Oral Supplementation of Chondroitin Sulfate to Facilitate Fracture Healing: A Pilot Study.

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Introduction

Fracture Callus formation occurs in a dynamic process. The initial hematoma provides a scaffold for the deposition of collagens types I, II, and III, glycosaminoglycans and proteoglycans. These proteoglycans are expressed in the extra-cellular matrix of the callus and comprise the main ground substance of this connective tissue.

Heparan sulfate, dermatan sulfate and chondroitin sulfate are three of the proteoglycans that are vital components of callus formation in the first to second week of fracture healing.

The first 9 days of callus
- chondrocytes produces chondroitin sulfate

By the end of the second week
- events involved in the production of cartilage switch off.

By the third week
- the amount of proteoglycans and their aggregates decreases and mineralization begins

Jackson et al. (2006):
- examined fracture healing using heparan sulfate.
- local application of 5g heparan sulfate to rat femoral fractures resulted in a significant increase in callus size

Rammelt et al. (2006):
- Implants coated with chondroitin sulfate facilitates bone healing.

We hypothesized that early administration of oral chondroitin sulfate will have similar effects on bone fracture healing and callus formation as the local direct application of chondroitin sulfate.

Materials/Methods

Eighteen male Sprague-Dawley rats
Mean weight 300g
The rats were randomized into six groups of three animals

	Experimental	Control
Group A	Anesthetized at 1 week	Anesthetized at 1 week
Group B	Anesthetized at 4 weeks	Anesthetized at 4 weeks
Group C	Anesthetized at 5 weeks	Anesthetized at 5 weeks

The eighteen rats were initially separated into experimental (#1-9) and control (#10-18) groups.
These were further subdivided into three groups of three rats each to be euthanized at 1 week (Group A), 4 weeks (Group B), and 5 weeks (Group C) time points.

All experimental rats were dosed once daily with a solution of 7mg Chondroitin Sulfate via oral gavage tubes.

The animals were dosed for nine days following the procedure (except for Group A animals which were sacrificed at 1 week.)

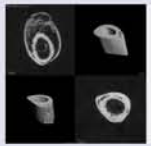
Animals were euthanized at their respective time points by CO_2 asphyxiation and the left legs were disarticulated at the hip joint.

Anesthetized using Ketamine and Xylazine.

After surgical dissection, a 1.6mm K-wire was inserted into the femoral canal. The distal end of the wire was cut to sit flush with the knee.

While still anesthetized, a blunt guillotine-like blade device was used to generate a transverse mid-femoral closed fracture.

Radiographs were immediately taken to confirm the fracture.



Micro CT Scanning

- The diaphysis of each femur was scanned via micro-CT, and 216 slices of the mid-diaphysis were obtained for each femur.

- CT Scan programming used to determine:
  - cross-sectional bone area
  - area of the periosteal envelope
  - area of the endocortical envelope
  - moments of inertia
  - transcortical thickness
  - cortical porosity
  - volumetric density of the mineralized tissue

Post Radiographs

- The femurs were arranged on an 11x17 large radiographic cassette and AP radiographs were taken.
- Radiographs were evaluated.
- Callus formation was quantified
- The widest transverse width and the longest longitudinal length were obtained.

Histology

- The rat femurs were decalcified in 5% formic acid and saturated with ammonium oxalate and agitated for 24 hours.

- The slides were initially stained with hematoxylin and eosin.
- Lastly, the slides were stained with Sfog to view any cartilage formation.
- A board certified blinded Pathologist reviewed all of the slides.

Results

During tissue processing, one of the femurs in the experimental group broke at the callus and was excluded from the analysis. Consequently we were able to fully analyze 14 (78%) of the initial 18 rats.

- Experimental Rats:
  - 8 experimental rats
  - 3 were euthanized at 1 week (Group A; rats number 1,2,4)
  - 2 were euthanized at 4 weeks (Group B; rats 5,6)
  - 3 were euthanized at 5 weeks (Group C; rats 7,8,9).
- Control Rats:
  - 6 control rats
  - 1 was euthanized at 1 week (Group A; rats number 10)
  - 2 were euthanized at 4 weeks (Group B; rats 11,13)
  - 3 were euthanized at 5 weeks (Group C; rats 14,15,18).

- **Micro CT Analyses**
  - One week after fracture, the amount of mineralized bone present was greater in the experimental group.
  - When bones from the 4wk and 5wk time periods were pooled, the periosteal area of experimental rats was 36% greater.
  - Similar to the one week time point, there was no difference in the endosteal area, indicating that the treatment effect was confined to the fracture callus.
  - Tissue mineral density, however, was similar between the groups.

MicroCT Results
Although no callus was appreciated in 2 (25%) of the experimental group, and 3 (50%) of the control group, there was an obvious trend towards more robust callus in the experimental group.

Radiographic Results
- No callus was appreciated in 2 (25%) of the experimental group
- No callus was appreciated in 3 (50%) of the control group
- Statistical significance was not achieved with the remaining femurs

Rat 15 of the control group: C showing all stages of bone healing at 5 weeks

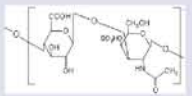
Rat 9 showing advanced stages of bone healing at 5 weeks.

Discussion

- In this study, chondroitin sulfate given during the initial hematoma phase showed the potential for fracture healing potential
- Histological studies demonstrated advances in marrow formation and bony callus compared to the control group.

Limitations:
- A pilot study has inherent limitations.
- Due to the small sample size in each group, statistical significance could not be obtained for each criteria measured.
- Radiographs demonstrated to be the least helpful tool in defining callus formation, and have been confirmed in the literature to be the least sensitive tool in addressing fracture healing in rats.
- Three dimensional imaging and histology have been promoted in the literature and was found to be useful in this pilot study.
- There was a trend towards increased callus formation in all of the experimental arms, most notably with the Micro CT scan.
- The pathology results also demonstrated a strong correlation between chondroitin sulfate and increased callus formation

Pharmaco-Kinetics
The pharmacologic response to chondroitin sulfate in patients with knee osteoarthritis was analyzed. It was discovered that chondroitin sulfate is a slow acting drug.
The study showed that pharmacologic response increases as a function of time until it reaches the maximal effect, even after cessation of treatment.
We believe that by taking advantage of the long acting effects of chondroitin sulfate, it could be used as a safe and effective tool to facilitate bone remodeling.



Conclusion

- Chondroitin sulfate accelerated the rate of bone healing as evidenced by the increased callus size and shorter healing span.
- The healing seen with chondroitin sulfate treated rats at the end of five weeks were the significant (36% higher).
- It is possible that a greater amount of repair may be noted with an elongated period of time.
- We would suggest that chondroitin sulfate has the potential to enhance the action of osteoblast genes important in the progression of osteogenesis and hence with further in-depth research.
- In the future, Chondroitin Sulfate could be recommended to be used as a drug in conjunction with other drugs to facilitate fracture healing.