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Early developmental dynamics of cell surface glycoconjugates in the rat left ventricle myocardium relate to the tissue stereological data

Abstract: The purpose of study was to reveal the connection between the dynamics of cellular surface glycoconjugates (detectable by binding plant lectins: PNA, LCA, RCA I, UeA, WGA) and stereological parameters (volume densities of cardiac muscle cells, capillaries and connective tissue) in the left ventricle myocardium of rat heart in early ontogenesis.

The weak RCA I binding was observed in the membranes of cardiac myocytes of left ventricle throughout 12-20th days of gestation. There was the moderate reaction with RCA I, LCA and WGA on cellular surface of vascular endothelium, pericardial mesothelium and endocardial luminal cells. LCA and WGA staining revealed the uniform pattern for cardiac muscle cells within the left ventricle myocardium. The weak UeA binding in the cardiac muscle cells appeared after 16th day of gestation in the subepicardial layer of myocardium.

PNA binding was not uniform during 14-18th days of gestation in cardiac muscle cells. It was corresponded the stereological differences in regions with the high and low receptors density. During this period, the volume densities of cardiomyocytes and capillaries were higher in areas with strong PNA binding (subepicardial and intramural layers). The volume density of connective tissue was high in areas with weak reaction (subendocardial region). The irregularity in PNA staining of cardiac muscle cells in the left ventricle myocardium disappeared to 20th day of gestation together with a loss of statistical difference in the volume densities of structural components in layers of myocardium.

Keywords: lectins, myocardium, stereological parameters, rat heart

Introduction. The further development of ideas about the formation of the heart needs to clarify the connection between quantitative and qualitative aspects of the cardiogenesis. The recently developed theory of the tissue unity through the molecular network passing from the actin cytoskeleton through transmembranous proteins to fibrils and other structures of the extracellular matrix brings to actuality of the study of the cellular surface glycoproteins.[1]

The developmental dynamic of the surface glycoconjugates is one of the phenomena, reflecting the changes of complex interactions between cellular and tissue components of organs, including developing heart.[2,3,4] It is known that surface glycoconjugates with the terminal carbohydrates being capable of binding the plant lectins determine the tissue structure, control the cell migration during heart development,[5,6] relate to some pathological processes in the heart.[7,8] The previous studies reveals the strong binding of some lectins in the parts of heart, which undergoes the important transformations such as the endocardial cushions and outflow tract.[5,6] It has not still cleared the relation the dynamics of cellular surface glycoproteins to other structural and functional characteristics of left ventricle myocardium of developing heart including stereological parameters, the latter can give the important information about the tissue reconstruction during ontogenesis or pathological processes.[9,10]

The aim of research was to study the relation between the dynamics of cellular surface glycoconjugates and stereological parameters of the left ventricle myocardium of rat heart in early ontogenesis.

Materials and methods. The rat hearts on 12th, 14th, 16th, 18th and 20th days of gestation were used. Whole embryos from 12th to 16th days of gestation and hearts of older embryos were fixed in Buen's fixative and used for paraffin embedding. Some sections were stained with haemotoxilin-eosin for morphological identification and stereological analysis. Selected slides were treated for 10 min in 3% hydrogen peroxide in methanol, washed for 5 min in PBS three times, treated for 5 min in 2% bovine serum albumin, rinsed for 5 min in PBS three times, and then incubated with lectins. We used PNA (Peanut Agglutinin), LCA (Lens Culinaris Agglutinin), RCA I (Ricinus Communis Agglutinin), UeA (Ulex Europaeus Agglutinin), WGA (Wheat Germ Agglutinin), conjugated with the horseradish peroxidase overnight at +4 °C. After visualization with DAB (Sigma), the slides were washed briefly in water, dehydrated, cleared in xylol, and mounted with Dpx.

The stereological quantitative analysis of the left ventricle myocardium: the volume densities of cardiac muscle cells ($V_v$cc), capillaries ($V_v$cap) and connective tissue ($V_v$ct) was estimated using the point-counting method. A point grid was overlaid on the images of myocardium and the density was obtained using the following formula: V_v(structure, reference)=Σ*P*(structure)/Σ*P*(reference)×100%, where «Σ(structure)» and «Σ*P*(reference)» were the total points hitting the favoured structure and the whole myocardial sections, respectively. The number of fields for stereological quantitative analysis was 40 on a section. The data were processed by standard statistical procedures.

