NF-κB Mediates Cartilage Degradation Induced by Trauma Injury and IL-1

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SIGNIFICANCE

IL-1 is a cytokine that mediates cartilage degradation in inflammatory diseases and after traumatic injury. Identifying the major signaling pathways (NF-κB versus p38) responsible for cartilage degradation after injury is important for understanding the onset of post-traumatic osteoarthritis (PTOA).

INTRODUCTION

- Chondrocyte death, induced by direct impact injury (necrosis) and/or apoptotic inducers (TNF-α, and high level of nitric oxide), is important for the development of post-traumatic osteoarthritis [1-3, 5-9].
- IL-1 induces the degradation of proteoglycan (PG) in cartilage through the NF-κB and Mitogen-activated protein kinases (MAPK: p38, ERK and JNK) pathways [1,2,6].
- IL-1 is highly upregulated in synovial joints after impact injury, but the role of IL-1 induced chondrocyte death and matrix/PG degradation in injured cartilage is not completely clear.
- Inhibition of p38 reduced chondrocyte death and PG loss in cartilage induced by blunt impact [4], but the role of NF-κB in chondrocyte death and PG degradation and the synergetic effects between IL-1 and injury was not clear.
- The objective of this study was to determine the contribution of NF-κB and p38 in IL-1 and injury induced PG loss and tissue remodeling genes.

HYPOTHESIS

NF-κB (versus p38) is the primary signaling pathway responsible for IL-1 induced PG loss in articular cartilage after trauma injury.

MATERIALS AND METHODS

- Bovine cartilage pre-cultured in DMEM was pre-treated with 10μM SB202190 against p38 (p38i), 50μM BAY117085 against NF-κB (IκBi), and .1% DMSO as control for 1 hour [2]
- Samples in Injury and Injury+IL-1 groups received impact injury using a drop-tower with impact energy of 15J/cm^2 (Fig. 1E) [4]
- IL-1 and Injury+IL-1 groups were treated with 1 ng/ml IL-1 for 48 hours
- Cell death was assessed using fluorescein diacetate and propidium iodine (Figs. 1A-1D)
- Medium was collected at day 0 (before pretreatment) and day 2, and analyzed for PG release/loss and nitric oxide (NO) using DMMB and Greiss assays, respectively.
- mRNA from cartilage was isolated and analyzed using real-time qPCR to determine pro-inflammatory cytokine and tissue remodeling genes (IL-6, MMP-3, TIMP-3). All gene expression was normalized to GAPDH.

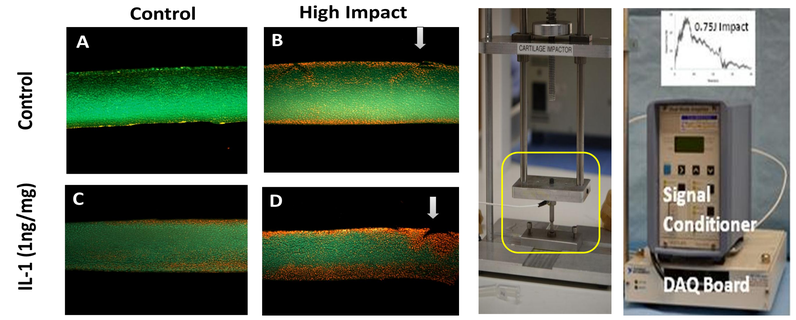

Fig 1. (A-D) Impact (white arrows) and IL-1 increased cell death (Red=dead cells, Green = live cells) in cartilage explant with impact energy of 15J/cm^2. (E) A drop-tower apparatus to create impact injury.

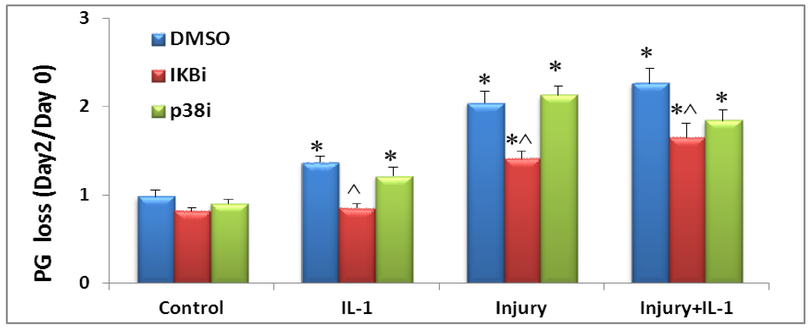

Fig 2. Effect of IKB and p38 inhibitors in PG loss/release in cartilage treated with (1ng/ml) IL-1 and/or blunt trauma injury (15J/cm2). (* indicate significant difference to control, p<0.05; ^ indicates significant difference to DMSO,p<0.05)

