscopic assessment of plaque

Macroscopic analysis revealed that by the 28th day after the balloon injury modeling, the lipid deposits characteristic of atherosclerosis were visualized in all aortic preparations stained with Oil Red O.

At the same time, the degree of damage varied greatly within the groups, which made it difficult to interpret the results obtained. As a result, no significant differences between the control group and the groups using the tested drugs have been found, although a certain tendency to reduce the area was observed in the group using P- α B1 (Fig.3).

Quantitative PCR

Using a molecular biological analysis of plaque tissues, it was found out that the studied peptides significantly reduce the expression of pro-apoptotic factors Bax, caspase-1 and caspase-3, and also slightly increase the expression of antiapoptotic factor Bcl-2. As the heatmap in Fig. 2 shows, the greatest effect was demonstrated by preparation P- α B1 (Fig.4A).

For an integral assessment of the pro-apoptotic orientation of tissues, the ratio of Bax expression to Bcl-2 was calculated. The average calculated Bax/Bcl-2 ratio was 0.67 in the group of the intact animals, 1.81 in the control group, 1.19 in the group treated with P- α B, 0.96 in the group treated with P- α B1 and 1.09 in the group treated with P- α B3 (Fig. 4B).

Along with the anti-apoptotic effect, the studied drugs decreased the expression of the genes for inflammatory marker Nos2, and molecules of intercellular adhesion Icam1, Vcam1, and E-selectin, which had been increased against the background of trauma. The most pronounced effect was obtained in the group treated with the P- α B1 compound (Fig. 5).

In addition, the studied drugs reduced the expression of factors VEGF-A, VEGFR, and HIF-1 α associated with angiogenesis (Fig. 6).

Finally, against the background of modeling atherosclerosis in *Polg-D257A/Cdh5-CRE* mice, it was found out that an increase in the expression of the genes of the antioxidant enzymes Pon2, Sod2 occurs. At the same time, in comparison with the control, the expression of the antioxidant system genes was reduced against the background of the use of the tested peptides. As well as in assessing the effect of peptides on pro-inflammatory

and pro-angiogenic genes, the most pronounced effect was obtained in the group treated with the P- α B1 compound (Fig. 7).

In vitro study of cytoprotective activity

When carrying out the MTT test on primary cultures of endothelial cells, it was found out that even without the addition of H₂O₂, endothelial cells expressing Polg-D257A are characterized by a lower signal intensity in comparison with the wild type. During the incubation with H₂O₂, most of Polg-D257A endothelial cells lost their signal intensity almost five times from 80.60 (95% CI 77.29-84.94) to 15.79 (95% CI 11.97-25.42) (Fig. 8).

The studied drugs, dose-dependently increased the cell survival under the conditions of the oxidative stress induced by the addition of H₂O₂. Moreover, the figure shows that modified peptides (P- α B1 and P- α B3) had a more pronounced effect compared to the base compound (P- α B) when added in equivalent doses.

DISCUSSION

Peptide agonists of the EPOR/CD131 heteroreceptor are activators of erythropoietin-associated cytoprotection cascades. Compounds P- α B1 and P- α B3, along with primary anti-apoptotic properties, also have an antiplatelet activity, which is achieved through the introduction of the KGD and RGD tripeptide motifs. In this study, as an experimental model for studying the anti-atherosclerotic activity of P- α B1 and P- α B3, transgenic mice were used; they had a mitochondrial dysfunction against the background of the tissue-specific knockdown of the Polg gene encoding gamma polymerase.

Polymerase gamma is an enzyme that plays a key role in mitochondrial DNA replication. This enzyme demonstrates a high accuracy of work and, at the same time, has its own 3'->5' exonuclease activity, due to which it is possible to correct polymerization errors. The inclusion of "wrong" nucleotides without subsequent correction leads to the accumulation of mitochondrial mutations and a mitochondrial dysfunction [19]. As a result, there is an increase in the production of active radicals and damage to the cell. Homozygous animals with a systemic *Polg* knockout do not survive, therefore, in this experiment an endotheliospecific gene knockdown was used [20]. This model reflects one of the key links in the pathogenesis of atherosclerosis – an oxidative stress against the background of a mitochondrial dysfunction. Nevertheless, in any animal model of atherosclerosis, individual differences in timing and a degree of plaque formation are so great that very large groups of animals are needed to test drugs [21–23]. In this regard, a decision to standardize the process of atherogenesis through the induction of atherosclerosis by a balloon trauma and a Western diet was made.

