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Research Article

MORPHOLOGICAL CHARACTERISTICS OF MYOCARDIAL CHANGES WHEN EXPOSED TO PESTICIDES

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ABSTRACT

The aim of our work is to identify features of morphogenesis in postnatal development, the study of the histological structure of different departments and membranes of rat heart at 1-22 days after birth. The object of the study was the heart of the 50 rats at 1, 6, 11, 16, 22 days after birth. Notes alternating periods of acceleration and deceleration of the growth rate increasing thickness of the atria and ventricles. Endocardial and epicardial thickness increases significantly less. The growth rate of the thickness of the ventricular myocardium were observed in rats 6 and 16 days of age. Structural changes occur due to the growth of the organism. Feature of the structure and topography of microvessels heart is their distribution in the course of cardiomyocytes and the relationship with fibrous connective tissue structures of cardiomyocytes.

KEYWORDS

Rat heart, postnatal ontogenesis, cardiomyocytes, fibrous structure atria and ventricles.

INTRODUCTION

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Very often one of the etiological causes of the heart disease is the toxic factor. Currently different chemical products used in agriculture for the treatment of grape, vegetable and especially cotton fields. Most of chemical compounds such as pesticides have adverse effects on the body. At the present time more than 1000 kinds of pesticides are being used around the worldwide and every year their number is increasing. It is well known, that prolonged exposure to even small doses of pesticides on the body increases frequency and enhances diseases of cardiovascular system, myocarditis of various etiologies. Therefore, of great interest is the study of pesticide effects on human and animals, in particular on the cardiovascular system. Significant relevance is acquired by the problem of pesticide influence on growing young organisms. Heart variability is not only general biological interest, but has a curtain significance in the disclosure of physiological processes developing therein, depending on the environmental conditions.

The purpose of the study to determine the characteristics of anatomical, microscopic, sub-microscopic and morphometric changes of different layers of the heart wall in rats at an early postnatal ontogenesis under the influence of pesticides through breast milk.

Material and research methods. In total, 205 rat hearts ages a day olds, 6 day olds, 11days olds, 16 days olds and 21days olds were used. The animals were divided into 3 groups. To obtain this goal several series of experiments were conducted. In the first series after the birth of rat pups, to a mother rats were administered pesticide cotoran at a dose 1/100 LD50 intra-gastrically through a catheter. In the first group of female mother rats starting from the day after giving birth cotoran was administered for 5 days, and rat pups died on the 6th day. In the second group cotoran was administered to mother rats after giving birth for the 10 days and the slaughter of animals made on day 11. In the third group cotoran was administered to the mother rats from the first day after giving birth for 15days, and rat pups died at 16days of age. In the fourth group pesticide was administered to mother rats also after giving birth and rat pups died after 21days of age. In the second series, pesticide β-tetramethyl at a dose of 5MDU was administered in the same way to the mother rats, and rat pups died in an analogical experiment days. To the mother rats in the control group, in the morning on empty stomach depending on the term injected 1 ml of distilled water daily. As a probe, for female rats subclavial cathetersN°1. were used. The animals were anesthetized with either at 1, 6, 11, 16, 21 days after birth. We used a range of methods including: general histological method, organometry, morphometry, ECG of the heart and mathematical modeling and prognosis of toxic myocarditis.

After removing the hearts from thorax measurements for the lengths, widths, thicknesses of the rat hearts were conducted. To determine the linear methods calipers with 0.05 mm scales were used. The length of the heart is determined from thee tip to the outermost part of the base of the heart. Heart width defined as a distance between the most projecting portions at atria-ventricular groove in a direction from left to right. The thickness of the heart is a distance between the most prominent parts on the level of atria-ventricular groove from front to back. Heart shape is determined visually. To determine the heart mass and mass of the rat electronic weight is used.

Histological sections were made in a plane perpendicular to the long axis of the heart. Paraffin sections with 8-10mcm thickness, made using a rotary microtones staining with hematoxyllin and eosin by standard methods. Collagen fibers in the connective tissue skeleton of the heart walls were detected with hematoxyllin and picrofuchsin by Van Gieson's staining method and reticular fibers by a foot in modification of N.A Yurina.

