PRELIMINARY DATA REPORT REGARDING THE EFFECT OF GROWTH HORMONE ON RAT CHONDROCYTES AS A FUTURE ALTERNATIVE NON SURGICAL TREATMENT FOR PECTUS EXCAVATUM

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INTRODUCTION

The surgical repair of pectus excavatum (PE) in childhood is a well-established procedure. Previously used operative techniques for the correction of PE were largely based on the Ravitch technique.¹ In order to avoid the relapse of the thorax after surgery, there are different procedures used for sustaining the thorax (metal boards, Steinmann nails, Kirschner threads etc.) and many other protocols, both interesting and innovative such as the sternum turnover technique proposed by Wada and the absorbable poly-L-lactic...
board for sustaining the sternum presented by Matsui and Kitano. Despite the large number of procedures, 50 so far, often very ingenious, the best solution is yet to be found. In 1999, Donald Nuss proposes an entirely new technique that does not require chondro-costal cartilage resection for the correction of PE and comes one step closer to the ideal surgical method of treatment of the condition, being nowadays considered the golden standard. The Nuss technique is minimally invasive, but it requires sternal fixation for at least 24 months. Any procedure has a bigger or lesser risk of recessive postoperative pectus excavatum. Choosing one method should be based on recurrence risk as well as on the potential complications of the procedure. Because of the surgical complications and because not all techniques can offer a desired result for the patient a great focus is nowadays given to the development of new alternatives to surgery.

MATERIAL AND METHOD

The present study was conducted at the “Pius Branzeu” Center for Laparoscopic Surgery and Microsurgery in Timisoara. A great deal of work has been conducted with the help of the Department of Pediatric Surgery at the “Luis Turcanu” Children Hospital, Timisoara, and with the help of the Hospital’s Laboratory, where the lab analysis of the collected blood were performed. All the conducted experiments on animals were conducted accordingly and with the approval of the Commission for Ethics and Animal Rights of the “Victor Babes” University of Medicine and Pharmacy Timisoara, following the Guide for the Care and Use of Laboratory Animals – National Research Council (1996).

A number of 16, two-week old rats divided into two groups, an experimental (E) and a control (C), were included in the study. We chose to use two week old rats because of the proliferating property of the cartilage at this age. The rats from the experimental group (E) received a human growth hormone (hGH), NorditropinTM PenSet, injected in the surface of intact chondro-costal cartilage of the right hemithorax, at two-day interval, for 21 days.

Animals and local hormone administration

The rats were anesthetized during the procedure with a mixture of Ketamin (human use) and Xylazin (veterinary use), administered in an individualized dose, calculated in correlation with the body weight. The animal was then placed on the operative table.

Figure 1. Placement of the rat on the operative table (A), followed by the exposure of the chondrocostal cartilage (B), injection of the human growth hormone, closure of the site of surgery (C) and then at 21 days harvest of the bony thorax (D).
and the anterior thorax was shaved. After a previous cleaning of the chest skin surface with a Betadine® solution, a 1-1.5 cm incision was performed. The surface of chondro-costal cartilage was reached after a careful dissection of the muscles and the surrounding tissue using microsurgical fine tools and the microscope. (Fig. 1)

The human growth hormone (hGH, dosage 0.05 IU hGH/kg-body weight) dissolved in physiological serum as vehicle was injected into the cartilages using an insulin syringe (100 IU). This operation was rather difficult because of the size of the chondro-costal cartilage at the two weeks old rat, despite the great help provided by the use of the microscope. Great deal of attention was given to the administration of the human growth hormone in the cartilage and not in the surrounding tissue, from where it could theoretically diffuse and have a systemic effect.