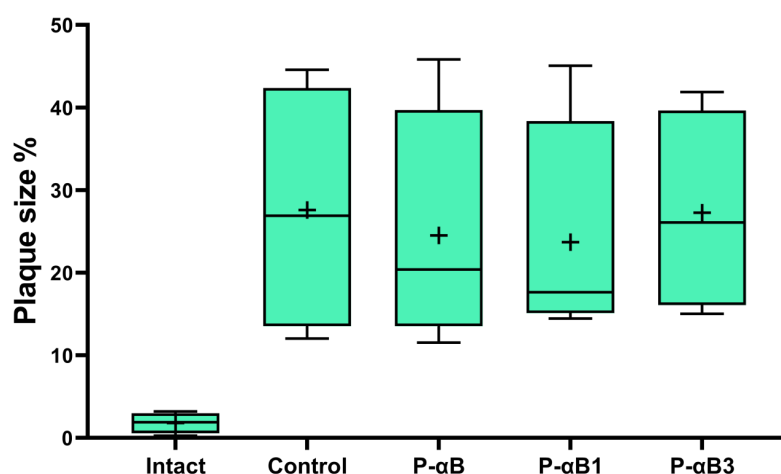


Figure 3 – Area of lipid deposits

Note: + is arithmetic mean

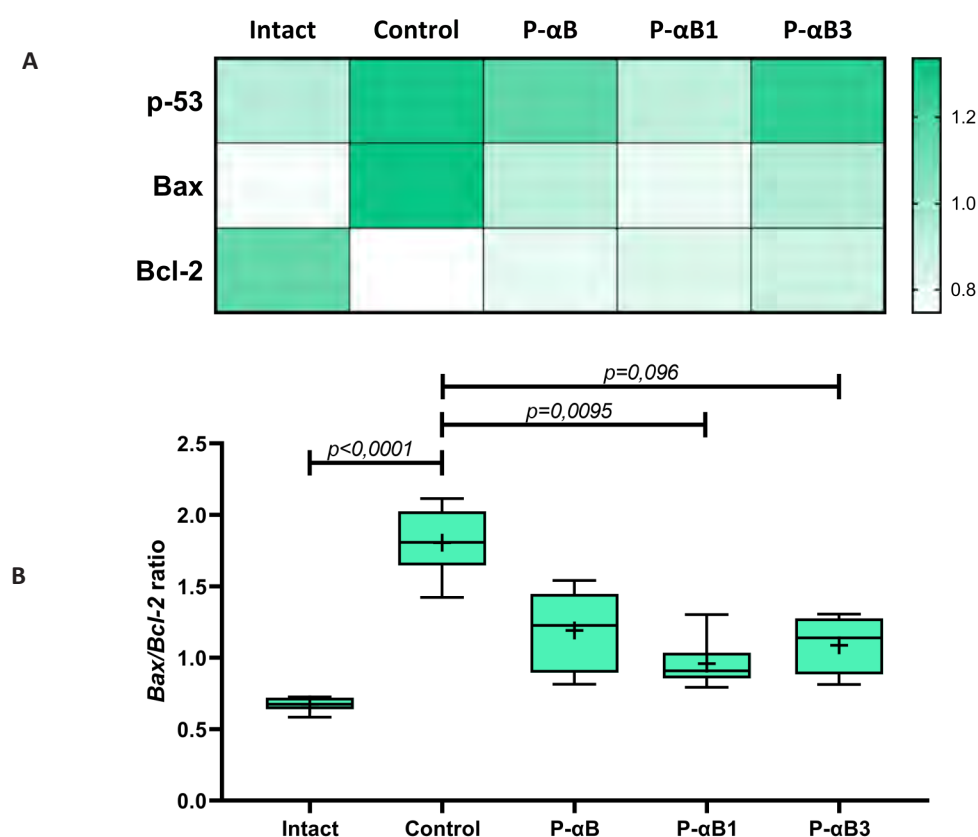


Figure 4 – Influence of the studied drugs on the relative expression of apoptosis markers