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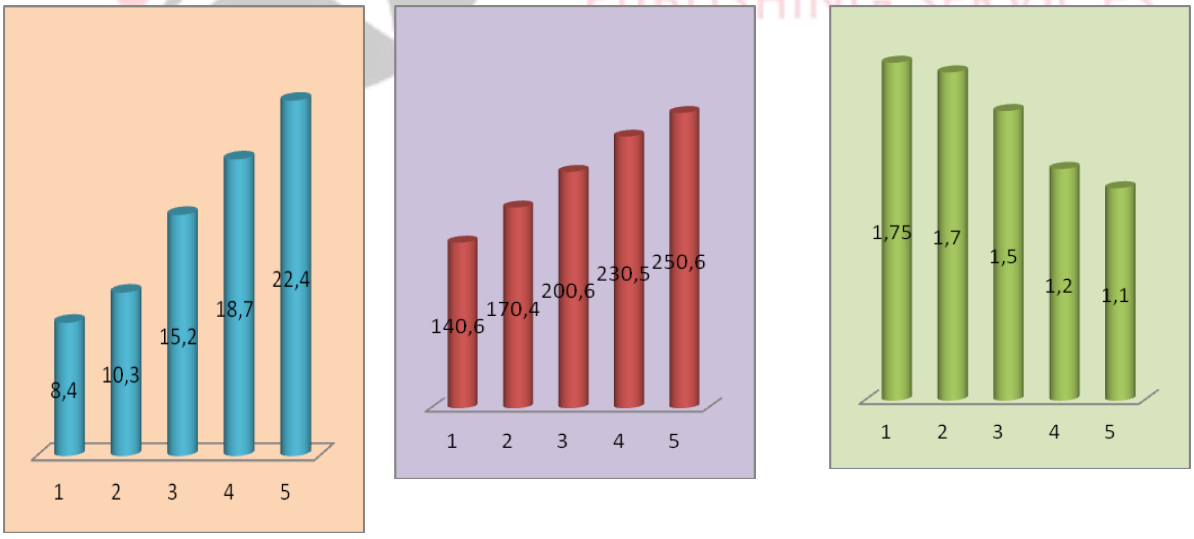
For scanning electron microscopy, tissue was fixed with 2.5% glutaraldehyde solution, with osmium tetroxide fixation to phosphate buffer, dehydrated in alcohol and acetone, and dried by the critical point in the HCP-2 device. Gold plated was carried out in IB-2 device and examined in electron microscopes JEOL JSM-6010 LV and Hitachi-S405 and photographing from the latest monitor screen using Canon digital camera.

Research and discussion results. Rat heart asymmetrically located in the thoracic cavity and occupies a large area on the left. The absolute heart weight is significantly increased with increasing body mass. Weight of a newborn ups is in average 8±0,4 gr, and the absolute heart weight averaged 140±1,7 mgr. The highest rate of weight gain in rats observed at 16 days of age up to 50%, and heart weight at 6days of age growth rate equals to 21%. From newborn to 22 days of old rat weight increased by 3 times, and the heart weight increases by 1.7 times. Our data analogic with the data of the authors that the relative weight of human heart and rat hearts are greatest during neonatal age. Heart weight of newborn rat pups are 1.75% from body mass. With age, the heart mass is reduced in relation to the body weight and on the 22 day of postnatal ontogenesis it equals to 1.1% (figure1). The results of the study show that the shape and size of the heart during postnatal ontogenesis at different ages is constantly changing. In newborn rat heart length in average is -4.5±0,02mm, width in average is-4,3±0,3mm, anterior posterior size is-3,8 ±0,2mm. Since newborn rats all anatomical parameters are almost the same and in most cases the heart shape newborn rats come close to spherical. During the early postnatal ontogenesis growth in length, width and anterior posterior size is irregular. Starting from day 6 rat heart length increases more than the width and anterior posterior size and shape of the heart approaches elongated form. The highest growth rate of anatomical parameters observed in rats is at 6 days of age. Anterior posterior size of the rat heart increases in 22 days of age, and heart approaches conical shape.



