CARDIAC MALFORMATIONS INDUCED AFTER "IN OVO" ADMINISTRATION OF OP-1 TO THE OUTFLOW TRACT IN THE DEVELOPING CHICK

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The trunco-conal region constitutes a complex segment of the developing heart that gives rise to the outflow tract of the ventricles and the root of the pulmonary and aortic arteries. Several morphological mechanisms are involved in this process, including the remodelling of the myocardial layer by cell death, the transformation of endothelial cells into mesenchyme, the selective growth of the cushion tissue mesenchyme to form the trunco-conal ridges, the fusion of the paired trunco-conal ridges, and the differentiation of the cushion tissue mesenchyme into the wall of the root of the great arteries (Hurle et al., 1990). However, possible cellular and molecular mechanisms involved in the early steps of the formation in the outflow tract have received little attention. Although a number of defined growth factors are expressed in the heart, their role in cardiac growth and/or morphogenesis is largely unknown. Bone morphogenetic proteins (BMPs) are a family of secreted signaling molecules which belong to the transforming growth factor beta superfamily, originally identified by their ability to induce ectopic cartilage and bone formation. Furthermore, recent studies have demonstrated OP-1, osteogenic protein-1 (BMP-7), to be implicated in additional morphogenetic processes. For example, it acts as an inducer of nephrogenesis, and required for eye development and skeletal patterning (Lyons et al., 1995; Lou et al., 1995).

In the present study we analyze the effect of "in ovo" administration of OP-1 during the morphogenesis of the outflow tract of the chick embryo heart.

Material and Methods

Experimental desing

Heparin acrylic beads (Sigma) were washed in PBS three times for 10 min and then soaked with BMP-7 (OP-1) at concentration ranging from 0.25 to 1 mg/ml. After 2h, beads were implanted "in ovo" to the anterior face of the trunco-conal region in the developing heart of chick embryos at stages 12 to 20 (Hamburger and Hamilton, 1951). Control embryos were obtained after implantation of beads soaked with PBS alone. After 2 to 7 days, incubation was stopped and embryos were videotaped and photographied before fixing (Figure 1).

Scanning Electron Microscopy

Several control and experimental embryos were fixed in 3% glutaraldehyde in 0.1M cacodylate buffer. After 24 h in fixative, the embryos were subsequently cut at informative planes with a fine scalpel blade, processed for scanning electron microscopy and examined with a Jeol T-100 scanning electron microscope.

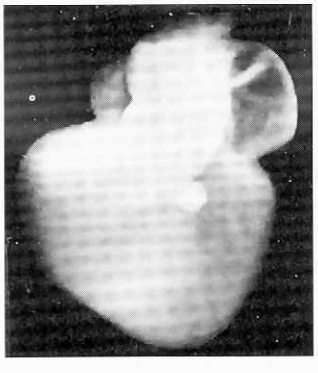

Figure 1.- Control embryo before fixation at stage 27 showing the location of a PBS heparin bead (implanted at stage 14) at the level of the atrioventriculoconal sulcus. x 25.

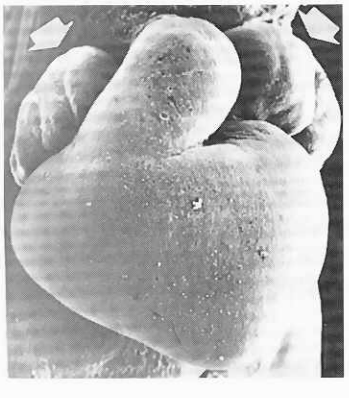

Figure 2.- Scanning electron micrograph of a control heart at stage 27. Arrows show the left and right atria. x 30.

