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3 knoll drive. London. N14 5LU United Kingdom

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DETECTION OF CD34 PROTEINS IN AORTIC PREPARATIONS OF EXPERIMENTAL ANIMALS BY IMMUNE FLUORESCENT METHOD

Abdullayeva Muslima Akhatovna
Bukhara State Medical Institute

Abstract: Specific changes in the lipid profile were found in rabbits of different ages. As the age of the rabbits increased, their total cholesterol, LDL cholesterol and triglycerides in blood serum increased, while we found a trend towards a decrease in HDL cholesterol. This led to a rapid increase in the atherogenic coefficient, especially pronounced in 12- and 24-month-old rabbits, which indicates the development of changes in the cardiovascular system and the risk of developing diseases. At the same time, studies have revealed intense staining in the endothelial cells of the intima and in the wall of vas vasorum at the border of t.muscularis and t.adventitia of the aorta of intact rabbits. Accelerated expression of CD34 in all layers of the aortic wall in young animals indicates their high regenerative and proliferative activity.

Keywords: endothelium, vasculogenesis, cell, regeneration.

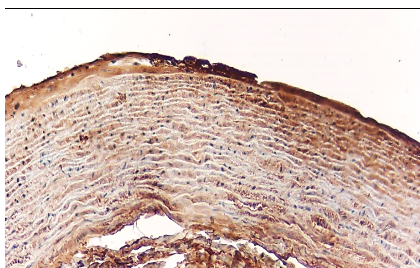
Actuality. Vascular walls are known to be rich in stem cells/progenitor cells that express the cell surface marker CD34 [1,7]. According to the authors, CD34 is a marker of endothelial and hematopoietic stem cells and is a glycoprotein in nature. Circulating CD34 cells are the most powerful factor activating vasculogenesis in the destruction of surrounding cells and are of great importance in the morphological regeneration of damaged foci. Most scientists believe that blood vessels are rich in CD34-expressing stem/progenitor cells [2,4,6], most of which are high in the endothelial layer and under certain circumstances lead to VEGF activation and promote angiogenesis. Tei et al. studies suggest that circulating CD34 cells may be used in the repair of damaged tissues [3,5]. But, according to the author, the potential of these cells can give good positive results in a young organism.

Based on the above considerations, we determined this factor using a semi-quantitative immunofluorescence method.

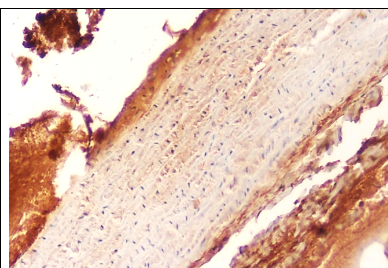
Aim of the study: Determination of CD34 proteins by immunofluorescence method in aorta preparations of experimental animals.

Materials and methods of investigation: The studies conducted were characterized by the normal staining of the intimal layer in the aorta of intact 3-month-old rabbits, and the absence of foci of thickening and hyperplasia in the vessels.

The obtained results and their discussion. The most active and intense staining is in the intima of endothelial cells and the wall of vas vasorum t. muscularis and t. Identified at the border of the adventitia. We used the IHC Profiler website recommendations from Sourceforge (<https://sourceforge.net/projects/ihcprofiler/>) to evaluate staining levels. According to the recommendations of IHC, the evaluation was carried out in a 4-point system: highly positive (3+), positive (2+), weakly positive (1+), and negative (0). According to the scoring system, CD34 expression in the aortic wall of intact rabbits was evaluated as high positive 3+ (Fig. 1).



Picture 1. Intact 3-month-old rabbit group aorta. No foci of staining, thickening and hyperplasia were detected in the intima norm. t. muscularis and t. Endothelial cells of the intima at the border of the adventitia and intense staining was detected in the wall of the vas vasorum. Expression CD34, 3+. Magnification x20.



Picture 2. Intact 9-month-old rabbit group aorta. Wavy staining of the middle layer of the aorta was observed, while smooth staining was found in the endothelial and subendothelial layers. Expression CD34, 3+. Magnification x20.

Notably, CD34 expression level decreased slightly as the rabbits aged. In particular, wavy staining was observed in the middle layer of the aortic blood vessel of 9-month-old intact rabbits, while strong staining was detected in the thin fibrous part of the endothelial layer and the thin subendothelial layer (Fig. 2). CD34 expression level was scored as high positive 3+.

Thus, CD34 expression in aortic wall layers of intact rabbits has been shown to decrease slightly as the animals age.

Irradiation of rabbits at a dose of 1 gray for 10 days caused structural disorders in the endothelial layer of vessels. In particular, irradiation of 3-month-old rabbits caused damage to the integrity of the inner wall of the vessels, roughening of the inner lining, and the appearance of bumps on the side of the cavity. This is based on the hypertrophy and hyperplasia of myofibroblasts in the muscular layer of blood vessels, and due to such changes, bumps are observed in the endothelial layer (Fig. 3). In the aorta of the animals, there were no muscular intervascular connections. In the vas vasorum, CD34 expression was minimally detected and its expression was scored as weakly positive, i.e. 1+.

