IV. PEDIATRIC SURGERY

LOCAL EFFECTS OF GROWTH HORMONE ON INTACT CONDRO-COSTAL CARTILAGE

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Abstarct

Pectus excavatum is the most common chest wall deformity seen in children.

We are trying to develop a new nonsurgical method of correcting the deformity.

The local and systemic effects of human growth hormone (Norditropin) injected at the surface of intact condro-costal cartilage in 9 two weeks old Sprague Dawley rats were investigated.

The cartilages were injected every two days for 21 days.

Every second day one of the subjects was sacrified and the injected condro-costal cartilage site, tibial bone and mandibula were histologicaly analyzed.

At the level of the tibial bone and mandibula no systemic effects of the growth hormone was found.

Histological analysis confirmed the presence of ossification and calus formation at the site of injection and modified shape of the cartilage.

A local increase in periosteal bone deposition and new bone formed was found after transversal sections of the condro-costal cartilage were performed.

Further studies will conclude if the local injection of growth hormone in the cartilage will open a new gate in the treatment of this malfomation.

Keywords: condro-costal cartilage, periosteal bone, pectus excavatum

Introduction

There have been a great variety of methods tried for the correction of funnel chest, but all of them can be divided in two main categories: the lifting or the overturn of the sternal bone. In both procedures recurrency is the most common complication by far, and it is determined by insufficient fixation of the sternum or by deterioration of the local blood flow.

However, there have been many procedures –50 – until nowadays, often very ingenious, it has not been found the best solution yet. Actually, the problem is not the elimination of pectus excavatum, but the maintenance of the newly created form after the surgical procedure. The sternum must be adapted for sustaining all the forces that appear – those resulted by respiratory movements, as well as those determined by growth – thus the stability of mechanism not to be put in danger at any time.

The self- repair capacity of the cartilage is poor. If there is a lesion on the cartilage and repair is begun, then this is done only by the perichondrium and usually only during the growing process, in young patients. In an adult, tipically, cells of the perichondrium proliferate to initiate repair, but proliferation is poor, resulting a few, insufficient cells. In this case, the process ends up with the production of merely dense connective tissue. However, not rarely, in adults, the development of new blood vessels at the site of healing enhances the growth of bones rather than that of cartilage. The limited capacity of the cartilage to self- repair may cause great difficulties for the surgeon, when the rib cartilages have to be cut in order to get into the thorax cage for a by-pass procedure or a sternochondroplasty, for instance.

At the site of repair**,** bone is produced instead of cartilage because chondrocytes spread in this area are not properly oxygenated.

The local and systemic effects of human growth hormone (Norditropin) injected at the surface of intact condro-costal cartilage in 9 two weeks old and 9 mature Sprague Dawley rats were investigated.

The aim of the study is to investigate the possibility to replace the surgical correction of pectus excavatum is a new non surgical method by local injection of human growth hormone at the surface of intact condro-costal cartilage.

Material and method

The present study was conducted at the "Pius Branzeu " Center for Laparoscopic Surgery and Microsurgery in Timisoara.

The histological analysis of the condro-costal cartilage was conducted at the Department of Pathology at

the Children Hospital "Loius Turcanu ", Timisoara, Romania.

The animal experiments were conducted on two weeks old Sprague Dawley rats in total a number of 18 subjects.

There are several reasons why we choose to use the Sprague Dawley rats a few of them beeing the fact that, it is an accepted animal model,the reproductive rate of these animals is high and last but not least the similarity with the humans.

Two groups were used each with 9 subjects as following; the first group received a human growth hormone (Norditropin) injection at the surface of intact condro-costal cartilage and the control group.

The rats were caged with 12 hours light and 12 hours darkness, and had free access to tap water and pellet food.

The drugs used in this study were Norditropin™ PenSet® 12 manufactured by NovoNordisk.

The rate of the injection of these substances was every two days for 21 days.

The first step was the anesthesia of the animals this beeing done with a mixture of Ketamin (for human use) and Xylazin (veterinary use) administered for each individual with regards to body weight.

The animal was them placed on the operative desk and the furr was shaved from the anterior thorax.After a previous cleaning of the chest skin surface with a Betadine® solution a 1.5-2 cm incision was performed and the muscular and bony structures of the chest were revealed.

The surface of condro-costal cartilage was reached after a carefull disection of the pectus major muscle on the right side using microsurgery fine tools and the microscope.

We delivered the hormone product at the desired site using a insuline seringe of 1 ml and the aid of the microscope.At this stage I would like to mention the fact that a great amount of pacience and skill is required to inject the dilution right at the condro-costal cartilage site since in two weeks old rats this structure is quite hard to reveal.

At the end of the procedure the skin of the chest was closed using a 4.0 coated Vicryl® suture.

Every second day one of the subjects was sacrified and the injected condro-costal cartilage site, tibial and femur bones and mandibula was harvested for histologicaly analysis.

