Ways of Formation and Development of Derivatives of Conotruncal Region of the Mouse Embryonic Heart at Normal Condition and Under Maternal Dehydration

Dyagovets Katerina

1. Introduction

Conotruncal defects are one of the main groups of the birth heart diseases. The world statistic shows that among 1000 births 6-8 newborns have cardiac defects[1]. Conotruncus (CT) of embryonic heart goes through a series of complex rearrangements to the magistral vascular field building for a very short term[2]. Namely short duration and complexity of histogenetic transformation of this region of the embryo heart to do this cardiogenesis stage such a critical, to be influence of pathological factors. Dehydration accompanied by a number of diseases of the first trimester of pregnancy, like preeclampsia and extragenital pathology. But it isn’t known influence of the maternal dehydration on the embryo cardiogenesis today[3].

2. Materials and Methods

There were compared the main histogenetic transformation of conotruncus region of embryo mouse heart at normal condition and under the maternal during of our research. There were used 317 embryo mice (C57BL/6) hearts at normal development and 118 under the maternal dehydration, which were obtained by PP “Biomodelservice”, Kiev and the prenatal period lasted from 10-th to 15-th embryonic days (ED) or 16-24 stages by K. Theiler[4]. We used the hyperosmolare model of maternal dehydration[5]. We used the complex of histological, histochemical, immunohistochemical and morphometric methods. There were created 3-dimenshional models of aorticopulmonary complex and septum, pharyngeal arches, swelling of the truncus and ridges of the conus. We carried
out biometrical and statistical analysis\(^6\). Immunohistochemistry reaction was conducted using visualization system LSAB (Labeled Streptavidin Biotin) (Lab Vision). This research conducted and interpreted on the base of Diagnostic center of Dnipropetrovsk under the supervision of professor, d. med. s. I. S. Shponka.

**Fig. 1**: Histological slice of embryonic heart, 12\(^{th}\) day of gestation, colored by iron hematoxilin, \(\times 100\). B-F – enlarge fragments of this slice. B, C – \(\times 400\). D, E, F – \(\times 1000\). The arrow signs the area of frontal slice of myocardial cuff.

3. Results and Discussions

The researches showed that transformation of CT region begins with migration of neural crest cells. So, it was initiation the septation of conus and truncus regios. Neural crest cells were detecting by the marker of glial fiber acid protein. The pericyticular space of these cells had the positive expression of present marker on the 10\(^{th}\) and 11\(^{th}\) days of gestation. The degree of expression of this marker was indicating in the cranio-caudal direction. Next day the septation of CT continued with the formation of aorticopulmonal septation complex (APSC). Neural crest cells population identified with condensed mesenchyma in present-day date\(^7\). The truncus fraction of this mesenchyma formed the concentrically sides, but in the conus ridges this sides had the eccentrically one (fig. 1).

To the definition of the neural crest cells migration we used the marker of triplets of
neurofilaments (NF). This marker showed the some degree of expression among cells from the cerebrospinal ganglion on 10th embryonic day. But on 12th day of embryogenesis NF+ there were detect these cells on the migration ways from the 4th and 6th aortic arches to aortic and pulmonary channels (fig. 2).

![Fig 2: 3-dimentional models of conotruncus and right ventricle at normal condition during 11th (A, B) and 12th (C, D). A, C – front projection; B, D - back projection. There is to indicate IV and VI aortic arches.](image)

The reaction of this marker was negative among mesenchymal cells. There was low level of expression of this marker among the subendocardial space of newly formed vessels. Septation complex was look like a horseshoe-shaped structure next day. It consists from the top, core and two prongs, left and right. Septation continued by wedge-shaped dives this prongs in same truncus swellings. So, the septation complex moved in cranio-caudal direction. It’s top down to core and divided truncus and then conus by the zipper type on two channels. Septation restructuring of CT at normal condition was comparing with experimental one and it was define some features. Firstly, it was observe constriction of APSC and elongation of its prongs (fig. 3). Secondly, cardiac jelly of truncus swelling was reduced near the myocardial cuff. Hence it wasn’t changes of quantitative indexes of mesenchyme of CT under the maternal dehydration.

The myocardialisation of the structural components of CT accompanied its septation and detected by forming myoid complexes. The quantitative of these complexes was growing in cranio-caudal direction too. There were showed all stages of myocardialisation and arterialization on the 12th day of gestation. Myocardial cuff and subendocardial stages were complete, but began arterialization of walls of great vessels. It consists of two periods, like septation and subendotelial. The phasing of this process was determined by the detecting a-SMA+ cells population. The same process of the conus ridges occurred in two stages during the 13th embryonic day. Trabecular stage ended, but subendocardial has just begun. Arterialisation of conus ridges didn’t occur. It was detect on all stages some elongation of
myoid complexes during the maternal dehydration (fig. 4). These changes of complexes phenotype probably were caused by changes of physical and chemical properties of cardiac jelly of endocardial structure of CT during the experimental condition.

It was set, that CT shortened almost half during its histogenetic transformations. This fact is confirmed in the data of the modern literature, but the reason remains an open question[8]. We suggested that this reason may be reduced proliferative activity of nucleus of some cells populations of CT structural components. In particular it was set, that nucleus of myocytes of myocardial cuff decreased in proliferative activity compared with the corresponding index of right ventricle. Myocardial cuff is delayed in growth, thus inhibiting CT elongation.

Rotation of this region of the heart began on the 10th day of gestation with formation of CT bend. This bend had valve function, because it was the primal valve structure. It formed conditional CT branch on the border between the conus and truncus. CT region of embryonic heart begin to rotate in counterclockwise direction during 11th embryonic day. Rotation continued until 13th day of gestation. Thus the angle of rotation the aortic channel about the pulmonary was 32.3±3.8º.

We showed maximum changes of quantititative parameters when it compared dates of normal rotation restructuring with experimental one. Namely the rotation angle of CT was increased significantly on 20.1% relatively normal condition. These changes were confirmed fact of steeper turn the aortic channel about the pulmonary. It was the presents of veracious changes of CT rotation restructuring of directed us to a more detailed consideration rearrangements after CT rotation under the maternal dehydration.

There were signs of the subaortic stenosis, which was forming during 13th day under the experimental condition. This state accompanied by the atrium dilatation (fig. 5). There were extension of the right coronary artery diameter twice and reducing the area of the exit aortic vestibule. Reason for this may have expansion of interventricular septum and myocardial fibrosis of left ventricle.
Fig 4: Horizontal histological slices of conus (A, B) and truncus (C, D) at normal (B, D) and experimental (A, C) condition, on 10th embryonic day. B, C – colored by iron hematoxilin, ×1000. A, D – by Stidmen, ×400. There is to indicate the myoid complexes.

Fig 5: Embryonic hearts at normal (A) and experimental (B) condition.

4. Conclusions
1. Conotruncus region of embryonic heart veracious enlarges on 56.7% and than it becomes shorter on 42.9% (p<0.05). Transformation of this region include the septation, rotation, formation a conotruncal branch, myocardialization, arterialization and definitive development of there derivatives.

2. Swelling of truncus are transforming in subvalvular part of aorticopulmonary septum and semilunar valves. Ridges of conus-in supravalvular part of aorticopulmonary septum and ventromedial part of interventricular septum.

3. Steeper turn the aortic channel about the pulmonary one to course by formation subaortic stenosis and extension of the right coronary artery under the maternal dehydration.

3. References