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Remodelling Through The Growth Plate- An Experimental Study In Rats

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Abstract

The ability of the skeleton to remodel itself has long been recognized and studied. There has been great controversy among scientists as to whether remodeling takes place mainly in the fracture site or in the growth cartilage. In this study the activity of the two halves of the growth plate of 56 laboratory rats is measured and compared and it seems that the half that is adjacent to the concave side of the fracture shows increased activity as compared to the opposite part.

Background

During the last two decades great progress has been made in understanding the development, growth and structure adaptation of the human skeleton in genetic and molecular level. Several studies suggest that remodeling occurs mainly at the fracture site while others support that the growth plate is the major contributor to remodelling (1-15).

The growth plate is known to play major role not only in bone growth but also in fracture healing by acceleration of its cellular activity in comparison to the normal side.The different parts of the growth plate show unequal activation that has been studied by cartilage angulation measurements (16). No reports in literature measuring the cellular activity in different parts of the growth plate cells after fracture are available and this is the purpose of this experiment.

Methods

Fifty-six white laboratory Wistar male rats three weeks old were used

after permission was taken from the Aristotle University of Thessaloniki. Under general anaesthesia with pentobarbitone 30mg/kg of weight assuming that the rats weighed an average of 100 gr, a fracture of the right femoral diaphysis was surgically inflicted after sterilizing the operative field with betadine solution. In a series of four rats it was possible to intramedullary nail the fracture using a curved k-wire and hold it in posterior angulation. All other fractures were left to heal freely and it seemed that due to the contracture of the posterior femoral muscles the fracture was distracted and assumed an anterior angulation of approximately 45 degrees at first that within days reduced to 25 degrees as shown in x-rays that were taken at time of surgery and sacrifice.

The rats were sacrificed at 48, 96 or 168 hours after the operation by medulla oblongata cross section after anaesthesia. Fourteen rats had to be excluded from the study because of clinical signs of infection. The femur was removed and cleared from the soft tissues in order to identify and isolate the growth plate .One left healthy femur of each group was used as control. With traction the growth plate was detached and prepared for histology or biochemical analysis

Biological analysis

Both side growth plates from twenty one rats were harvested with manual epiphysiolisthesis and separated in anterior and posterior halves. They were homogenized with TritonX-100 of Sigma Chemicals, centrifuged and prepared in a soluble form.

The DNA activity was determined indirectly using fluorometry as described by Labarca and Paiger (17).

Using the same samples the quantity of protein present was measured as described by Bradford (18). The measurement is based on the engagement of Coomasie Brilliant Blue G – 250 with the amino-acid Arg and the residues of aromatic unions of proteins.

We compared the content in dna/mg of tissue and protein/mg of tissue between the anterior and the posterior half of the growth plate in each group, that is at 48, 96 and 168 hours after the fracture and also contrasted all anterior halves to posterior halves regardless of time of sacrifice after the fracture. In all the measurements of the groups with the fracture in anterior angulation there is statistically important difference in the content of dna/mg of tissue as well as in protein/mg of tissue between the anterior and posterior part of the growth cartilage, the posterior side showing increased content of dna/mg of tissue and protein/mg of tissue compared to the anterior side.

We also contrasted the anterior halves to the posterior

halves of the growth cartilage of the femurs that were stabilised in posterior angulation 168 hours after the fracture In these measurements there is a statistically significant increase in the content of dna/mg of tissue as well as in protein/mg of tissue in the anterior side of the growth cartilage.

Histological analysis

The remaining twenty one right side femurs were prepared for histology -after fixation in formalin, demineralization in mirmicic acid and microcut coronal section and haematoxylin eosin stain and were studied for morphological differences between the two half-parts- anterior and posterior of the growth plate.

Statistical analysis was performed using the SPSS for Windows (v. 11.5) in order to determine the significance of the differences in dna/mg of tissue and protein/mg of tissue between the anterior and posterior half sides of the growth cartilage. The level of significance was set at $p \leq 0.05$.

Results

Biological analysis

At 48 hours after the fracture there seems to be a statistically significant difference in the content in dna/mg as well as in the content in protein/mg between the anterior and the posterior half of the growth plate, the posterior part showing increased content as compared to the anterior part.

At 96 hours after the fracture there is also statistically significant increase in the content of dna/mg of tissue and in the content of protein/mg of tissue in the posterior part of the growth plate compared to the anterior half.

At 168 hours after the fracture and for the femurs that healed in anterior angulation the content in dna/mg of tissue as well as the content in protein/ mg of tissue is again higher in the posterior part of the growth plate when contrasted to the anterior half of the growth plate showing a statistically significant difference.