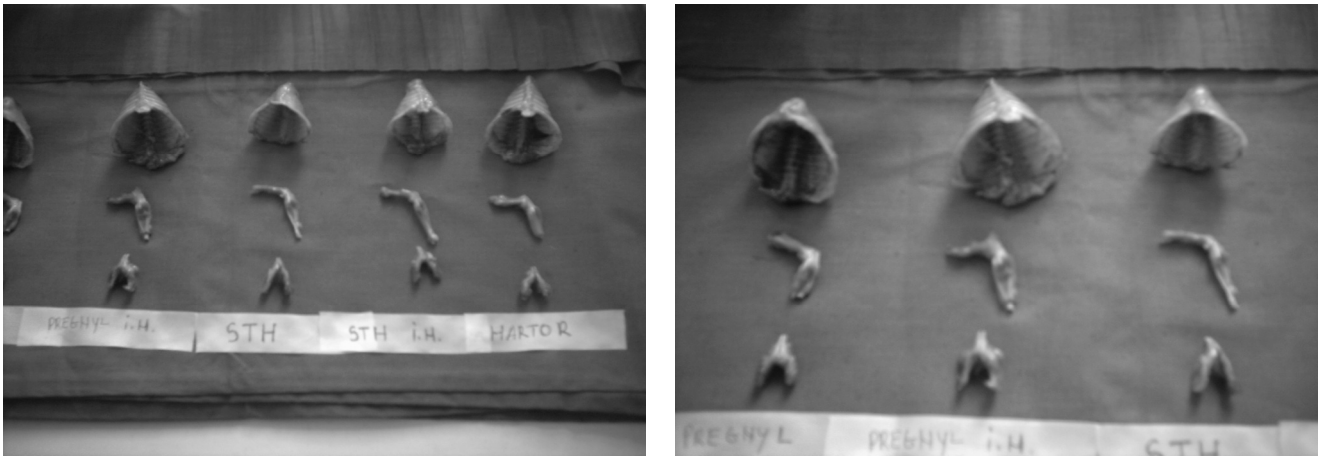

Fig.1 The thorax,femur and mandible harvested from the rats at the end of the study for histological analysis.

Subsequently, 3.5-um-thick sections were cut perpendicular to the longitudinal direction of the bone using a microtome.

The tissue formed during the injection period was identified using a trichrome stained sections (light microscopy) and unstained sections (polarization microscopy).

Results

It is well known that systemic administration of growth hormone (GH) increases diaphysial bone mass by periosteal bone formation in both young and old rats.

The aim of the study is to investigate the possibility to replace the surgical correction of pectus excavatum is a new non surgical method by local injection of human growth hormone at the surface of intact condro-costal cartilage.

At the injected site on the right side of the thorax, the doses of GH induced new bone formation at the periosteal surface, and the responses were located at the medial and lateral surfaces corresponding to the areas where GH was injected.

Local GH treatment of the right hemithorax did not influence the left hemithorax bony structures dimensions, volume, length.

At the GH-injected location, increased external bone dimensions were seen, and the responses to rGH was noted to be dose dependent. The new bone formed at the periosteal surface was woven bone.

External callus dimensions at the site of the administration of hormones was increased in the GH group compared with the control group, whereas body weight changes during the healing period were also present.

The light microscopy aspects of the cartilage correspond to those found in electronic microscopy and are suggestive for the adaptive nature of both chondrocytes and matrix to mechanic injuries, inflammations, hypoxia.

The obtained modification were similar to those reported in the case of the study of the cartilage in pectus excavatum.

Particulary similar patternes were seen since in both cases we deal with a form of hyperthrophy.

Electron microscopy study of rib cartilage in pectus excavatum reveals particular aspects of cartilaginous matrix components as well as of cellular ones (chondroblast –chondrocyte).

Thus, the cellular lacuna is covered with an osmiophil material (MOs). Around the lacunae there are collagen fibers (Fs) and extracellular material with calcium (Ca) deposits. In contrast to the matrix from the connective tissue of the perichondrium, the cartilage matrix (Mx), with the exception of calcium deposits, looks homogenous in a lower power view, containing fine fibers (5-20μ). These fibers are collagen type 2 with smaller diameter and less obvious periodicity compared to collagen type 1.The free spaces of the matrix network are irregular and large. Lipids (L) and secretion granules accumulate at the periphery of the cartilaginous cell.

Chondrocytes have an irregular cellular surface, presenting microvili (Mv), and are covered with matrix with a fibrillar aspect. These surfaces covered with microvili account for the resorptive activity of the chondrocyte.