Note: A) Fig. A shows that against the background of the balloon damage modeling, the expression of the programmed cell death markers p53 and Bax increases significantly, and the expression of the anti-apoptotic marker Bcl-2 decreases. In almost all cases, the studied drugs return the expression of p53, Bax and Bcl-2 to the level of the values in the intact group; B) Fig. B reflects the Bax/Bcl-2 ratio. The ratio characterizes the Pro-apoptotic orientation of the cell: the higher it is, the more pronounced the activation of cascades of the programmed cell death. Fig. B shows that the P-αB1 peptide significantly reduces the Bax/Bcl-2 ratio.

+ is arithmetic mean; the statistical significance of the intergroup differences was detected using the Kruskal-Wallis test and the post-hoc analysis according to Dunne's method.

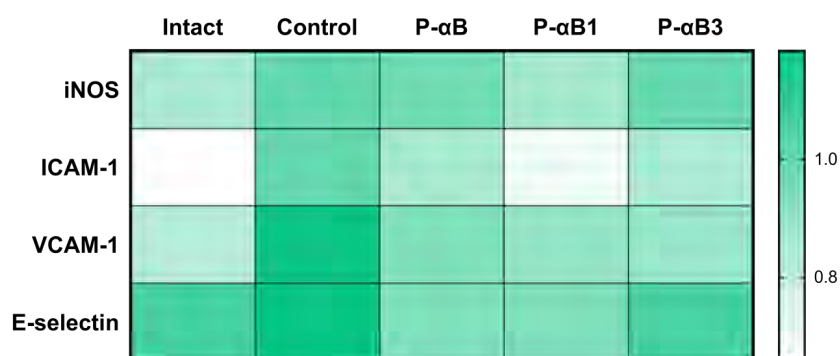


Figure 5 – The expression level of genes *Nos2* (iNOS), *Icam1*, *Vcam1* and *Sele* (E-selectin)

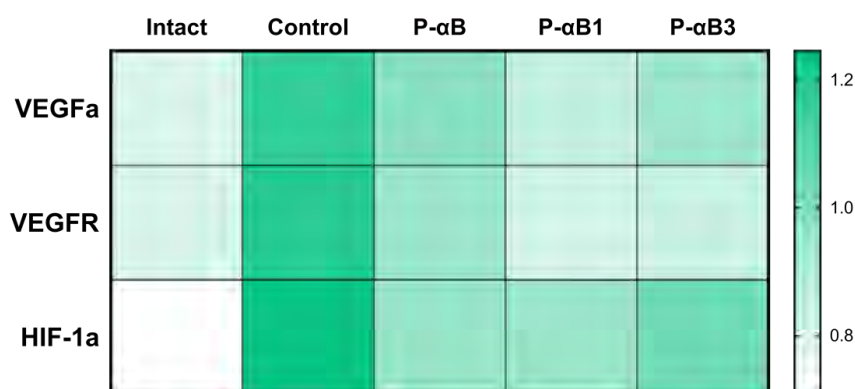


Figure 6 – Expression level of *Vegfa* (Vegf-A), *Kdr* (VEGFR-1), and *HIF1alpha* (HIF-1α)

