THE EFFECT OF COX-2 INHIBITORS ON THE FORMATION OF HETEROTOPIC BONE IN A NEW RAT MODEL

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INTRODUCTION:
Heterotopic ossification (HO) induced by trauma or surgical procedure is a common clinical problem. Prophylactic options include radiation and/or pharmacological therapy with NSAIDs. Although efficacious in decreasing HO, especially the more severe forms, nonspecific NSAIDs, which inhibit both cyclooxygenase 1 (COX-1) and COX-2 enzymes, can have serious side effects on the gastrointestinal tract (GI) and can lead to increased bleeding. Lately a novel class of COX-2 inhibitors, including celecoxib, has been popularized. Due to their selective effect on COX-2-dependent prostaglandin formation, COX-2 inhibitors would be expected to be equally effective in the prophylaxis of HO with fewer side effects. Unfortunately two major challenges were encountered when we planned to test our hypothesis. Firstly, no reproducible animal model to study HO with absolute certainty exists and secondly quantitative evaluation of HO by plain radiographic analysis is imprecise. Our objectives were (1) : To create a new and reproducible animal model to produce HO ; (2) : To be able to exactly quantify the amount of HO using a microCT scan and (3) : To prove our hypothesis that COX-2 inhibitors are efficacious in the prevention of HO.

METHODS:
We have developed an IACUC-approved Lewis rat model. The right lower limb of each rat was surgically prepared and a lateral incision was made to access the entire diaphysis of the femur. After elevation of the vastus lateralis and vastus intermedius, the ventral part of the femur was scraped to mechanically disrupt the periosteum. Kocher clamps were placed across the vastus intermedius for 10 min. to produce ischemic injury to the muscle to enhance HO. Finally, homologous bone marrow, removed from one femur and one tibia of a donor rat, was placed upon the anterior surface of the femur. On the left side, the surgical procedure was identical except the periosteum was covered with bone wax after scraping. Each study group consisted of 8 animals. Half of the study group (COX-2 group) received a 4-week postoperative oral administration of celecoxib (10 mg/kg/day) mixed with rodent chow while the other half (control group) was fed normal rodent chow. After 6 weeks, animals were sacrificed, femurs were removed and imaged by microCT scan. For each sample, 400 serial cross-sectional views of the femur from proximal (hip) to distal (knee) end were viewed in rapid succession (with the possibility to stop at any one view). Grading was based on the thickness of ectopic bone, which covered the anterior surface of the cross-section of the femur expressed as a percentage of the cortical bone thickness. The highest grade given to the bone with the most ectopic bone was 4 and the lowest was 1 (Grade I: 0-25%, Grade II: 26-50%, Grade III: 51-75, Grade IV: 76-100%). MicroCT scan images were graded in a blinded fashion by 3 independent observers. Cohen’s kappa test for interobserver reliability yielded a score of 0.4; p < 0.04.

RESULTS:
All of the animals studied developed bilateral HO. Right limbs from rats in the COX-2 group developed statistically significant less ectopic bone (grade = 2.1 +/- 0.32; Mean +/- S.E; N = 8) compared to right limbs from control group rats (3.1 +/- 0.38; N = 8); p< 0.05 (student’s t test). Left limbs from rats in the COX-2 group developed statistically significant less ectopic bone (grade = 1.7 +/- 0.18; Mean +/- S.E; N = 8) compared to left limbs from the control group rats (2.8 +/- 0.27; N = 8); p< 0.003 (student’s t test). There was no significant difference in grade of HO between the left (periosteal disruption and bone wax) and right side (periosteal disruption).

DISCUSSION:
All of the animals studied developed bilateral HO. These results suggest that we have created a very reliable (100% in our study) reproducible model to form “true” ectopic bone in rats. Using the microCT we are able to quantify precisely the amount of HO with an acceptable degree of intra/interobserver variability. The process of grading which is based upon our criteria is much less subjective than that previously utilized in earlier HO models, which relied on 2-D plain X-ray imaging. COX-2 has been shown to be important in the cascade of bone formation through its regulation of prostaglandin synthesis. Recent data on COX-2 inhibitors demonstrate that they inhibit/delay bone healing. We know that nonspecific NSAIDs (such as indomethacin) are effective in the prevention of severe HO. A selective COX-2 inhibitor should thus be effective as an inhibitor of heterotopic bone development, with minor or no effects on the GI tract and bleeding. Although our study group was small, we have been able to show that a COX-2 inhibitor significantly decreases the amount of heterotopic bone formation. Due to the reproducibility of our rat model, we will be able to study the basic pathophysiological mechanism of HO from early time points up to the endpoint of heterotopic bone formation.

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