THE MECHANISMS OF REORGANIZATION OF BLOOD VESSELS OF MYOCARDIAL RIGHT ATRIA AT HEMODYNAMIC CHANGES

Kukurtchyan, N. S.[a] and Karapetyan, G. R.[a]

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It was shown that pulmonary arterial valve stenosis moderate changes in hemodynamic parameters of patients lead to a change in the structure of cardiomyocyte and blood vessel. Strengthening of plastic processes, lead to hypertrophy and proliferation of endothelial cells as well as to increase of blood vessels' lumen diameter. Quite interesting is the fact that new capillaries formed mostly by intuscusseptive angiogenesis. In the same way new arteries are formed. Hemodynamic changes of heart right compartment accompanied by structural vascular and cardiomyocyte remodeling. In the later stages of disease vascular remodeling goes by the mechanism of intussusceptions forming vessels of different caliber.

* Corresponding Authors: Karapetyan, G. R.
Fax: +374 -10-28 19 51
E-Mail: sapootraa_a@yahoo.com
[a] H. Buniatian Institute of Biochemistry, of National Academy of Sciences of Republic Armenia, Paruir Sevag str. 5/1, 0014, Yerevan, Republic of Armenia.

INTRODUCTION

In vertebrates the cardiovascular system is the first to function for the same purpose throughout the entire lifespan of the individual. It generates pressure for the displacement of blood within it and of metabolites through its walls. It has long been recognized that in fluid transport systems in which only on pressure source the heart provides the driving force for transport through billions of minuscule capillaries one optimizing feature is a system of hierarchical bifurcations.[1] Cardiac hypertrophy is formed as an adaptive response to increased work load to maintain cardiac function.[2] An increase in heart tissue must be matched by a corresponding expansion of the coronary vasculature to maintain.[3] Myocardial hypertrophy, secondary to increased hemodynamic demands, required to provide adequate oxygen and nutrients to the increasing cardiac mass.[4] When deregulated the formation of new blood vessels contributes to numerous malignant ischemic inflammatory infections and immune disorders.

There are two forms of vascular remodeling associated with physiological and pathological processes: angiogenesis and arteriogenesis. Angiogenesis is a potent physiological process that underlies the natural manner in which our bodies respond to a diminution of blood supply to vital organs namely the production of new collateral vessels. However under pathological conditions angiogenesis can be found as a part of pathological process such as in diabetic retinopathy, tumor growth and wound healing. Increased capillaring also can be found in the heart with coronary artery occlusion in young adult animals.[5] Angiogenesis is a process of growing of new capillaries from the existing capillaries trough capillary sprouting or intussusceptions.[6,7] Variant of angiogenesis different from sprouting is intussusceptive angiogenesis. Intussusceptive microvascular growth is fast process that can take place within hours or even minutes, because it does not need proliferation of endothelial cells and with a little amount of energy.[8,9,10] The physiological mechanisms that underlie the coordination of angiogenesis and cardiomyocyte growth are unknown.[3]

Prolonged cardiac hypertrophy causes heart failure, and its mechanisms are largely unknown.[11] Right ventricular failure is an important clinical problem with no available therapies, largely because its molecular mechanism is unknown. Lack of coordination between the myocytes – driven hypertrophic response and the production of angiogenic growth factors hallmarks the transition to heart failure.[4] Inhibition and suppressed angiogenesis and the resultant ischemia may contribute to the rapid deterioration of right ventricular function upon entrance to a decompensation phase.[11]

We have previously shown the relationship of structural changes of cardiomyocytes and blood capillaries in the development of hypertrophy of the right compartment of heart.[12,13,14,15]

The aim of this study is to investigate the reorganization of the blood vessels of myocardium at hemodynamic changes.

MATERIALS AND METHODS

Reagents

Crystalloid cardioplegic solution (Na-147 meq L^{-1}, K-19 meq L^{-1}, Ca-4 meq L^{-1}, Cl-155 meq L^{-1}, HCO_3- 25 meq L^{-1}, Glucose-0,2% , pH-7,4, Mg –2 meq L^{-1}); powdered paraformaldehyde; OsO_4; Sodium cacodylate trihydrate; 96º ethyl alcohol, acetone, Epon 812, Epon Hardener MNA, Epon Hardener DDSA, Epon accelerator DNP-30, uranyl acetate, citrate Na, nitrate Pb, photoplates.

