OXIDANT STATUS AND ENDOTHELIAL DYSFUNCTION (EXPERIMENTAL RESEARCH)

Research article

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Abstract

The research assessed the oxidant status and NADPH-diaphorase content in blood serum and in arteries in 30 rats during 6 months of experimental hyper-lipoidemia. The oxidative coefficient in blood serum was increased by rats’ 6 month age. The low level of AOA and AOA by 6 months of hyperlipidemia in the aorta of experimental rats, the presence of a direct correlation between the AOA indices and the level of aortic NADPH-diaphorase confirms that prolonged hyperlipidemia causes inhibition of endothelial function, possibly apoptotic endotheliocyte death, contributes to a decrease in the activity of their antioxidant ferments and NO-synthase family ferments.

The prolonged hyperlipoidemia causes suppression of endothelial NADPH.

Keywords:
hyper-lipoidemia, oxidant status, NADPH-diaphorase.

Introduction

From modern positions the key link in the pathogenesis of atherosclerosis is considered to be endothelial dysfunction (ED), which is an imbalance between the main functions of endothelium: vasodilatation and vasoconstriction, inhibition and promotion of proliferation, antithrombotic and prothrombotic, antioxidant and prooxidant [1], [2], [4], [7]. Recent studies have shown that in atherosclerosis there is both an intensification of lipid peroxidation processes and a decrease in the level of antioxidant protection [3], [4], [6], [8]. Endothelial damage is accompanied by the activation (priming) of polymorphonuclear leukocytes (neutrophils), increased production and secretion of reactive oxygen species (singlet oxygen) O and hydrogen peroxide (H2O2), and intensification of protein and fatty acid peroxidation [12].

Nitric oxide (NO) is a signaling molecule that carries out intercellular interactions, and regulates lipid peroxidation. Under physiological conditions, NO acts as an antioxidant, inhibits radical oxidative reactions by binding with free Fe2+ ions that are part of the heme. Under hyperproduction of reactive oxygen species, lipoprotein oxidation occurs, which contributes to the increase of cavelin-1 synthesis and leads to a decrease in NO production by endothelium [1], [11], [13].

When free radical activity is activated, superoxide oxygen anion O2- reacts with NO and peroxynitrite (ONOO-) is formed, which is a trigger factor in the development of inflammation and tissue damage, is significantly superior to NO in toxicity and deprives it of its biological action as a relaxation factor [3], [6]. Peroxynitrite stimulates apoptosis and necrosis of endothelial cells and increases their sensitivity to other damaging factors. Peroxynitrite exerts its effect by initiating lipid peroxidation (LPO) in membranes and lipoproteins [3]. NO synthesis in the body is catalyzed by the NO synthase (NOS) family. NOS, use L-arginine as a substrate and NADPH-diaphorase as a cofactor. NADPH-diaphorase is involved in the transport of electrons to the prosthetic group of the enzyme. Of the family of NADPH-dependent dehydrogenases, only NO synthase is a membrane-bound enzyme, which accounts for its greater stability during aldehyde fixation. The determination of...
NADPH-diaphorase is based on the formation of diformasan in the presence of endogenous β-NADPH and tetrazolium salts. The density of the diformasan precipitate precipitated in the cytoplasm after histochemical staining for NADPH-diaphorase is adequate to the content and activity of NO synthase in the cell [9], [13].

The aim of the study was to evaluate the oxidative status and arterial NADPH-diaphorase content during long-term experimental hyperlipidemia in rats.

**Research methods and principles**

To develop hypercholesterolemia in 15 Wistar rats weighing 200-250 g, we used the method of K.A. Meshcherskaya and N.P. Koroleva in modification, with cholesterol, methylthiouracil and vitamin D added to animal food for 6 months (180 days) [5]. The experiment was carried out in strict compliance with the requirements of the European Convention on the maintenance, feeding and care of experimental animals, as well as their withdrawal from the experiment and subsequent disposal. Experimental animals were guided by the "Guidelines for work with experimental animals, approved at the meeting of the Ethical Commission of P.K. Anokhin Research Institute of Normal Physiology of RAMS (Minutes № 1, September 3, 2005), requirements of the World Society for the Protection of Animals (WSPA) and the European Convention for the Protection of Experimental Animals. Fifteen healthy rats (eating the usual diet) served as the control during the experiment (Group II). In the dynamics of the study (after 2, 4, and 6 months), 5 animals from each group were removed from the experiment under ether anesthesia by decapitation. Blood and vessels were sampled: aorta and femoral artery.

The study was approved by the interdisciplinary ethical committee (protocol № 4, case № 21, 24.01.11). Total oxidative (TOA) and total antioxidant (TAA) activities of blood plasma and vascular biopsy specimens (aorta and femoral artery) of rats were determined by spectrophotometric method developed in the laboratory of noninfectious immunity chemistry TIBOKh FEB RAS using indicator – sea urchin Scaphechinus mirabilis pigment – histochrome [4]. To judge the imbalance of antioxidant and oxidant systems, the oxidative index (OI) was determined. Calculation formula: OI=TOA/TAA.

NADPH-diaphorase was examined on a Vickers-M85 microdensitometer (mask size -2, wavelength 550 nm, magnification 400) using the program "ImageJ1.37 v", the results were expressed in units of optical density.

SPSS v. 16 was used for mathematical processing of the obtained data. 16. Comparison of mean values in the samples was performed using nonparametric Wilcoxon-Mann-Whitney U-criterion. Correlation analysis was performed by the Spearman rank correlation method.