Being a poorly nourished tissue, cartilaginous cells contain lipid and rich glycogen inclusions within their cytoplasm. This aspect is common in chondrocytes in young tissues as well as in degenerated cartilage, a fact we could also confirm in the control group**.**

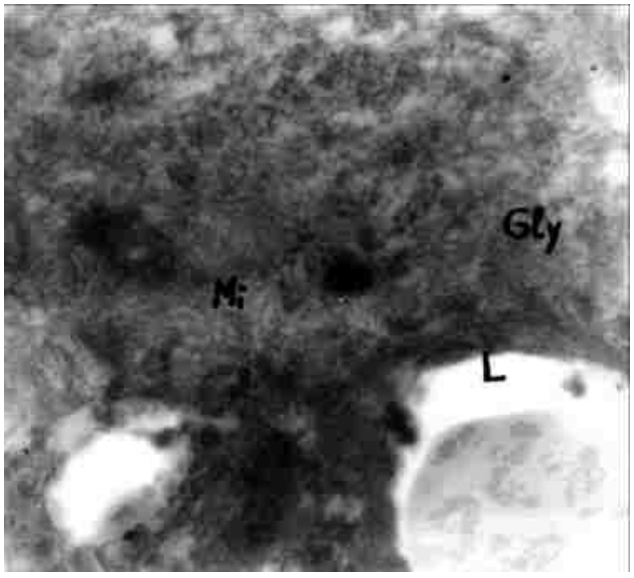

Fig. 2 Lipids and glycogen deposits in the cytoplasm of a chondrocyte; density of mitochondrias within the cytoplasm diferres from one territory to another; x 4000.

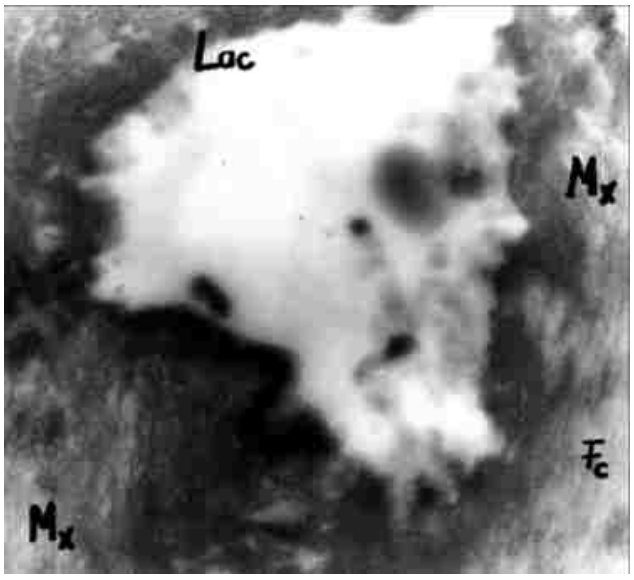

Fig. 3 Ultrastructural aspect of a lacuna (lac), surrounded by territorial matrix (Mx) and condensed colagen fibers in perilacunar capsule; this lacuna contains an electrodense waste material after chondrocyte degradation; x 12930.

A more detailed histological analysis of the ribs and sternum togheder with the tibial bone and the mandibula is still in progress and futher results will be available in a short amount of time.

Discutions

Endochondral bone formation during vertebrate genesis is a highly regulated process resulting, in the case of long bones, in increased length. [1]

During this process,young chondrocytes initially undergo rapid proliferation; then cease proliferation to become mature chondrocytes, producing a large amount of extracellular matrix, and subsequently become hypertrophic.

At the stage of hypertrophy, the cells exhibit a number of changes (Nurminskaya and Linsenmayer, 1996), including de novo synthesis of collagen type X. [1]

These events change the composition and, conceivably, the properties of the cartilage matrix in the hypertrophic zone (Chen et al., 1992), allowing the invasion of blood vessels and the ultimate replacement of the cartilage matrix by bone. Thus, the proper control of chondrocyte development, i.e., appropriate regulation of cell proliferation and the subsequent differentiation to hypertrophy, is of critical importance to the formation of a normal bone. [1]

Studies of transgenic mice with local GH expression in either the osteoblasts or the erythroid tissue of

the bone marrow have revealed increased tibial and femoral cortical thickness in the transgenic animals. [1]

This strongly indicates a local effect of GH on intact cortical bone, although the transgenic mice lines developed in these experiments also showed increased weight gain
and linear growth.

The GH receptor has been found on osteoblast-like cells, and in vitro experiments have shown that GH directly induces proliferation in a number of osteoblastic cell lines. However, this proliferative activity induced by GH can be abolished if antiserum to IGF-I is added to the cell system.GH also stimulates the production of type I collagen, osteocalcin and alkaline phosphatase in osteoblastic cells. [1]

In two previous experiments, the local effect of GH on healing bone defects have been investigated by different authors.

In rat mandibular defects covered with osteopromotive membranes, GH was applied locally by mini-osmotic pumps. [1]

The defects were analyzed after 4 weeks by semiquantitative histomorphometry, and the results revealed an enhanced bone formation (bone union, bone maturity) when GH was given in doses of 2 and 20 ug/day, respectively. [1]

Macroporous biphasic calcium phosphate implants loaded with GH were inserted into rabbit bone defects located at the distal end of the femurs.

After 3 weeks, a dose-dependent increase in bone ingrowth and ceramic resorption was found in the GH-loaded implants.[1]

A number of studies with regards to the effect of the human growth hormone on the costal cartilage are ongoing at the monent and there is a clear indication that this form of theraphy will someday be able to substitute extensive surgical procedures specially in the field of orthopedics.

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