NF-kappa B Mediates Cartilage Degradation Induced by Trauma Injury and Interleukin-1

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Introduction: Joint inflammation is common after blunt trauma injury and closely related to cartilage degradation. Several studies of synovial fluid of injured joints showed that degradative enzymes and related pro-inflammatory cytokines, including IL-1 and IL-6, and were upregulated after joint injury [1-3, 5-9]. IL-1 is one of major pro-inflammatory cytokines responsible for the degradation of proteoglycan (PG) in cartilage through NF-kB and Mitogen-activated protein kinases (MAPK: p38, ERK and JNK) pathways [1,2,6]. A recent study showed that an inhibition of p38 reduced chondrocyte death and PG loss in cartilage induced by blunt impact [4], but the role of NF-kB in chondrocyte death and PG degradation and the synergic effects between IL-1 and injury was not clear. The objective of this study was to determine the contribution of NF-kB and p38 in IL-1 and injury induced PG loss and tissue remodeling genes. We hypothesize that NF-kB (versus p38) is the primary signaling pathway responsible for IL-1 induced PG loss in articular cartilage.

Methods: Full-thickness cartilage were removed from trochlear groove of bovine knees (>18 months). Samples were pre-cultured in DMEM at 37°C and 5% CO2. The signaling pathways of intact chondrocytes in cartilage were inhibited by a pretreatment of small molecule inhibitors: 10µM SB202190 for p38 (p38i) and 50µM BAY117085 for NF-kB (IκBi) for 1 hour [2]. The controlled sample was treated with 0.1% dimethyl sulfoxide (DMSO), an organic solvent used for the inhibitors. Samples in Injury and Injury+IL-1 groups received impact injury using a drop-tower with impact energy of 15J/cm2 (Fig 1E) [4]. IL-1 and Injury+IL-1 groups were treated with 1 ng/ml IL-1. Medium was collected at day 0 (before pretreatment) and day 2. Cell death was assessed using fluorescein diacetate and propidium iodine. The mRNA from cartilage was isolated using Trizol and RNeasy Mini kit (Qiagen), reversely transcribed, and analyzed using Real-time qPCR (CFX96- BioRad) to determine pro-inflammatory cytokine and tissue remodeling genes (IL-6, MMP-3, TIMP-3) as previously described [9]. All gene expression was normalized to GAPDH. Medium was analyzed for PG release/loss and nitric oxide (NO) using dimethylmethylene blue (DMMB) and Greiss assays, respectively. Statistical analysis (Student’s t test or two-way ANOVA) was performed with Excel and Systat with significance level at 0.05.

Results: Increased cell death was found in the Injury and Injury+IL-1 groups, especially at the edges of impaction where the maximal shear stress was located (Fig. 1A-1D). There were 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). Blunt injury also significantly increased PG loss (104%). In the p38 inhibitor group, similar increases were found at IL-1, Injury and Injury+IL-1 groups (22%, 113% and 84%, respectively). Significant decreases of PG losses were found in all IkBi 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,) This indicated that IkB mediated IL-1 induced PG degradation and loss (Fig. 2). This finding was supported by the results from qPCR analysis where MMP-3 upregulation induced by IL-1 and injury was reduced by IkB inhibitor (Fig 3). There was little or no effect in MMP-3 expression in the treatment of p38. These findings were also consistent with the results from nitric oxide production which was decreased in the IkBi treated groups (Fig. 4).

Discussion: These findings support our hypothesis that NF-kB signaling pathway plays a role in PG loss and NO production in articular cartilage after trauma injury [1,2]. Our study suggests that NF-kB is the major pathway responsible for injury and IL-1 induced PG degradation/loss. Future studies are needed to determine the time-course responses and specific NF-kB mediators for downstream regulation, as well as the effects in long-term therapeutic treatments to reduce the progress of posttraumatic arthritis [4,6].

Significance: IL-1 is a main cytokine that mediates cartilage degradation in inflammatory diseases and after trauma injury. To identify the major signaling pathways responsible for cartilage degradation after injury is important for understanding the onset and progression of posttraumatic arthritis.

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Fig 1. (A-D) Impact (white arrow) and IL-1 increased cell death (Red = dead cells, Green = live cells) in cartilage explant with impact energy of 15J/cm². (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 (1 ng/ml) IL-1 and/or trauma injury (15J/cm²). * = Difference to Control, ^ = 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 IkB treatment. (* = Difference to Control, \(^{*}\) = Difference to DMSO, p<0.05)

Fig 4. Results of nitric oxide production. Significant decrease was found in the groups treated with IkB (* = Difference to Control, \(^{*}\) = Difference to DMSO, p<0.05)

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