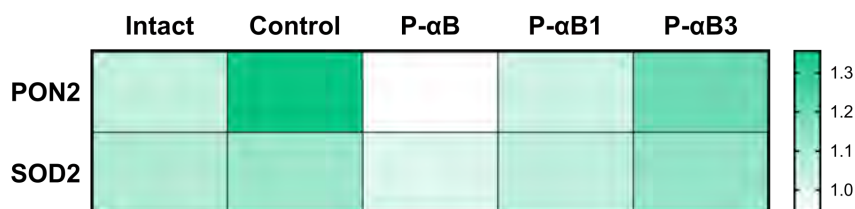


Figure 7 – Expression level of genes PON2, SOD2

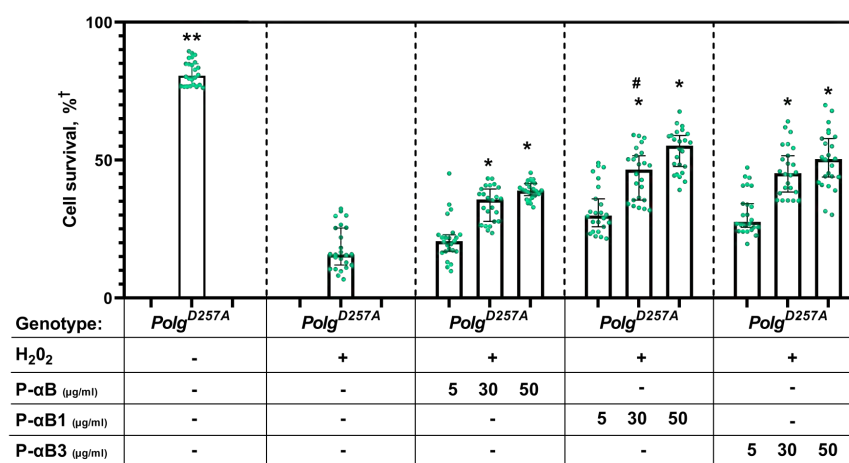


Figure 8 – Influence of H₂O₂ and tested peptides on the survival of endothelial cells with the *Polg*-D257A genotype

Note: † – relative to intact wild-type endothelial cells; * – p < 0.0001 when compared with the group treated only with H₂O₂; # – p = 0.0783 when compared with the group treated with supplemented P-αB 30 μg/ml

In this model, atherosclerosis is associated with a traumatic effect on the vessel against the background of damage to endothelial cells due to a mitochondrial dysfunction. To confirm the in vivo effects, an in vitro study of the effectiveness of the selected peptides on a primary culture of endothelial cells *Polg-D257A* was also carried out. To enhance the oxidative stress, the cells in the presence of 200 μM H_2O_2 were incubated.

The studied peptides have demonstrated a pronounced endothelioprotective effect in the oxidative stimulus the in vitro model. It has also been found out that the drugs have a pronounced reducing effect on the expression of pro-apoptotic markers. These results are consistent with the concept of the basic mechanism of the action of the erythropoietin derivatives. When the cytoprotective heteroreceptor EPOR/CD131 is activated, Jak/STAT-mediated signal transmission to the nucleus occurs, leading to "survival" signaling by reducing the expression of Pro-apoptotic genes [24, 25]. Similar effects of erythropoietin receptors stimulation have previously been shown in modeling of atherosclerosis [26].

In addition, it has also been found out that the expression of the genes of the antioxidant system PON2 and SOD2 decreased in the treated animals compared to the control. The observed effect is associated with the fact that a strong oxidative and toxic stress develops in the vessels of the control group animals, stimulating an increase in the expression of genes of the antioxidant system. At the same time, against the background of the treatment, pathological phenomena in the cells were reduced, and the stimulating activity against the PON2 and SOD2 genes also decreased.

It has also been found out the peptide agonists EPOR/CD131 have a pronounced anti-inflammatory activity, reducing the expression of pro-inflammatory cytokines and intercellular adhesion molecules. Inflammation is an active factor in the development of atherosclerosis and it contributes to the destabilization of atherosclerotic plaques [27]. VCAM-1, ICAM-1, IL-6, TNF- α molecules play a special role in regulating inflammatory cascades and vascular infiltration by immune cells [28-

30]. In general, the anti-inflammatory effect of erythropoietin and its derivatives is a widely studied phenomenon [31, 32]. Therefore, our data fit into the general idea of the pharmacodynamics of EPOR/CD131 agonists.

Further on, a decision to evaluate the effect of the studied peptides on the expression of genes encoding angiogenic factors was made. Angiogenic factors play an important role in the atherosclerosis progression, and erythropoietin is known to be able to stimulate angiogenesis [33]. In atherosclerosis areas, specific local conditions (relative anoxia, inflammation, oxidative stresses) increase the expression of classical and nonclassical angiogenic factors that promote the proliferation of pre-existing vasa vasorum [34]. Neovascularization increases the local flow of nutrients and O_2 and thus may contribute to the progression and remodeling of plaques [35]. The obtained results demonstrated that, in contrast to erythropoietin, peptide agonists EPOR/CD131 exhibit antiangiogenic effects, at least against atherosclerotic plaques.

