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Differential Functions of Cyclooxygenase 1 and 2 in Bone Repair

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INTRODUCTION: Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for pain control after orthopaedic surgeries. They inhibit the enzyme cyclooxygenase (COX), of which two isoforms exist (COX-1 and 2). Non-selective COX inhibitors have been shown to delay bone healing, suggesting possible roles of COX-1 and COX-2 in bone repair. However the distinct functions of COX-1 and COX-2 during bone repair and formation are largely unknown. This study aims to investigate functions of the two enzymes by comparing the effects of different COX inhibition in fracture repair, using 2 well established mouse femoral fracture models with different mechanical environments. Further investigation using COX-1(-/-) and COX-2(-/-) mice is also carried out.

METHODS: To achieve different COX inhibition, mice were treated with ketorolac (non-selective COX inhibitor), valdecoxib (selective COX-2 inhibitor) or placebo (control). 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. Histological analyses and mechanical testing were 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. Histologically, ketorolac showed delayed healing in both fixed and unfixed fractures; while valdecoxib showed impaired healing in unfixed fractures only.

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

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

	Fixed fracture				Un-fixed fracture			
	K	**V**	**A**	**D**	**K**	**V**	**A**	**D**
x-ray	/	/	/	/	+	—	+	++
Histology	+	—	—	—	+	+	+	++
Mechanical testing	+	+	—	+	/	/	/	/

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)
++: severe inhibitory effect
—: no effect
/: data not available

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. These results 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.