All reagent used were of analytical grade and purchased from Sigma Chemical Co. (USA).

Human Subject

All procedures involved human subject were approved by institutional review board/bioethical committee (Erevan State Medical University, RA) conformed to the Legal Aspects of Research Ethics and Science in European Community directive (2001/20/EC), (IRB Approval YSMU Bioethical committee N7 by 26.04.2011).

In this study the myocardium of right atria of 10 patients with pulmonary arterial valve stenosis (PAVS) was investigated. The patients were divided into 3 groups depend on the systolic pressure of right ventricular: I group - mercury column pressure was up to 60mm (2 patients); II group - mercury column pressure was from 60 to 100mm (3 patients); III group - mercury column pressure was more than 120mm (5 patients).

Collecting biopsy material during cardiosurgical procedure of patients was performed during canulation.

Treatment of material

The bioptates taken during canulation (small pieces of the right atrii) have immediately put in cold 4 ºC mix of paraformaldehyde in a sodium cacodylate buffer and glutaraldehyde for 12 hours with following post fixation in 1% OsO_4 solution during 2 hours; dehydration in ascending series of spirits; saturation in a mixture of acetone and Epon resins of different proportions and pouring in gelatinous capsules into epon.

Obtaining of ultrathin slices and its treatment

The ultrathin slices (up to 500 Å) were made using ultracut LKB (Swedish) and Reichert (Austria). Ultrathin slices were double contrasted with uranyl acetate and citrate Na and nitrate Pb solutions.

Observation under TEM

Obtained ultrathin slices were observed under the transmission electron microscope (Phillips CM 10) with resolution X 20.000.

Observation under light microscope

Obtained semithin epoxy sections stained with Azur 2 and observed under the light microscope with resolution X 1.000.[16]

RESULTS

It was shown that pulmonary arterial valve stenosis (PAVS) moderate changes in hemodynamic parameters of patients lead to a change in the structure of cardiomyocyte and blood vessel.

In the first group patients with right ventricular blood pressure up to 60mm of mercury column the process of enhancing of the plastically processes in cardiomyocytes as well as in blood vessels without cardiomyocytes organelles hyperplasia and hypertrophy takes place. Changes in the endothelial lining go in two directions. In one case, it is a relatively thin and uniform thickness, the other marked irregularity of its thickening. Strengthening of plastic processes, the presence of many ribosome, rough reticulum hypertrophy lead to hypertrophy and proliferation of endothelial cells (Fig. 1) as well as to increase of blood vessels' lumen diameter.

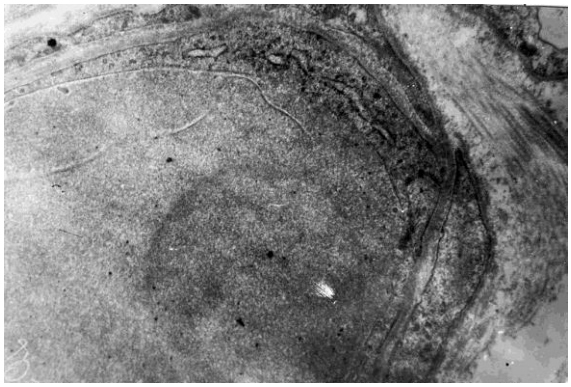

Figure 1. Plastic processes in endothelial cells. X 20.000

In the second group of patients with blood pressure in the right ventricular up to 60 -100mm of mercury column the processes of myofibrils hypertrophy and Mch hyperplasia take place. The plastically processes are enhanced lead to reorganization of cardiomyocytes and blood capillaries as well. Blood vessels are presented in significant number. The studies performed by TEM showed proliferation of cellular elements in the walls of small arteries of patients with PAVS. Endothelial cells were increased not only in quantity but also in size (hypertrophic).

Proliferation of smooth muscle cells lead to its unevenly localization. Proliferation of contractile fibers inside of these cells are expressed (Fig. 2).