The surgical wound at the end of the procedure was closed using a 4.0 coated Vicryl® suture. (Fig. 1) The rats were caged and tagged, being held in a 12 hours light and 12 hours darkness condition environment and had free access to tap and pellet food. During the 21 days of the study all individuals were measured, weighed, every two days and kept under observation for any possible modifications that could occur. At the end of the study the rats were anesthetized, as described previously and sacrificed by exsanguinations. The rib cage with the sternum was freely dissected with special care being given to the injected cartilage. The tibia, femur and the mandible were also harvested and stored in buffer 10% phosphate formalin for future analysis. (Fig. 2)

Figure 2. The harvested bony thorax, femur bone and mandibula for comparison in two subjects of the group injected with human growth hormone (A, B) and a subject from the control group (C). The tissue samples were obtained after dissection and were preserved in buffered formalin before being embedded and sectioned for histological analysis.

Hormone concentration analysis

The blood concentration of growth hormone was monitored with the aid of blood samples taken before administration of the hGH and one hour after the procedure using a RIA kit for hGH, (DRG Instruments GmbH, Germany). The blood samples were obtained from the femoral vein, after an inguinal incision and puncture of the vessel.

Histological analysis

The chondro-costal cartilage was separated from the rib cage and then embedded in methacrylate, in order to be sectioned. Subsequently, 5 μm thick sections were cut in a perpendicular and longitudinal direction of the cartilage using a microtome. (Leica Instruments GmbH, Germany)

The tissue formed during the injection of the human growth hormone was identified using a hematoxilin-eozin staining. (Fig. 3) The specimens from the (E) group were then compared with the samples from the (C) group in order to determine the modification that may be the result of the local injection of hGH.

Figure 3. Up: Longitudinal sections in (E) group showing a thickened periosteal surface pointed out by the right arrow and a defect in the continuity the rib (left arrow), produced by the injecting needle. Down: The normal image obtained from a (C) group rat. The chondrocyts appear to be healthy and there were rarely signs of the chondronecrosis.

Statistical analysis

The mean and standard deviation together with the probability value and t for the body weight and
the hGH concentration were calculated and compared by means of Student’s t test and ANOVA for multiple mean analysis (SPSS 6.1 for Windows, SPSS Inc. Chicago, USA).

RESULTS

There were no significant differences regarding the body weight between individuals that received the human growth hormone (E), and the control group (C). Based on the measurements that we did during the study, mean weight of the rats in the experimental group (E) was 26.12 ± 25.52 grams vs. 22.87 ± 34.98 grams for rats in the control group (C), with, a two-tailed probability value of 0.7953 and t = 0.26 (not significant). The results underline the fact that the hGh had no systemic distribution, based on the direct results of growth hormone administration (weight gain) and that the local injection was able to target only the cartilage.

The measured hormone levels of the human growth hormone one hour after local injection were significantly increased in the experimental group (E). During 21 days of the study, 3 measurements were performed at 7 days intervals, for all the rats from both groups. At the first measurement the mean value of the hGH for the (E) group was 61.14 ± 35.87 μU.I./ml vs. 0.15 ± 2.56 μU.I./ml in the (C) group, with a p value of 0.003 and t = 3.73. These results clearly indicate the fact that hGH levels were significantly increased in the (E) group rats. The second measurement showed 41.18 ± 41.68 μU.I./ml for the (E) group vs. 0.020 ± 0.024 μU.I./ml for the (C) group with p = 0.14 and t = 1.65. The third measurement, provided the following data: 0.53 ± 1.36 μU.I./ml for the (E) group vs. 0.27 ± 0.37 μU.I./ml for the (C) group with p = 0.81 and t = 0.24. The second and the third determinations show that the hGH levels returned to normal range in the (E) group rats. These results, as well as body weight values, are strong indicators that systemic distribution of the hGH was not present.

Regarding the local effect of the injection of growth hormone, histological analysis confirmed the presence of calus formation at the site of injection and modified shape of the cartilage observed at gross inspection. A local increase in periosteal bone deposition and new bone formation were found after sections of the chondro-costal cartilage were performed. At the injected chondro-costal cartilages, the hGH induced new bone formation at the periosteal surface and the responses were located at the lateral surfaces corresponding to the site where the hormone was injected. Local hGH treatment of the cartilages on the right hemi thorax did not influence the left hemi thorax bony structure parameters, assessment made by gross inspection. When comparing the chondrocytes from both groups, there was marked hypertrophy in the (E) vs. (C) rats.