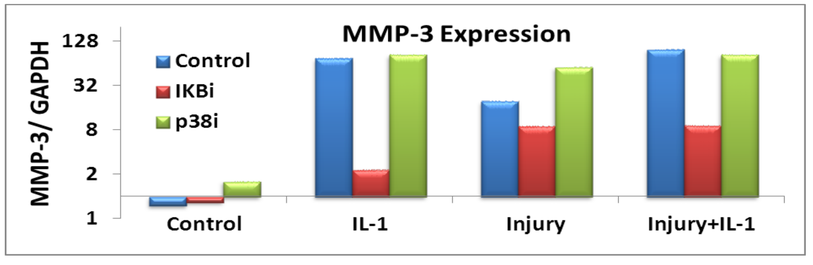

Fig 3. Real-time qPCR showed that the upregulation of MMP-3 induced by load and injury was reduced by the IκBi treatment.

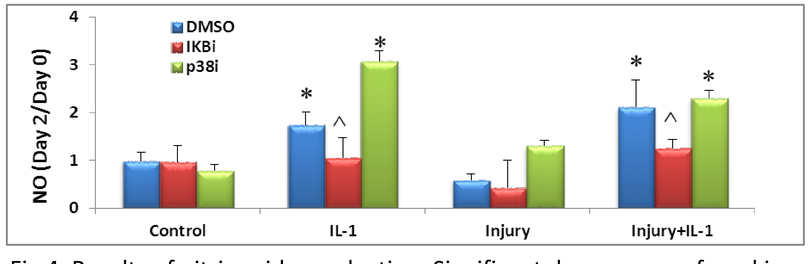

Fig 4. Results of nitric oxide production. Significant decrease was found in the groups treated with IκB inhibitor (^, p<0.05). * indicates difference between Control and treatment groups (*, p<0.05).

RESULTS

Impact and IL-1 increase Cell Death and PG loss

- Increased cell death in the Injury and Injuy+IL-1 groups, especially at the edges of impaction where the maximal shear stress, was located (Fig. 1A-1D).
- 37% increase of PG loss (p<0.05) in IL-1 treated group. A further increase of PG loss (126%) was found in the Injury+IL-1 treated groups (Fig 2). Injury significantly increased PG loss (104%).

IKBi (not p38i) reduces PG loss and MMP-3 expression

- In the p38i group, similar increases were found in IL-1, Injury and Injury+IL-1 groups (22%, 113% and 84%, respectively).
- Significant decreases of PG losses were found in all IκBi treatment groups where PG loss in Control and IL-1 treated groups was reduced to below that of DMSO-Control levels. A similar prevention was also found in the Injury and Injury+IL-1 groups (69% and 73% of reduction, respectively.)
- MMP-3 (a PG degradative enzyme) upregulation induced by IL-1 and injury was reduced by IκB inhibitor in qPCR analysis (Fig 3). There was little or no effect in MMP-3 expression in the treatment of p38i.

IKBi reduces Nitric Oxide production

- Nitric Oxide production was decreased in the IκBi groups (Fig. 4).

DISCUSSION AND CONCLUSION

These findings support our hypothesis that the NF-κB signaling pathway plays a role in PG loss and NO production in articular cartilage after trauma injury [1,2]. Of particular interest, we did not observe the inhibitory effects in p38 inhibitors as reported in previous studies using isolated chondrocytes and osteochondral block [2,4]. Since there was no measurement for PG loss and degradation in the isolated chondrocyte studies, the role of tissue remodeling enzymes (MMPs and aggrecanases) was not clear. Our study suggests that NF-κB is the major pathway responsible for injury and IL-1 induced PG degradation/loss. Future studies are needed to determine the time-course response and specific NF-κB mediators for downstream regulation, as well as the effects in long-term therapeutic treatment to ameliorate the progress of OA [4,6].

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