INTRODUCTION

According to current scientific data, endothelial cells can be attributed to the endocrine organ, which provides vascular homeostasis, vascular tone and anatomical structure of the vessel wall [1, 2, 3]. Hypercholesterolemia (HC), in particular excessive concentrations of low density oxidative lipoproteins in the blood, as well as local non-specific systemic inflammation, are the main risk factors for endothelial cell damage in cardiovascular disease [4]. Hypercholesterolemia enhances the arterial wall reaction to vasoconstrictors and reduces endothelium dependent vasodilation [5] as a result of inhibition of synthesis and reduction of nitric oxide activity. Endothelial dysfunction is considered one of the important prerequisites for the development of cardiovascular pathology, in particular atherosclerosis, coronary heart disease, arterial hypertension, thrombosis, microcirculatory disorders in various organs and systems [6]. This is confirmed by manifestations of endothelial dysfunction, which are described in case of diabetes mellitus [7], hepatitis [8], chronic obstructive pulmonary disease [9], and in conditions of experimental hyperuricemia [10, 11]. The morphological marker of endothelial dysfunction is an increase in the number of circulating desquamated endothelial cells (DEC), but this situation needs to be clarified in the development of this pathology in the age aspect.

THE AIM

To find out the degree of morphofunctional remodeling of endothelial cells and arteries of hind limbs in rats of pre-reproductive and reproductive age with experimental hypercholesterolemia.

MATERIALS AND METHODS

The studies were conducted on 32 white rats. The experimental group consisted of 16 animals with biochemically confirmed HC which were divided into 2 groups: Group 1 – 8 animals, aged 2–3 months, weighing 150–170 grams; and Group 2 – 8 rats aged from 11 to 11 months weighing 230–250 gram. The control group consisted of rats of the same age of 8 animals in each.
230 to 250 grams. The control group consisted of rats of the same age of 8 animals in each.

Determination of the number of desquamated endothelial cells circulating in blood was carried out according to the method of J. Hladovec (1978) in the modification of V.V. Sivak [12].

Hypercholesterolemia was modeled by feeding cholesterol in a dose of 0.5 g/kg with warmed vegetable oil. In order to inhibit the function of the thyroid gland, Mercazolilum was used in a dose of 10 mg/kg. The mixture was administered with a help of a catheter intravenously [13, 14, 15].

Rats’ holding and all experiments were performed in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Animal Experiments adopted by the First National Congress on Bioethics (Kyiv, 2001), the Helsinki Declaration of the World Medical Association (2000).

The removal of laboratory rats from the experiment was carried out by intra-abdominal injection of large doses of thiopental sodium in 15, 30 and 45 days of the study.

For histological examination, soft tissues were excised with vascular-nerve beams up to 0.5 cm in thickness from the femoral, knee, and tibia of the hind limbs of the rats. The obtained tissue, after removing the bone fragments, was fixed in 10% neutral formalin solution and sealed with paraffin according to the standard method. Depart-

**Figure 1.** The level of cholesterol in the blood of experimental animals under experimental hypercholesterolemia.

**Figure 2.** Thickening of the artery wall (1) with pronounced narrowing of the lumen (2). Histologic cut of the popliteal artery of the rat of pre-productive age. 30th day of experimental hypercholesterolemia. Coloring with hematoxylin and eosin. Amplification: × 200.
Figure 3. Positive reaction to glycosaminoglycans. Histologic cut of the popliteal artery of the rat of pre-reproductive age. 30th day of experimental hypercholesterolemia. Alcian blue coloring. Amplification: × 200.

Figure 4. A histological cut of the vein of the femur segment of the posterior limb of the rat of pre-reproductive age. 30th day of experimental hypercholesterolemia. Reaction with Alcian blue. Alcian blue coloring. Amplification: × 200.