At 168 hours after the fracture the femurs that where stabilized with a k-wire and healed in posterior angulation show the opposite results- in this series there is a statistically significant difference in the content of dna/mg of tissue and in the content of protein/mg of tissue between the two halves , the anterior half showing increased content compared to the posterior half.

In all fractures that healed in anterior angulation regardless of timing after fracture there is a statistically significant difference in the content of dna/mg as well as in protein/ mg of tissue, the posterior halve showing increased content in both.

Histological analysis

At 48 hours after the fracture and fivefold enlargement (X5) there is clear difference in the epiphysis of the fractured side as compared to the witness. At a x25 magnification there is increased thickness in the posterior surface of the growth cartilage. Growth cartilage and joint cartilage seem to have merging borders. At x 100 magnification there is a characteristic chondroblastic activity of cells and the chondrocytes appear in columnar structure.

At 96 hours after the fracture there is great proliferation in the x25 magnification in al the extend of the growth plate but at x100 magnification there is marked reformation with soap like cells in columns in the anterior part of the growth plate while in the posterior part mitoting cells present in columns.(photographs 1-3).

Discussion

The wonderful ability of the growing skeleton to remodel and correct fracture angulation has long been recognized and studied. Remodeling seems to occur not only at fracture site according to Wolff's Law (19) but also at the growth cartilage that alters its activity so as to correct the deformity.

In our knowledge the interphysial angle has been measured in order to prove the growth plate's contribution to remodeling but no quantified measurement of its activity has been published.

DNA presents an indirect proof of cell proliferation and protein is an indirect measurement of cell activity. In our study we were able to demonstrate that the side of the growth cartilage that is adjacent to the concave side of the fracture was more active than the side that was adjacent to the convex side probably in an early attempt of the body to correct the deformity of the displaced fracture.

Conclusion(s)

The growth plate alters its activity so as to remodel the angulation of the healing fracture.

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Illustrations

Illustration 1

Right femoral epiphysis at 48 hours-A:thinner anterior half,B:overactive posterior half

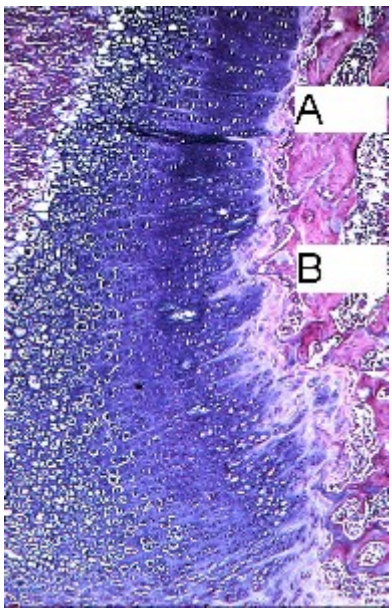


Illustration 2

Right femoral epiphysis at 96 hours-A:thinner anterior half,B:overactive posterior half

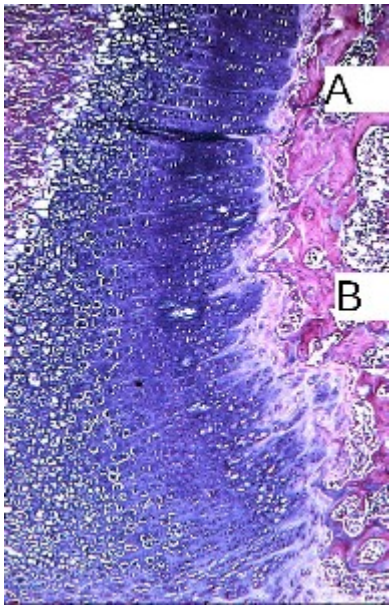
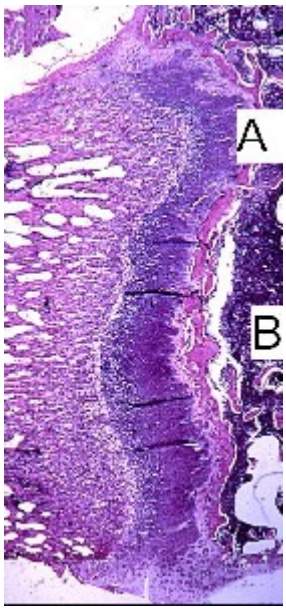


Illustration 3

Right femoral epiphysis at168 hours-A:thinner anterior half,B:overactive posterior half



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