**Results and discussion**

It was found that the values of the TAA in the rats with hypercholesterolemia in the 2nd and 4th months of the study were lower than in the control rats (pts<0.05) (Table 1). By the 6th month, the level of TAA in the blood increased and insignificantly exceeded the control values. In the experimental rats, a dynamic change in antioxidant activity was determined; at month 2 it was significantly higher than in the control rats, by month 4 it decreased and remained at this level by month 6 of the experiment (Table 1).

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Dynamics of the experiment</th>
<th>I group (diet)</th>
<th>II group (healthy rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOA</td>
<td>2 mo. 0.27 (0.18-0.32) •</td>
<td>0.55 (0.51-0.59)</td>
<td></td>
</tr>
<tr>
<td>AOA</td>
<td>4 mo. 0.32 (0.24-0.36) •</td>
<td>0.52 (0.47-0.53)</td>
<td></td>
</tr>
<tr>
<td>OI</td>
<td>6 mo. 0.59 (0.52-.63) ××</td>
<td>0.48 (0.32-0.50)</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** data presented as Median (LQ-UQ).

• - reliability between the experimental groups and the control group (pts<0.05);
× - reliability of differences in indicators between 2 and 4 months of the experiment (pts<0.05);
×× - reliability of differences in the indices between 4 and 6 months of the experiment (pts<0.05);
×××× - reliability of differences in the indices between 2 and 6 months of the experiment (pts<0.05)

The dynamics of the oxidative index (OI) of rat serum also revealed its decrease in the experimental group in the 2nd and 4th months of the experiment compared to the control animals, whereas by the 6th month of the study, its values, on the contrary, began to exceed those of healthy rats (pts<0.05) (Table 1).

The study of TAO and AOA in arterial biopsy specimens recorded that in the aorta of the experimental group of rats the values of TAO and AOA were lower than in the control group of animals (Table 2). In femoral artery biopsy specimens the AOA values had no significant differences with the control group. The determination of AOA showed an equal picture: in the
arteries of the experimental group it was lower than in the control group of animals, while in the aorta of hyperlipidemic rats it was also lower than in the femoral arteries (pts<0.05) (Table 2).

Table 2 - Indexes of total oxidative and antioxidant activity in the wall of the aorta and femoral arteries of rats (experiment 6 months)

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>TAO</th>
<th>AOA</th>
<th>OI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The aorta</td>
<td>Femoral arteries</td>
<td>The aorta</td>
</tr>
<tr>
<td>Group I (diet)</td>
<td>0.28 (0.21-0.32) **</td>
<td>0.53 (0.48-0.55)</td>
<td>0.33 (0.31-0.35) **</td>
</tr>
<tr>
<td>Group II (healthy)</td>
<td>0.69 (0.62-0.73)</td>
<td>0.61 (0.58-0.64)</td>
<td>0.51 (0.46-0.54)</td>
</tr>
</tbody>
</table>

Note: data presented as Median (LQ-UQ).
** - reliability between experimental groups and control group (pts<0.05).
* - reliability of differences between indices in aortic and femoral artery ioptates (pts<0.05)

NADPH-diaphorase was decreased in aortic and femoral artery biopsy specimens in hyperlipidemic rats (pts<0.05) (Table 3). At the same time, the content of NADPH-diaphorase in the femoral arteries was lower than in the aorta in both experimental and control animals, which can be explained by the anatomical features of the walls of these vessels (the muscular component is more pronounced in the femoral arteries) (pts<0.05).

Table 3 - NADPH-diaphorase content in rat arteries

<table>
<thead>
<tr>
<th>Dynamics of the experiment</th>
<th>Group I (diet)</th>
<th>Group II (healthy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NADPHdiaphorase of rat aorta</td>
<td>NADPH-diaphorase rat femoral arteries</td>
</tr>
<tr>
<td></td>
<td>(units of optical density)</td>
<td>(units of optical density)</td>
</tr>
<tr>
<td>2 mo.</td>
<td>38 (31-44) •</td>
<td>16 (14-18)</td>
</tr>
<tr>
<td>4 mo.</td>
<td>33 (30-41) •</td>
<td>15 (13-16)</td>
</tr>
<tr>
<td>6 mo.</td>
<td>27 (24-31) •</td>
<td>11 (11-13) *</td>
</tr>
</tbody>
</table>

Note: data presented as Median (LQ-UQ).
• - reliability between experimental groups and control group (pts<0.05).
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A direct correlation between AOA indices and NADPH-diaphorase level in the aorta of experimental rats was established r=0.74 p<0.05.

Conclusion
Thus, in the dynamics of long-term experimental hyperlipidemia, there is a change in oxidative activity both in the blood serum and in the vascular wall. The decrease of antioxidant activity in the 2nd month of the study is probably caused by the inclusion of compensatory metabolic processes, the increase of antioxidant enzymatic activity. The increase in the oxidative index in the serum of experimental rats, by the 6th month of the experiment, indicates depletion, or a decrease in the function of the enzymes of the antioxidant system. The low level of AOA and AOA by 6 months of hyperlipidemia in the aorta of experimental rats, the presence of a direct correlation between the AOA indices and the level of aortic NADPH-diaphorase confirms that prolonged hyperlipidemia causes inhibition of endothelial function, possibly apoptotic endotheliocyte death, contributes to a decrease in the activity of their antioxidant ferments and NO-synthase family ferments.