Figure 5. PAS-positive reaction of membranes of smooth myocytes. Histological cut of the artery of the lower leg segment of the reproductive age rat. 30th day of experimental hypercholesterolemia. PAS-reaction. Amplification: × 200.
RESULTS AND DISCUSSION

The level of cholesterol in rats of both age groups in terms of experimental hypercholesterolemia increased significantly and in 15 days it was \((2.15 \pm 0.03) \times 10^4/l\) in animals of pre-productive age (PRA), and in rats of reproductive age (RA) – \((2.20 \pm 0.03) \times 10^4/l\), after 30 days – respectively \((2.68 \pm 0.04) \times 10^4/l\) and \((2.82 \pm 0.03) \times 10^4/l\), and after 45 days – \((2.83 \pm 0.05) \times 10^4/l\) and \((3.03 \pm 0.03) \times 10^4/l\).

In the group of intact rats, the level of cholesterolemia was in animals of PRA \((1.30 \pm 0.04) \times 10^4/l\) against \((1.37 \pm 0.04) \times 10^4/l\) in animals of RA, as shown in Fig. 1.

The number of desquamated endothelial cells (DEC) in intact PRA rats was \((3.13 \pm 0.35) \times 10^4/l\), and in RA animals \((4.38 \pm 0.26) \times 10^4/l\).

On the background of the growth of cholesterol indexes, the number of desquamated endothelial cells in 15 days of the experiment in the blood of PRA rats increased to \((4.00 \pm 0.33) \times 10^4/l\), or 27.80 %, which was not authentic, and in RA animals – \((5.63 \pm 0.38) \times 10^4/l\), which was 28.53 % (p <0.05).

After 30 days of the study, the number of DEC increased in 2.11 times and amounted to \((9.25 \pm 0.56) \times 10^4/l\) in animals of reproductive age (p <0.001) and in 1.95 times in animals of pre-productive age, reaching \((6.13 \pm 0.44) \times 10^4/l\) (p <0.01). After 45 days of experimental hypercholesterolemia, the level of DEC in rats of RA increased in 2.56 times and amounted to \((11.25 \pm 0.70) \times 10^4/l\) (p <0.001), and in PRA animals – in 2.35 times and it was \((7.38 \pm 0.42) \times 10^4/l\) (p <0.01).

After 15 days of the experiment, thickening of the vessel wall was observed, primarily due to the subendothelial layer. In this case, the endothelial cells became heterogeneous – in the unchanged cells there appeared the rounded, swollen with a light cytoplasm and predominantly pyknotic nuclei. A part of the cells was mutilated. The subendothelial layer was unevenly thickened and sealed, especially in the areas of denudation, due to plasma irradiation and proliferation of collagen fibers. Sometimes the xanthoma cells were detected and small PAS-positive deposits were visualized. The reaction with Alcian blue was poorly positive. Occasionally, powdered inclusions of neutral fat were detected. The inner elastic membrane retains its usual appearance. Smooth myocytes of the tunica media are unevenly hypertrophied. Extracellular matrix was and swollen. The coloration with Alcian blue in some cases gave a weakly positive reaction. The outer envelope looked loose, rash edema.

The mentioned set of morphological changes revealed by us was more pronounced in the popliteal artery. The phenomena of disrupting the extracellular matrix grew and there were weak signs of lipidation of intima.

In rats of reproductive age, at the same time of the experiment, structural reorganization affected, first of all, the arteries of the shin. The most significant changes occurred in intima and tunica media. The number of damaged and desquamated endothelial cells increased and the areas of denudation of intima expanded, which contributed to a more profound infiltration of blood plasma proteins. The cells of plasma coagulation appeared. The sub endothelial layer sometimes lost any structuring. More often, “loaded” macrophages with lipids and isolated lymphocytes were found out. Artery lumen, as a rule, without the contents of formed elements of blood however, in the places of desquamation of endothelial cells, aggregation of erythrocytes was revealed. Elastic membranes usually retain their integrity, but the corrugation was uneven, often a multiplication of elastic fibers was observed, and their fragmentation was rarely visualized.

Like previous observations, smooth myocytes in the tunica media of the arteries were hypertrophied, but in this study group, these changes became uniform. The number of collagen fibers in the extracellular matrix increased. Unlike vessels of animals of PRA, lymphocytes appeared in insignificant quantities. The coloration with Alcian blue gave an uneven, slightly positive perception of the dye. In some cases, PAS-positive regions were also detected. The histoarchitecture of the adventitia was similar to the described changes, but isolated lymphocytes were found among the thin collagen fibers.