Body mass, in grams *Heart mass, in mgs* *Coefficient*

Figure-1. Ratio of heart weight to the weight of the rats.

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Results for morphometric studies on thickness of ventricular and atrial heart walls, in control group animals showed dynamic growth on the thickness of walls for both the left and right atriums and ventricles of the heart at different times of postnatal ontogenesis and it occurs uneven. Growth dynamics of the wall thickness for both left and right ventricles of heart at different terms of development depend from the parts of the heart wall. Growth rate of the atrial wall thickness greater in rats 6 days of age. Ventricular wall thickness mostly increases in rats 6 and 22 days of ages. The thickness of atrial endocardium is greater on the walls of atrium than in the ventricles. The thickness of myocardium and epicardium is greater in the left ventricle on the posterior part. The thickness of the ventricular wall on posterior part increases more than the atrial wall and on the anterior part. The thickness of the epicardium in age aspect compared with other layers varies slightly.

Microscopic examination can be traced a certain pattern of development and differentiation in the dynamics of early postnatal ontogenesis in the histological structure of the structural elements of the layers of the wall of the atria and ventricles of the heart in intact rats.

This provision is the conclusion of the development and differentiation of the structural elements of the atrial wall and ventricular dynamics in early postnatal ontogenesis. In the early period (6 th and 11 th days) study the structural elements of all layers of the heart wall remain undifferentiated, especially in the myocardium, where cellular elements predominate over the myofibrils. The left ventricle distinguish subendocardial, subepicardial and intermediate layers. Subendocardial infarction layer is more differentiated than the other layers and is composed of parallel beams cardiomyocytes that are parallel to the endothelium. Subepicardial myocardial layer has a loose structure and puchkoobraznoe, cardiomyocytes it arranged randomly and are larger. In individual cardiomyocytes myofibrils thicker in others - a vague and vacuolated. The intramural layer left ventricular myocardial muscle cells are arranged perpendicular to the subendocardial layer. The interventricular septum myocardium heart denser, thicker form cardiomyocytes parallel beams. Around cardiomyocytes and around the vessels are arranged bundles of collagen and elastin fibers. Reticular fibers are arranged between the cardiomyocytes as a winding dark brown fibrous structure that envelop the individual muscle bundles, forming melkopetlistuyu, and around the vessels in the epicardium - krupnopetlistuyu network.

In the later stages of research, to the 21 th day, the structural elements of all layers of the heart wall is completely formed, gaining their true morphological and functional features. In the wall of the left ventricle of the heart, all three layers are clearly distinguishable as compared with the previous study periods well developed subendocardial layer which is thicker than the other layers. From the endocardial penetrate deep cracks and Tebeziya vessels that reach the intramural myocardium layer. This layer of the myocardium loose and consists of multiple individual beams of cardiomyocytes, between which there are enlightened cracks, small vessels and soft connective tissue stroma. Intramural myocardial layer is presented in parallel reaching cardiomyocytes myofibrils which significantly prevail over the nuclear structures. Subepicardial layer compared to other layers composed of thin and cut transversely cardiomyocytes that exist between arterial and venous vessels. This venous sinus have different shape and size, some of them form large elongated blood lake. In histochemical staining according to the method of Van Gieson in the walls of

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arteries, arterioles revealed large bundles of collagen fibers, and in the walls of the veins and in the stroma infarction - the gentle collagen and discontinuous fibers. When painting PAS-reaction compared to previous periods of research in the walls of blood vessels, and connective tissue in epicardium defined decrease in PAS-positive substance. In the walls of blood vessels increases the amount of elastic fibers. Especially thickens subendothelial inner elastic membrane, which is represented by a thick winding material of uneven thickness deep purple color. The muscular and adventitial layers of artery walls elastic fibers is small, and they are presented in the form of bluish shadow structures.