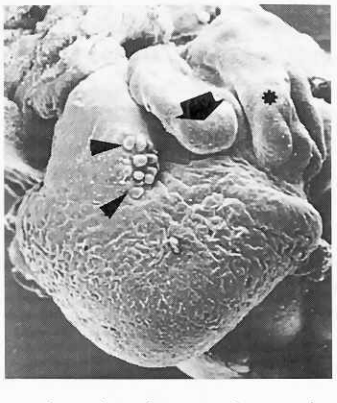

Figure 3.- Scanning electron micrograph of an experimental embryo at stage 27 showing the spherical protuberances at the level of the outflow tract (arrowheads). Note the presence of left (asterisk) and right (arrow) atria on the left side of the outflow tract. x 30.

Figure 4.- Scanning electron micrograph of an experimental embryo at stage 35, showing the ventricular septal defect (arrow) at the level of the membranous interventricular septum. x 18.

Light microscopy

The rest of hearts were treated in one of two ways. Some hearts were fixed in 4% formaldehyde, dehydrated in ethanol, embedded in paraffin and cut into sections 7-10μm thick. Deparaffinized sections were stained with hematoxylin-eosin. In a second group, small fragments of the outflow tract were fixed in 3% glutaraldehyde, dehydrated in a series of acetones, cleared in propylene oxide and embedded in Araldite. Serial semithin sections stained with 1% toluidine blue were employed for light microscopy.

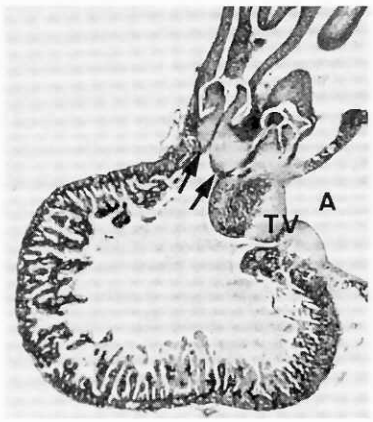

Figure 5.- Longitudinal section of an experimental heart at stage 34 after hematoxilyn-eosin staining showing the double outlet right ventricle (arrows). Note the inmature muscular trabeculae of the myocardial ventricular mass. TV: Tricuspid valve. A: Atrium. x 15.

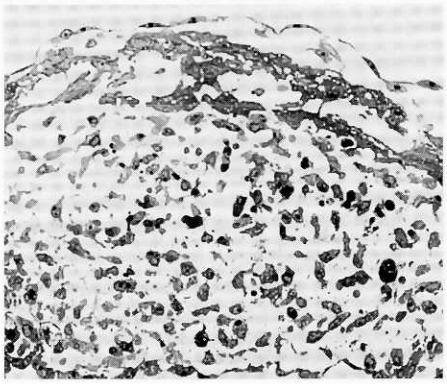

Figure 6.- Semithin section of the outflow tract at stage 26 showing intense areas of cell death at the level of the mesenchymal tissue. x 200.

Results and Conclusions

Our results show that after implantation of OP-1 (but not control) beads, a series of abnormalities is induced in the developing heart. In contrast to the morphology of control hearts (Figure 2), the OP-1 treated hearts have spherical protuberances in the lower part of the outflow tract, just where the beads were implanted (Figure 3). In addition, the atria were increased in size, and located on the left side of the outflow tract (Figure 3), instead of occurring bilaterally. The internal configuration of the OP-1 treated hearts show a ventricular septal defect, affecting the membranous portion of the interventricular septum (Figure 4), combined with either partial or total double outlet right ventricle (Figure 5). Furthermore, paraffin sections show a significant retardation in growth and maturation in the muscular trabeculae of the myocardial ventricular mass (Figure 5). Semithin sections show intense areas of cell death, mainly located at the level of mesenchymal layer of the outflow tract (Figure 6).

The intrinsic mechanisms implicated in the alterations produced by administration of OP-1 are difficult to elucidate from this study. Cell death and degeneration in the embryonic heart have been correlated with the morphogenesis of cardiac defects (Pexieder, 1975). It is possible that the intense areas of cell death observed in our experiments after OP-1 administration may be responsible of the heart malformations. Additional experiments to ascertain the role of OP-1 in cardiac development are underway.

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