From a macroscopic point of view, the changes obtained were not significant and did not mimic the modifications reported in the cases of pectus excavatum.

The local effects of the growth hormone were based on the presence of the growth hormone receptor at the level of the cartilage previously demonstrated by other investigators. GH receptors have been found on osteoblast-like cells, and in vitro experiments have shown that GH directly induces proliferation of such cell lines. GH, also stimulates the production of type I collagen, osteocalcin and alkaline phosphatase in osteoblastic cells.

DISCUSSION

Many conservative alternative non surgical methods of treatment for the PE have been imagined nowadays. Conservative treatment with the aid of a vacuum bell, to elevate the funnel in patients with PE was introduced by Haeker et al. (2006). A suction cup is used to create a vacuum of the anterior chest wall. A patient-activated hand pump is used to reduce the pressure up to 15% below atmospheric pressure. Three different sizes of vacuum bell exist which are selected according to the individual patients age. When creating the vacuum, the lift of the sternum is obvious and remains in that position for a different amount of time. The device should be used, for a minimum of 30 minutes, according to Haeker et al. and may be used up to a maximum of several hours daily. In addition, the device was used intraoperatively during the MIRPE procedure (Nuss) to enlarge the retrosternal space to ensure safer passage of the introducer bar in a few patients.

As PE is a condition determined by an excess growth of the modified chondro-costal cartilage, a new approach could be to try to control the growth of this structure using substances that may interfere with its abnormal growth pattern. By controlling the growth patterns of the cartilage, the severe carinatum and excavatum deformities could be successfully prevented by applying early detection and treatment in a local manner targeting the chondro-costal cartilages. Nowadays, it has been recognized that receptors for many hormones such as estrogen, growth hormone...
and glucocorticoids are present in or on the growth plate chondrocytes, suggesting that these hormones may influence processes in the growth plate directly.\textsuperscript{11} Moreover, many growth factors like IGF-I, Indian hedgehog, parathormone, fibroblast growth factors, bone morphogenetic proteins and vascular endothelial growth factor are now considered as crucial regulators of chondrocyte proliferation and differentiation.\textsuperscript{11}  

Andreassen et al. investigated the local effects of rat growth hormone (rGH), injected at the surfaces of intact tibial diaphyses and healing tibial diaphysial fractures in 10-month-old female rats.\textsuperscript{12} At the rGH-injected location, increased external diaphysial bone dimensions and increased calcine-labeled area were seen the responses to rGH being dose dependent.\textsuperscript{12} According to Andreassen et al., the new bone formed at the periosteal surface was woven bone. At the opposite left tibia, no systemic effect of rat growth hormone (rGH) was found; furthermore no influence on body weight or muscle mass was found during the treatment.  

In our study we did not measure the response of the chondro-costal cartilage in a dose-dependent manner, the doses administered to the rats from the (E) group were the same in regard with the IU/gram body weight ratio.  

The results that we obtained from this study show that the injection of the hGH in the cartilage does not produce a dramatic effect that will in the near future eliminate the necessity for surgical correction of PE. However, we are aware that it can be very helpful in different techniques of pectus excavatum correction that use stabilizing bars. By injection of the growth hormone in the cartilage, the bony thorax can achieve a much quicker stability after surgery and the time necessary for the maintenance of the metal bar to support the sternum can be reduced significantly.\textsuperscript{13}  

We consider that further investigation of the effect of hormones on the cartilage correlated with a better injection method and the use of rGH, for the rat animal model, could provide an increased effect vs. the use of hGH and may reveal new answers opening the gate to new alternative therapies for conditions such as pectus excavatum.  

REFERENCES