CONCLUSION

Previously, the following hypothesis had been formulated and confirmed: by adding the tripeptide motifs KGD and RGD, cytoprotective peptide derivatives of erythropoietin can acquire antiplatelet properties. In the course of this study two innovative peptides and a base compound were demonstrated. In this study, it has been demonstrated that two innovative peptides and a basic compound P- α B (pHBSP) protect endothelial cells in vitro; they also reduce pro-apoptotic, pro-inflammatory, and angiogenic activation of vascular wall cells in the model of atherosclerosis combined with a mitochondrial dysfunction. Such a pharmacological activity of the studied drugs seems to be very promising in combination with the information on the presence of an antiplatelet activity in them. Thus, the observed effects complement the information on the cardiovascular activity of innovative peptides P- α B1 and P- α B3, as well as new prospects in the development of peptides combining atheroprotective and antiplatelet properties.

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AUTHORS' CONTRIBUTION

O.A. Puchenkova – administration of drugs to animals, collection of organs for molecular biological and macroscopic studies, article writing; **S.V. Nadezhdin** – isolation of the primary culture of endothelial cells, in vitro study of the cytoprotective activity of erythropoietin derivatives, article writing; **V.O. Soldatov** – article writing, development of the research design; **M.A. Zhuchenko** – peptide synthesis and literature analysis, article writing; **D.S. Korshunova** – isolation of RNA, conversion of RNA to cDNA, analysis of the expression of targeted genes; **M.V. Kubekina** – isolation of RNA, conversion of RNA into DNA, the analysis of the target genes; **E.N. Korshunov** – handling and caring for animals, preparation of the experimental group of animals; **L.V. Korokina** – article writing, developing a research design; **A.L. Kulikov** – pharmaceutical service, statistical processing and work with graphic materials; **P.A. Golubinskaya** – article writing, formalizing the bibliography; **V.M. Pokrovsky** – observation and care of animals, animal handling, administration of drugs; **E.A. Patrakhanov** – observation and care of animals, animal handling, administration of

