**THE ROLE OF CYCLOOXYGENASE 1 AND 2 IN FRACTURE REPAIR**

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**INTRODUCTION:**
Fracture repair is a complex process which requires the coordination of sophisticated biological and physiological events. During the inflammatory stage of fracture healing, prostaglandins have been suggested as one of the important cytokines that initiate the bone repair process. The rate limiting step of prostaglandin synthesis is carried out by the enzyme cyclooxygenase (COX), of which two forms exist. COX-1 is constitutively expressed and COX-2 is highly inducible following inflammation, although it also has some constitutive function. The differential and well regulated expression patterns of COX-1 and COX-2 imply that they may carry different functions in bone metabolism. Previous evidence on the functions of COX-1 and COX-2 has mainly come from animal studies using various COX inhibitors, which are a class of drugs named non-steroidal anti-inflammatory drugs (NSAIDs) and used widely for post-operative pain control after orthopaedic surgeries. Although non-selective NSAIDs have been shown to inhibit bone healing and formation, how COX-2 selective inhibitors affect bone formation and healing is rather controversial.

This study aims to investigate functions of COX-1 and COX-2 in fracture repair, using both pharmacologic COX inhibition approach and mice with genetic absence of COX-1 / COX-2 ("knockout"). This knowledge when applied to human practice may have significant implications to the use of COX inhibitor drugs in conservative management of fracture healing.

**METHODS:**
To achieve different COX inhibition, mice were treated with ketorolac (non-selective COX inhibitor), valdecoxib (selective COX-2 inhibitor) or placebo (control) by oral gavage. In order to validate the drug dose and delivery method used in mice, serum drug levels were measured using high performance liquid chromatography (HPLC) after a single oral dose of 5 mg.kg\(^{-1}\). The analgesic activity of ketorolac, and valdecoxib was also characterized at the selected dose in mice, using standard nociception models such as hot-plate and formalin tests.

In all treated mice, fractures were created on both femora while one side was externally-fixed and the other side left unfixed. In another set of experiment, the same type of fracture surgeries were done in COX-1(-/-), COX-2(-/-) and wild-type mice (without any further treatment).

For all the operated mice, radiographs were taken weekly. Three time points were chosen as 7, 14 and 21 days post-surgery. X-ray microcomputed tomography (\(\mu\)CT) analysis was done on both fractures at 14 and 21 days after healing. Histological analyses and mechanical testing were also performed.

**RESULTS:**
In the COX inhibition experiment, x-ray showed reduced callus formation in ketorolac treated mice, while mechanical testing showed significantly decreased stiffness of fractured femora from both NSAID groups. \(\mu\)CT data showed that ketorolac treatment caused reduced bone formation in both fixed and unfixed fracture callus. Histologically, ketorolac showed delayed healing in both fixed and unfixed fractures; while valdecoxib showed impaired healing in unfixed fractures only (Fig.1).

Interestingly, in COX-2(-/-) mice the impairment on bone healing was more severe. Radiographs showed reduced callus formation in COX-1(-/-) mice at 14 days, and in COX-2(-/-) mice at both 14 and 21 days. From \(\mu\)CT analysis, COX-1(-/-)mice showed delay in new bone formation in fracture callus in the early stage of un-fixed fracture healing, whereas COX-1(-/-) mice showed impaired new bone formation in both the early and late stages of un-fixed fracture healing. Mechanical testing at 21 day showed significantly decreased stiffness of fractured femora in COX-2(-/-) mice. Histology at 21 day showed severe delay in bone healing of unfixed fracture in COX-2(-/-) mice, and minor delay in COX-1(-/-) mice (Fig.1).

The results of the two sets of experiments were summarized in table 1.

**DISCUSSION & CONCLUSIONS:**
This is the first study to fully investigate the effect of various COX inhibition, as well as the effect of COX-1 or COX-2 gene deficiency, in both fixed and unfixed fracture repair. Differences in previously reported effects of COX inhibitors in bone repair may be because of pharmacokinetic diversity between mice (a commonly used animal model) and humans, and a lack of standardization of dosage in the animal models. Therefore a scientific validation of the drug dose and efficacy was carried out.

The results from two sets of experiments suggest that COX-1 and COX-2 are both involved in bone healing, yet carrying differential functions. COX-1 has minimal function in fixed fracture healing and minor function in early stage of unfixed fracture healing. COX-2 has minor function in fixed fracture healing, but plays an essential role throughout unfixed fracture healing. As fixed fractures heal predominantly by direct bone formation, while unfixed via endochondral ossification, this study has significant implications on the mechanism of bone healing which is the subject of further research.

**Table 1.** The effect of COX inhibition (as compared to placebo), and the effect of COX gene knockout (as compared to wild-type) on bone repair

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<th>Fixed fracture</th>
<th>Un-fixed fracture</th>
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<tr>
<td></td>
<td>K</td>
<td>V</td>
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<tr>
<td>x-ray</td>
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<td>Histology</td>
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<td>Mechanical</td>
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K: ketorolac (both COX-1 and COX-2 inhibition)
V: valdecoxib (COX-2 inhibition)
A: COX-1(-/-) mice
D: COX-2 (-/-) mice
+: inhibitory effect (reduced callus formation, delay in healing, or reduced mechanical stiffness)
=: no effect
/: data not available

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