Results and discussion. The small amount of RCA I (sugar specificity: Galβ1-4GlcNAcβ1) receptors was observed in the membranes of cardiac myocytes of left ventricle throughout all period (12-20th days of gestation). The somewhat more intensive reaction with RCA I we observed on cellular surface of endothelial cells of vessels, as well as pericardial and endocardial luminal cells. As for LCA (Manα and Glcα) and WGA (GlcNAcβ1-4GlcNAcβ1, Neu5Ac) staining, we did not noticed the difference in the staining pattern in layers of myocardium. The reaction was moderate during all stages researched in cardiac muscle cells, endothelial and mesothelial cells. We did not observed UeA (Fucα1-2Gal) binding in the myocardium of left ventricle of rat heart before 16th day of gestation. The glycoconjugates containing terminal fucose appeared in the membrane of cardiac muscle cells mostly in the subepicardial layer of myocardium. The reaction with UeA in that region was weak throughout 16-20th days of gestation. The marked binding of fucose specific lectins was found in the outflow tract and directing the migration of neural crest cells into the heart6. In our study the UeA staining in the subepicardial layer of myocardium can point to the main pathway of the migration of the cell of extracardial origin within left ventricle.

The weak regular binding of cardiac muscle cells with the lectin PNA (Galβ1-3GalNAcα1) in the ventricular part of the heart on 12th day of gestation pointed to the small amount of β-galactose determinants of cellular surfaces. The endocardial and epicardial endothelium demonstrated the moderate reaction with this lectin. On 14th day of gestation the difference in PNA staining of cardiac muscle cells appeared clearly in the layers of left ventricle. The irregular pattern of PNA binding on this term gave us the backgrounds for the stereological analysis. The maximum of receptors production was observed in the compact layer of myocardium, where $V_v$cc was 70,3 ± 5,51%, $V_v$cap – 1,6 ± 0,02%, $V_v$ct – 28,1 ± 2,23%. In contrast to this we found the relatively low ability of PNA binding in the trabecular layer of myocardium, where the stereological parameters were: $V_v$cc – 56,1 ± 4,32% ($p < 0,05$); $V_v$cap – 0; $V_v$ct – 43,9 ± 3,81% ($p < 0,05$), indicating the less developed tissue structure in this region.

On the 16th day of gestation the difference in PNA binding of the cardiac muscle cells of left ventricle remained the same. The cells with strong staining were present in the subepicardial areas of the left ventricle whereas the intramural and subendocardial layers demonstrated the weak PNA binding. The stereological analysis revealed the higher volume density of cardiac muscle cells and low volume densities of connective tissue in subepicardial layer compared to the rest areas of left ventricle myocardium ($V_v$cc – 75,2 ± 6,34% and 61,9 ± 5,76%, respectively ($p<0,05$); $V_v$ct – 21,7 ± 1,95% and 37,2 ± 3,31%, respectively, $p<0,01$). The volume density of capillaries was also much higher in the intensively PNA stained subepicardial zone.

On the 18th day the PNA staining pattern of cardiac muscle cells became more uniform, however, the subendocardial layer demonstrated less intensive reaction. The stereological analysis showed a greater volume density of connective tissue in subendocardial area compared to the myocardial mass with strong staining of cardiac muscle cells ($V_v$ct 19,6 ± 1,81% and 14,8 ± 1,23% respectively, $p < 0,05$). There weren't the statistical difference between the other parameters ($V_v$cc – 77,2 ± 8,13% and 81,3 ± 8,45%; $V_v$cap - 3,20 ± 0,32% and 3,90 ± 0,40%).

The irregularity in PNA staining of cardiac muscle cells in the left ventricle myocardium almost disappeared to 20th day of gestation. Stereological analysis revealed the absence of statistical difference in the volume densities of structural components in subepicardial and subendocardial areas ($V_v$cap 5,8 ± 5,53% and 5,6 ± 5,94%, respectively; $V_v$cc - 82,3 ± 7,88% and 81,5 ± 8,07%; $V_v$ct 11,9 ± 1,86% and 12,7 ± 1,30%). The endocardial and epicardial layers of the left ventricle, endothelium of all the vessels demonstrated the moderate to strong reaction with PNA throughout 14-20th days of gestation.

We conclude that the most important transformations of the myocardium have finished to 20th day of gestation and they have been related to β-galactose determinants of cellular surfaces of cardiac muscle cells whereas the in other regions of the heart such as endocardial cushions the PNA binding decreased markedly after 14th day of gestation[5].

In summary, the reaction with PNA on the cell surface of the cardiac muscle cells related to more matured histological structure of left ventricle myocardium in rat heart between 12th and 20th days of gestation.

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