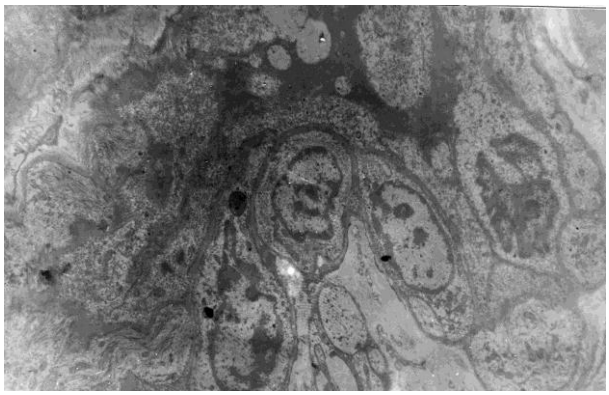

Figure 2. Proliferation of cellular elements in the walls of small arteries. X 20.000

When blood pressure in right ventricular is more than 120mm myofibrils are not hypertrophied. The process of hyperplasia of Mch takes place. The plastically processes are enhanced in cardiomyocytes and blood vessels. Endothelial lining has different thickness, took place the process of proliferation of endotheliocytes as well as sharp expansion of the capillaries lumen. It must be noted that some patients of this group have interruption of myocardial blood flow, lead to ultrastructural destruction of cardiomyocytes and blood vessels.

By the method of light microscopy used semithin epoxy sections of patients with PAVS were found formation of new blood vessels from preexisted (Fig. 3, 4).

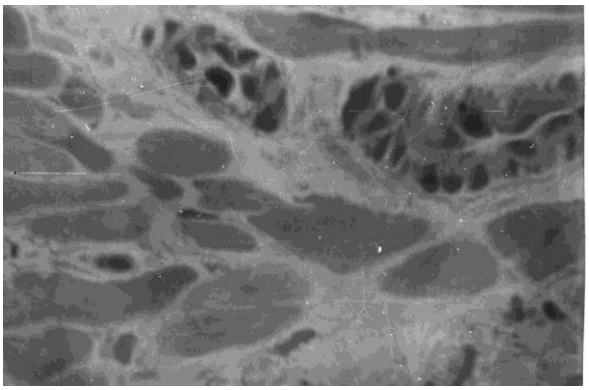

Figure. 3. Intussusceptive angiogenesis of small arteria. X 1000

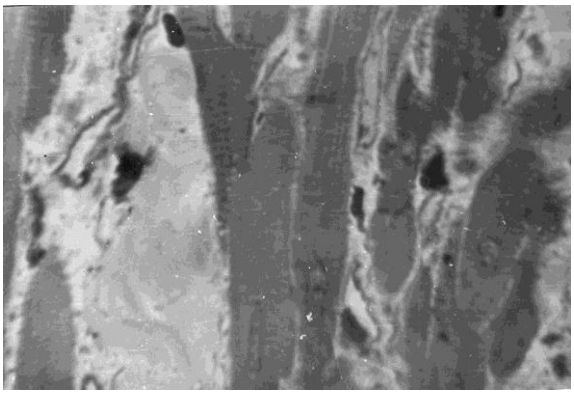

Figure 4. Intussusceptive angiogenesis of capillaries. X 1000

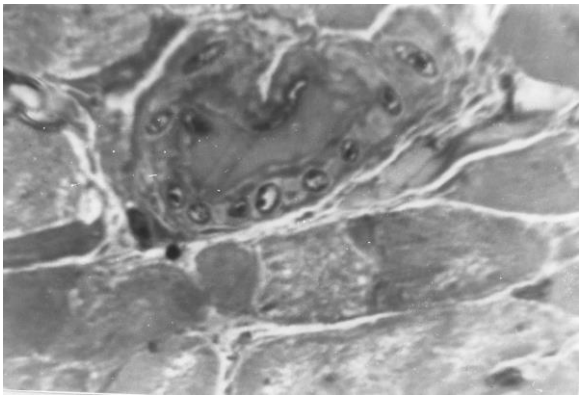

Figure 5. Pillar formation in small arteria. X 1000

In some capillaries transluminal bridges dividing the lumen of the capillary are observed. In most cases took place the invagination of the opposing capillary walls on both sides into the lumen forming pillar.