drugs; **P.R. Lebedev** – observation and care of animals, administration of drugs. Animal necropsy; **V.V. Gureev** – article writing, consulting on planning, methodology and implementation of the experiment, modeling of balloon injury; **T.A. Denisyuk** – statistical processing, article writing; **V.S. Belyaeva** – analysis of the graphic images and measurement of the area of atherosclerotic plaque, isolation of the primary culture of endothelial cells, study of the cytoprotective activity of erythropoietin derivatives in vitro; **E.A. Movchan** – isolation of the primary culture of endothelial cells, in vitro study of the cytoprotective activity of erythropoietin derivatives, preparation of aorta samples for graphic analysis; **E.I. Lepetyukha** – isolation of RNA, quantitative PCR; **M.V. Pokrovskiy** – creation of the idea, planning of research, consultation on the implementation of individual stages of experimental work, quality assurance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Zárate A, Manuel-Apolinar L, Basurto L, De la Chesnaye E, Saldivar I. Cholesterol and atherosclerosis. Historical considerations and treatment. Arch Cardiol Mex. 2016; 86(2):163–9. DOI: 10.1016/j.acmx.2015.12.002.
2. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation; 2004; 109(23):27–32. DOI: 10.1161/01.CIR.0000131515.03336.f8.
3. Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. Nat Clin Pract Cardiovasc Med. 2009; 6(1):16–26. DOI: 10.1038/ncpcardio1397.
4. Sorokin A, Kotani K, Bushueva O, Taniguchi N, Lazarenko V. The cardio-ankle vascular index and ankle-brachial index in young Russians. Journal of atherosclerosis and thrombosis. 2015; 22(2):211–8. DOI: 10.5551/jat.26104.
5. Polonikov A, Bykanova M, Ponomarenko I, Sirotina S, Bocharova A, Vagaytseva K, Shvetsov Y. The contribution of CYP2C gene subfamily involved in epoxygenase pathway of arachidonic acids metabolism to hypertension susceptibility in Russian population. Clinical and Experimental Hypertension. 2017; 39(4):306–311. DOI: 10.1080/10641963.2016.1246562.
6. Bennett M.R, Sinha S, Owens G.K. Vascular Smooth Muscle Cells in Atherosclerosis. Circ Res. 2016; 118(4):692–702. DOI: 10.1161/CIRCRESAHA.115.306361.
7. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative Stress in Atherosclerosis. CurrAtheroscler Rep. 2017; 19(11):42s DOI: 10.1007/s11883-017-0678-6.
8. Quintero M, Colombo SL, Godfrey A, Moncada S. Mitochondria as signaling organelles in the vascular endothelium. Proc. Natl. Acad. Sci. U.S.A. 2006; 103:5379–5384. DOI: 10.1073/pnas.0601026103.
9. Brines M, Patel NS, Villa P, et al. Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. Proc Natl AcadSci U S A. 2008; 105(31):10925–10930. DOI: 10.1073/pnas.0805594105.
10. Korokin MV, Soldatov VO, Tietze AA, Golubev MV, Belykh AE, Kubekina MV, Puchenkova OA, Denisyuk TA, Gureyev VV, Pokrovskaya TG, Gudyrev OS, Zhuchenko MA, Zatolokina MA, Pokrovskiy MV. 11-amino acid peptide imitating the structure of erythropoietin α -helix b improves endothelial function, but stimulates thrombosis in rats. Pharmacy & Pharmacology. 2019; 7(6):312–320. Russian. DOI: 10.19163/2307-9266-2019-7-6-312-320.
11. Korokin M, Gureev V, Gudyrev O, Golubev I, Korokina L, Peresypkina A, Pokrovskaya T, Lazareva G, Soldatov V, Zatolokina M, Pobeda A, Avdeeva E, Beskhmel'nitsyna E, Denisyuk T, Avdeeva N, Bushueva O, Pokrovskii M. Erythropoietin Mimetic Peptide (pHBSP) Corrects Endothelial Dysfunction in a Rat Model of Preeclampsia. Int. J. Mol. Sci. 2020; 21:6759. DOI: 10.3390/ijms21186759.
12. Golubev IV, Gureev VV, Korokin MV, Zatolokina MA, Avdeeva EV, Gureeva AV, Rozhkov IS, Serdyuk EA, Soldatova VA. Preclinical study of innovative peptides mimicking the tertiary structure of the α -helix B of erythropoietin. Research Results in Pharmacology. 2020; 6(2):85–96. DOI: 10.3897/rrpharmacology.6.55385.
13. Trifunovic A, Wredenberg A, Falkenberg M. et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature. 2004; 429:417–423. DOI: 10.1038/nature02517.
14. Kujoth GC, Hiona A, Pugh TD, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science. 2005. 309(5733):481–484. DOI: 10.1126/science.1112125.
15. Zvartsev RV, Korshunova DS, Gorshkova EA., et al. Neonatal Lethality and Inflammatory Phenotype of the New Transgenic Mice with Overexpression of Human Interleukin-6 in Myeloid Cells. DoklBiochemBiophys. 2018; 483(1):344–347. DOI: 10.1134/S1607672918060157.
16. Stubbendorff M, Hua X, Deuse T, et al. Inducing myointimal hyperplasia versus atherosclerosis in mice: an introduction of two valid models. J Vis Exp. 2014; 87:51459. DOI: 10.3791/51459.
17. Tediashvili G, Wang D, Reichenspurner H, Deuse T, Schrepfer S. Balloon-based Injury to Induce Myointimal Hyperplasia in the Mouse Abdominal Aorta. J Vis Exp. 2018; 132:56477. DOI: 10.3791/56477.
18. Molina-Sánchez P, Andrés V. Isolation of Mouse Primary Aortic Endothelial Cells by Selection with Specific Antibodies. Methods in Mouse Atherosclerosis. Methods in Molecular Biology. Humana Press, New York, NY. 2015; 1339. DOI: 10.1007/978-1-4939-2929-0_7.
19. Stumpf JD, Saneto RP, Copeland WC. Clinical and molecular features of POLG-related mitochondrial
20. Kusov P, Deikin A. Developing Novel Transgenic Mice Model Of Atherogenesis With Conditional Oxidative Stress By Introduction Of Epithelium-Specific Inducible Mitochondrial Polg With Mutagenic Activity. Atherosclerosis. 2019; 287:99 s. DOI: 10.1016/j.atherosclerosis.2019.06.287.
21. Poznyak AV, Silaeva YY, Orekhov AN, Deykin AV. Animal models of human atherosclerosis: current progress. Braz J Med Biol Res. 2020. 53(6):9557 s. DOI: 10.1590/1414-431x20209557.
22. Mushenkova NV, Summerhill VI, Silaeva YY, Deykin AV, Orekhov AN. Modelling of atherosclerosis in genetically modified animals. Am J Transl Res. 2019.11(8):4614–4633.
23. Volobueva AS, Orekhov AN, Deykin AV. An update on the tools for creating transgenic animal models