The blood vessels of the rat heart are presented by arterioles, capillaries, venules and sinusoids. The myocardial blood vessels are directed along the bundle of cardiomyocytes. Around the cardiomyocytes and vessels located bundles of collagen and elastic fibers. Parallel arrangement of muscular fibers and capillaries in the myocardium may create conditions for even pressure on the walls of capillaries thus preventing its clamping in systole. There is no significant difference in diameters of the blood vessels walls of a right and left ventricles.

Arterioles are characterized by thin inner layer, clearly pronounced middle layer, and outer layers. Inner layer of arterioles are presented by the nuclei of endothelial cells with round shape. They are located at a slight distance from each other. Well pronounced middle layer consist of circular bundles of muscular fibers. They form two layers. The outer layer is formed by loose fibrous tissue and adventitial cells. The inner diameter of arterioles of newborn rats of control group on average varies11.7±0,6mcm, up to the 6th day there is the greatest pace of growth up to 40% in diameter of arterioles, in the next age group there is an insignificant growth rate for inner diameter of arterioles.

Capillaries have a diameter in average 4.7±0,6microns. Capillaries of myocardium consist of 3 layers. The inner layer is formed by endothelial cells. Middle layer is from basal membrane and outer layer from elastic membrane. Mainly capillaries of myocardium are found in subepicardial layer. In subepicardial layer blood vessels are rare.

The wall of the venules is presented by the endothelial cells which are located at a great distance from each other. Muscular layer in venules are underdeveloped. Venules thickness on average varies 16.7±1,2mcm. In the walls of left ventricles sinusoids are found. The walls of the sinusoids by structure do not differ from the capillaries. By the diameter sinusoids are bigger than capillaries and reach up to 35-42mcm. Sinusoids usually are oval or round shape.
The study of morphometric data of the left and right atrium and ventricles inexperimental group show that under the exposure to cotoran observed a decrease in all parts of the heart wall.

Analysis of the table shows that rats 6days of age have decreased thickness down to 10% on the walls of left and right atrium under the exposure to cotoran. The thickness of the left and right ventricles are less in atrium part by 11-13% compared with control group under the exposure to cotoran. On the 11th day there is an 18% decrease of the thickness of the left and right ventricles are less in anterior part by 23% compared with control group under the exposure to cotoran.. The thickness of the left and right ventricles are less in posterior part by 16-20% compared with control group under the exposure to cotoran.

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At 16days of age there is a 13% decrease of the thickness of the left and right atria under the exposure to cotoran. The thickness of the left and right ventricles are less in anterior part by 19% and in posterior part 17% compared with control group under the exposure to cotoran .

In rats at 21days of age there is a 9% decrease of the thickness of the left and right atriums under the exposure to cotoran. The thickness of the left and right ventricles in anterior part are less in anterior part by 9% and posterior part by 11% compared with control group under the exposure to cotoran.

Findings

1. In postnatal ontogenesis changes in heart position of rat pups occur from cranial to caudal direction. The greatest increase in length, width and anterior posterior sizes occur from the moment of birth to 6 days of age, further mostly the length of the heart grows. From birth to 21 days of age, the ratio relative heart weight to the body weight is reduced from 1.75 to1.1.During the postnatal development of rat pups heart position is displaced from cranial to caudal part. Comparative changes occur in heart, body mass and in their rate, and also there are changes in the shape of the heart and the development of the chest.
2. Traced a definite pattern of development and differentiation in the dynamics of early postnatal ontogenesis in the microscopic structure of the structural elements of the wall layers of the atria and ventricles of the heart in control rat hearts.
3. The toxic effects of pesticides on the heart of rats manifested pathologic-morphologic changes developing in the wall of arterioles and capillaries in the form of disorganization of fibrous structures, the development of proliferative processes of proper cell walls of blood vessels as well as changes in myocardial muscle fibers in the form of protein dystrophy, disintegration of myofibrils and dis-complexion of nuclear-cytoplasmic ration.

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