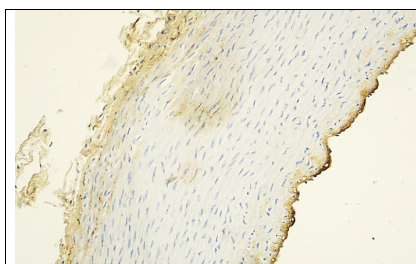


Figure 3. Aorta of irradiated rabbits at 3 months of age. Violation of the integrity of the inner layer of blood vessels, the appearance of bulges in the space due to hypertrophy of myofibroblasts in the muscle layer (shown by an arrow). Vascular bundles are not identified between muscle fibers. Vas vasorum expression is minimal. Expression CD34, 1+. Magnification x20.

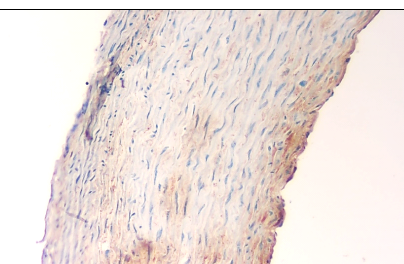


Figure 4. Aortas of irradiated rabbits at 9 months of age. Vas vasorum expression is slow, atrophy is observed, t. In the intima, the expression is also very weak, the endothelial layer is divided into pieces. The inner layer of the veins has a wavy appearance. Expression CD34, 1+. Magnification x20.

Immunohistochemical examination of the aorta of 9-month-old irradiated rabbits revealed that the expression of Vas vasorum was slow, their atrophy was observed, and strong changes were observed in the aorta (Fig. 4). The obtained results indicate that CD34-expressing endothelial cells are strongly damaged by radiation. Such changes can cause insufficiency in vas vasorum and gradually lead to the development of hypotrophic and hypoxic disturbances in the cells of the vascular wall.

Administration of ASD to 3-month-old irradiated rabbits for 10 days resulted in a slight reduction of the above-mentioned changes. Histochemical examinations showed thickening and swelling of muscle and collagen fibers in the middle part of the vessel wall. Microvesiculation foci remained in some places. But despite this, the proliferation of capillary-type vessels was detected among the swollen myofibroblasts (Fig. 5). This indicates that recovery processes have developed in the aorta. CD34 expression on endothelial cells was scored positive as 2+

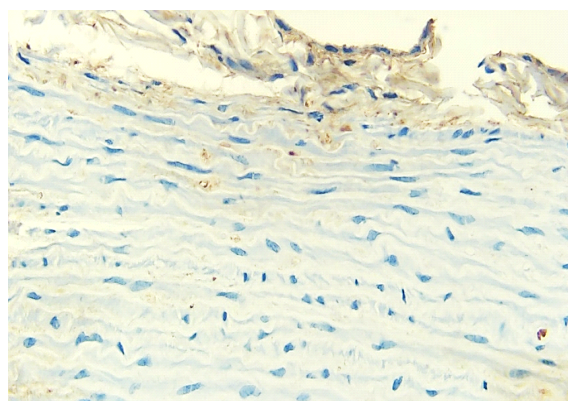
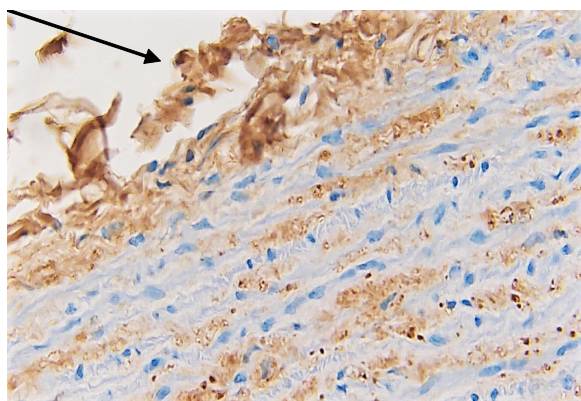


Figure 5. Aortas of 3-month-old rabbits irradiated and treated with ASD drug. In the middle part of the vessels there is a breakdown of muscle and collagen fibers and microvesiculations. Proliferation of capillary-type vessels among swollen myofibroblasts (indicated by arrow). Expression CD34, 2+. Magnification x40.

Figure 6. Aortas of 9-month-old rabbits irradiated and treated with ASD drug. Endothelial cell sloughing and desquamation, decreased CD34 expression. T. media and t. a decrease in the amount of vas vasorum at the border of the adventitia, weakening of vascularization (shown by an arrow). Expression CD34, 1+. Magnification x40.

In the treatment of irradiated 9-month-old rabbits with ASD drug, the changes detected in aortic endothelial cells were preserved (Fig. 6). The obtained results show that CD34 expression can be observed in certain parts of the vascular endothelium when irradiated rabbits are treated with ASD. The expression of CD34 in vascular endothelial cells damaged by ASD can be predicted from the activation of stem cells in them. In our opinion, ASD shows that there is a positive effect on regenerative and reparative processes in the body of irradiated animals.

Conclusions:

1. In rabbits of different ages, exposure to 1 Gray for 10 days resulted in a decrease in serum cholesterol and its transport forms. Strong changes were observed in 12-24-month-old rabbits and indicated the developing endothelial dysfunction in the vessels.
2. Treatment of irradiated rabbits with ASD drug increased serum total cholesterol and cholesterol in ZYuLPs, but no complete normalization was observed. Such positive changes reduced the atherogenic coefficient and prevented the development of atherosclerotic changes in the endothelium.

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