After 30 days of the experiment, the manifestations of morphological changes significantly increased. Microscopic examination in these conditions in rats of pre-reproductive age also showed the predominance of these changes in the arteries of the femoral and knee areas. At this time the narrowing of the lumen of the vessels became systemic. Centripetal contraction was conditioned by further thickening of the intima, which acquired sufficiently clear outlines of a thick homogeneous or fibrous sub endothelial ring. Here also appeared focal clusters of xanthoma cells and free lipids. Among the stored endothelial cells proliferating cells appeared. Endothelial conglomerates and sludge erythrocytes were found in the lumen of the vessels (Fig. 2). Correspondingly, the elastic frame of the vessels changed. The most characteristic manifestation was hyperplasia of elastic fibers. The fibrils themselves acquired an uneven thickness and sinuation, fragmentated, straightened, and fluttered, which led to a change in the configuration of the intima, giving it a clear corrugation. Smooth myocytes...
the synthesis of biologically active substances involved leads to their damage by neurohumoral stimulation of the barrier between blood and vascular wall and hyperuricemia. It should be noted that the peculiarity was the accumulation of lipids, xanthoma cells and acid mucopolysaccharides in the intima of the arteries. The reaction with Alcian blue and PAS-reaction were positive. (Fig. 3).

After 45 days of experimental hypercholesterolemia in rats of pre-reproductive age, we revealed further, but significantly delayed thickening of the intima of the arteries of the femur, knee, and tibia caused by the swelling of the endothelial cells, their desquamation and proliferative changes in the places of the preserved vascular endothelium and the sub endothelial layer. In response to the lipid deposition and PAS-positive substrates, cellular reactions appeared as a weak lymphocytic infiltration. In addition to hyperelastosis, fragmentation of elastic fibers was revealed. Corrugation of intima contributed to a further narrowing of vascular lumen. This type of structural adjustment was most characteristic for arteries of the femoral and knee regions of these arteries The processes of concentric remodeling combined with signs of a violation of lipid and carbohydrate metabolism dominated in these arteries. The reaction with Alcian blue and PAS-reaction were positive. The adventitia was thickened, collagen fibers merged into thicker beams.

The same changes in 45 days of the experiment were detected in the arteries of rats of reproductive age, but more closely related to the ankle arteries and regions of the arterial branches. It should be noted that the peculiarity was the accumulation of lipids, xanthoma cells and acid mucopolysaccharides in the intima of the arteries. Lymphocytic infiltration, increased hyper elastosis of the arteries of the femoral and knee regions were characteristic compared to those of animals of pre-reproductive age. Elastic fibers were thickened. At the same time their fragmentation and multiplication were noted. In addition to lymphocytic infiltrates, the amount of collagen fibers in adventitia increased. Often, the manifestations described were revealed in the areas of branching, or the withdrawal of additional arterial branches.

CONCLUSIONS

1. Hypercholesterolemia causes damage to the vascular endothelium of the arteries, characterized by an increase in the number of desquamated endothelial cells in the peripheral blood, which significantly increases until the 30th day of the experiment, somewhat slows down on the 45th day of the experiment and predominates in rats of reproductive age.

2. In animals of pre-reproductive age, manifestations of hypertrophic remodeling were observed in the arteries of the femoral and knee regions, and in animals of reproductive age, along with manifestations of hypertrophic-neoplastic remodeling, there were sclerotic and inflammatory changes that were most pronounced in the tibia.

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Presented article is a fragment of the initiative research of Ivan Horbachevsky Ternopil State Medical University “Biochemical mechanisms of metabolic disorders under the influence of toxicants of various origin” (state registration number 0116U003353) 

Authors’ contributions: 
According to the order of the Authorship. 

Conflict of interest: 
The Authors declare no conflict of interest. 

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Received: 21.03.2019 
Accepted: 29.04.2019