The cytoskeleton itself is actively involved in this process. Quite interesting is the fact that new capillaries formed in this way have visually identical profiles. In the same way new arteries are formed. This process is different from capillaries by involving only one side of artery wall in the process of invagination and pillar formation (Fig. 5). It should be noted that these new vessels are different by sizes and their lumen diameter varies from very small to larger. The hyperplasia of the cellular elements of the wall is observed.

Increasing number of transluminal bridges in blood vessels and pillars, as well as in the same capillary 2 or more invaginations of the opposing walls of the capillary take place. This process indicates that the new formed vessels will be of different caliber.

DISCUSSION

Progressing changes of hemodynamic parameters at PAVS accompanied by the inclusion of growth stimuli, increase plastic processes which lead to remodeling of blood capillaries in the direction of increasing of their caliber.

One of the conditions for the success of remodeling process is proliferation of endothelial and smooth muscle cells.[17] However, the proliferation and hypertrophy of endothelial cell, proliferation of smooth muscle cells and the randomness of their location in the wall of collateral vessels at the changes in the severity of hemodynamic parameters of patients with PAVS leads to the formation of blood vessels look like giant structures with a wide lumen.

The accepted point of view is that arteriogenesis and angiogenesis have been through to be distinct processes mediated by different mechanisms.[18,19,20,21] Our study indicate that strengthening of the growth stimulus leads to an increase of cell elements in the wall of collateral blood vessels and capillaries, as well as to most expressed change in their caliber in the later stages of the disease.

Studies of angiogenesis induced by adeno Vpf have shown that angiogenic response in skin was much more intense than that with developed in either skeletal or heart muscle.[22] The kinetics of new blood vessel formation and the structure and functional capacities of the newly formed vessels induced by VPF/ VEGF or other cytokines in ischemic tissues have not been carefully investigated. At adeno Vpf many mother vessels divided into smaller "daughter vessels" by sprouting, or by projection of EC cytoplasmic processes into and across mother vessel lumens, forming translumenal EC 'bridges'. These bridges divided blood flow into smaller sized channels. The course of several days separated from each other to form smaller caliber daughter vessels.[22]

Mother vessels in ear skin evolved along yet another pathway, that of intussusceptions a process distinct from translumenal bridging. Intussusception occurs in embryogenesis when 'pillars' of connective imprignge from without on hollow tubular structures causing focal invagination.[23] Although the mechanism of intussusceptions is not fully understood, there are several keypalyers that cold influence pillar formation.[24] Alteration in blood flow dynamics in arterial branches could stimulate this process.[25] Pillar formation and remodeling is not observed in capillary plexuses, but also within smaller arteries and veins.[26]

Intossusception is a relatively new mechanism of angiogenesis in biology. It should be noted that there are conflicting views regarding the mechanism. Some studies combined the process of intussusceptions with forming transluminal endothelial bridges.[27] However, other researchers believe that transluminal endothelial bridges formation and intussusceptions are different processes. Intussusception causes to pillar formation and then to separation and formation of new blood vessel.

At the expressed alteration of hemodynamic parameters of myocardium formation of new blood vessel take place on the basis of preexisting capillaries increased in size. In the myocardium of right atrium at PAVS the presence of intussusception process with transition to pillar formation in the capillaries, as well as formation of transluminal bridges in small arteries are observed. The type of blood vessel formation was closely dependent on the pathology.

It could be mentioned that because of the fact that intussusception is a faster process and compared with the formation of transluminal bridges is more preferably in the compartments of heart with hypertrophy.

However, it should be noted that for myocardium these processes are not positive, because there is an uneven caliber in formed blood vessels, especially concerning the small collateral vessels, as there are risks of failure to ensure some regions of the myocardium with oxygen and nutrients that may lead to postoperative complications. Such structural reorganization of the blood vessels in parallel with changes in cardiomyocytes themselves can be the cause of chronic heart failure.

CONCLUSION

The alterations of SS structures as well as in mitochondria of cells could be one of the main reasons leading to postsurgical damages of cardiomyocytes.

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