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THE ROLE OF CYCLOOXYGENASE 1 AND 2 IN FRACTURE REPAIR – IMPLICATIONS FOR ATROPHIC NONUNION

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INTRODUCTION: 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. 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").

METHODS: Fractures were created on both femora while one side was externally-fixed and the other side left unfixed. Radiographs were taken weekly. Time points of 7, 14, 21 and 60 days post-surgery (dps) were examined. X-ray microcomputed tomography (μCT) analysis was done on both fractures at 14 and 21 days after healing. Histological analyses and mechanical testing were also performed. Molecular markers in chondrogenesis were examined.

RESULTS: COX-2(-/-) mice showed severe impairment in non-stabilized bone healing. Radiographs showed reduced callus formation in COX-1(-/-) mice at 14 days, and in COX-2(-/-) mice at both 14 and 21 days. From μCT analysis, COX-1(-/-)mice showed delay in new bone formation in fracture callus in the early stage of un-fixed fracture healing, whereas COX-2(-/-) 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).

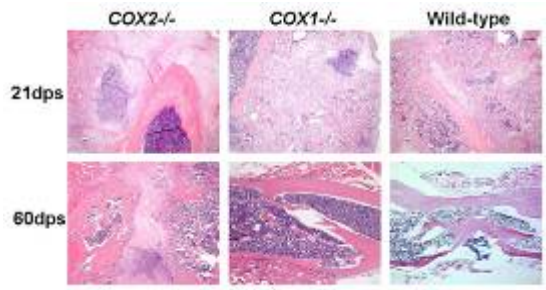

Fig.1 Histology of mice at 21and 60 dps (un-fixed fracture).

Interestingly radiographs showed reduced callus formation in COX-2(-/-) mice even at 60 dps, when compared with COX-1(-/-) mice and wild-type mice. Histology at 60 dps showed nonunion of unfixed fractures in COX-2(-/-) mice. Furthermore, although Cox2-/- mice showed mesenchymal cell condensation morphologically, they failed to express early chondrogenic marker Sox9. Also no pre-hypertrophic chondrocyte (indicated by presence of Ihh signal) was detected in COX-2(-/-) mice.

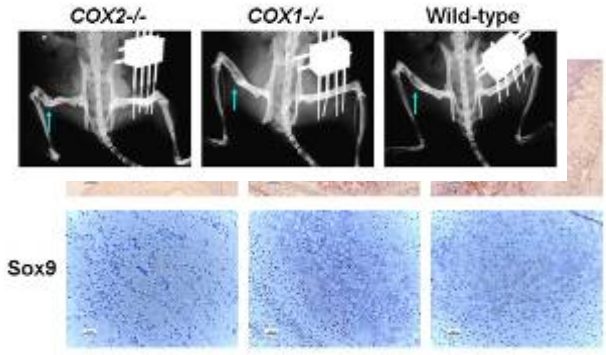

Fig.2 x-ray of mice at 60 dps. The un-fixed fracture was indicated by blue arrow.
Fig.3 Immunohistochemistru of Ihh and Sox9 on fracture sections at 7 dps (un-fixed fracture).

DISCUSSION & CONCLUSIONS:

This study suggests 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.

Interestingly, these results also show that COX-2 has a significant effect in endochondral ossification and absence of COX-2 may cause atrophic nonunion of fracture healing. The cessation of reparative process concurred with failure in initiation of chondrogenic cell differentiation. These data may have significant implications on the mechanism of atrophic nonunion and may contribute to the conservative management of fracture healing and prevention of nonunion.