- of human diseases – focus on atherosclerosis. *Braz J Med Biol Res.* 2019; 52(5):8108. DOI: 10.1590/1414-431X20198108.
24. Bittorf T, Jaster R, Lüdtkke B, Kamper B, Brock J. Requirement for JAK2 in erythropoietin-induced signalling pathways. *Cell Signal.* 1997; 9(1):85–89. DOI: 10.1016/s0898-6568(96)00121-0.
 25. Peng B, Kong G, Yang C. et al. Erythropoietin and its derivatives: from tissue protection to immune regulation. *Cell Death Dis.* 2020; 11(79). DOI: 10.1038/s41419-020-2276-8.
 26. Warren JS, Zhao Y, Yung R, Desai A. Recombinant human erythropoietin suppresses endothelial cell apoptosis and reduces the ratio of Bax to Bcl-2 proteins in the aortas of apolipoprotein E-deficient mice. *Journal of Cardiovascular Pharmacology.* 2011; 57(4):424–433. DOI: 10.1097/fjc.0b013e31820d92fd.
 27. Bäck M, Yurdagul A, Tabas I. et al. Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. *Nat Rev Cardiol.* 2019; 16:389–406. DOI: 10.1038/s41569-019-0169-2.
 28. Ley K, Huo Y. VCAM-1 is critical in atherosclerosis. *J Clin Invest.* 2001; 107(10):1209–1210. DOI: 10.1172/JCI13005.
 29. Fatkhullina AR, Peshkova IO, Koltsova EK. The Role of Cytokines in the Development of Atherosclerosis. *Biochemistry (Mosc).* 2016; 81(11):1358–1370. DOI: 10.1134/S0006297916110134.
 30. Fotis L, Agogiannis G, Vlachos IS, Pantopoulou A, Margoni A, Kostaki M, Verikokos C, Tzivras D, Mikhailidis DP, Perrea D. Intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 at the early stages of atherosclerosis in a rat model. *In Vivo.* 2012; 26:243–250.
 31. Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G. The pleiotropic effects of erythropoietin in infection and inflammation. *Microbes Infect.* 2012; 14(3):238–246. DOI: 10.1016/j.micinf.2011.10.005.
 32. Liu Y, Luo B, Shi R, et al. Nonerythropoietic Erythropoietin-Derived Peptide Suppresses Adipogenesis, Inflammation, Obesity and Insulin Resistance. *Sci Rep.* 2015:515134. DOI: 10.1038/srep15134.
 33. Kimáková P, Solár P, Solárová Z, Komel R, Debeljak N. Erythropoietin and Its Angiogenic Activity *Int J Mol Sci.* 2017; 18(7):1519. DOI: 10.3390/ijms18071519.
 34. Michel JB, Martin-Ventura JL, Nicoletti A, Ho-Tin-Noe B. Pathology of human plaque vulnerability: mechanisms and consequences of intraplaque haemorrhages. *Atherosclerosis.* 2014; 234(2) 311–319.
 35. Camaré C, Pucelle M, Nègre-Salvayre A, Salvayre R. Angiogenesis in the atherosclerotic plaque. *Redox Biol.* 2017; 12:18–34. DOI: 10.1016/j.redox.2017.01.007.

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