The Effect of Inhibitors of Brd4 and CDK9 on Early Phase of Post Traumatic Osteoarthritis

Tomoaki Fukui, M.D., Ph.D., Jasper Yik, Ph.D., Dominik R. Haudenschild, Ph.D.
Lawrence J. Ellison Musculoskeletal Research Center, Department of Orthopaedic Surgery, University of California Davis Medical Center, Sacramento, CA, USA.


Introduction: Osteoarthritis (OA) is a slowly progressing degenerative disease of the whole articular joints characterized by cartilage degradation and changes to subchondral bone. Joint trauma is a risk factor for OA, and approximately 50% of patients with ACL or meniscal injury develop posttraumatic OA (PTOA) within 10-20 years (Ref.1). The acute response to joint trauma includes increased transcription of pro-inflammatory cytokines and proteinases, which cause the catabolic destruction of cartilage and trigger the onset and progression of osteoarthritic changes. Bromodomain protein-4 (Brd4) and cyclin-dependent kinase 9 (CDK9) control the expression of primary response genes (Ref. 2, 3), including most pro-inflammatory genes, by positively regulating transcriptional elongation. In response to an acute stress such as traumatic injury, Brd4 and CDK9 act to release the RNA polymerase II complex from promoter-proximal paused state, to enable transcriptional elongation of the pre-mRNA. Because this is the rate-limiting step for the transcriptional activation of primary response genes, we hypothesized that inhibition of Brd4 or CDK9 will prevent the acute inflammatory response in chondrocytes and the resulting damage to cartilage. Inhibition of transcriptional elongation represents a novel therapeutic target in arthritis. The objective of the current study is to investigate the effects of small molecule inhibitors of Brd4 (JQ1) and CDK9 (Flavopiridol) on the activation of inflammatory genes using chondrocytes and cartilage tissue under inflammatory stimuli, and a mouse model of traumatic joint injury.

Methods:

Treatment of human chondrocytes in vitro
Primary chondrocytes were isolated from cartilage of healthy human donors (n=4, IRB approved) and cultured 5 hours with inflammatory stimuli (either 10ng/ml IL1b, 10ng/ml TNFa, or 100ng/ml IL6 and 60ng/ml IL6 receptor), in the presence or absence of Brd4 and Cdk9 inhibitors. Treatment conditions were: 1) vehicle only (Ctrl), 2) no drug with cytokine, 3) Hi JQ1 (1200nM) with cytokine, 4) Hi Flavopiridol (250nM) with cytokine, 5) Combination of Lo JQ1 (250nM) and Lo Flavopiridol (60nM) with cytokine. Total RNA was extracted and analyzed by quantitative real time RT-PCR (n=X), and Affymetrix GeneChip Human Gene 2.0ST arrays using GeneSpring software (n=2).

Treatment of cartilage explants
Cylindrical cartilage explants (6mm x 2mm, d x h) were isolated from femoral articular surface of bovine stifile knee (n=6) and randomly assigned to 5 groups cultured as described above, with the cytokine being 10ng/ml IL1b. Glycosaminoglycan released into the culture media was measured on day 3 and 6 using dimethylmethylene blue dye assay.

Animal model of joint injury
The right knees of adult male C57BL/6 mice (n=6) were injured with a single mechanical compression, which causes a transient anterior subluxation of the tibia and rupture of the anterior cruciate ligament, and leads to PTOA within 8 weeks(Ref. 4). The contralateral uninjured knees served as controls.
Immediately after injury, mice were treated with intraperitoneal injections of Brd4 and/or Cdk9 inhibitors. Treatment conditions were: 1) vehicle only (Ctrl), 2) Hi JQ1 (50mg/kg), 3) Hi Flavopiridol (7.5mg/kg), 4) Combination of Lo JQ1 (17mg/kg) and Lo Flavopiridol (2.5mg/kg) Flavopiridol. Four hours after injury, joints were dissected and total RNA was extracted for real-time RT-PCR analysis. All animals were maintained and used in accordance with NIH guidelines, and with IACUC approval.

Results:
The effects of drugs on mRNA expression in chondrocytes under inflammatory stimuli
The mRNA expression levels of pro-inflammatory genes (iNOS, Cox2) and catabolic genes (MMP-1, -3, -9, and -13, and ADAMTS4) were significantly induced by all 3 inflammatory cytokines, and this induction was suppressed by all 3 drug treatments. The combination of both drugs at lower doses suppressed gene expression similarly or more strongly than single high doses of each individual drug.

Distribution of genes induced by IL-1b and suppressed by the drugs
Compared to baseline expression, 873 genes were induced >1.5-fold by IL1b, as indicated in a heat map (Fig.1). IL-1b treatment in the presence of either JQ1 or Flavopiridol alone prevented the induction of many genes, with Flavopiridol being somewhat more effective. However, a combination of both drugs prevented the induction of most IL-1b response genes, as shown by cluster analysis. The combination drug treatment indicates a synergistic interaction between Brd4 and CDK9, since the drugs were active at a much lower dose in combination than when used individually.

Measurement of GAG released from cartilage
IL1b treatment of cartilage explants induced significant release of GAG within 3-6 days. GAG release was effectively prevented (not different from baseline) when IL-1b treatment in the presence of either JQ1 or Flavopiridol, or both drugs combined. (Fig2. ***p<0.001)

Suppression of pro-inflammatory gene expression in early phase after trauma by drugs
In PTOA mouse model, knee injury caused significant increases of IL1b and IL6 expression in the injured joint. Systemic administration of JQ1 and/or Flavopiridol prevented injury-induced increases of these cytokines, and in some groups even suppressed them below the basal level (Fig3).

Discussion: JQ1 and Flavopiridol are each able to effectively repress a panel of pro-inflammatory and catabolic genes in chondrocytes induced by inflammatory stimulus. We found that the combination of the 2 drugs showed a synergistic interaction, with similar or better repression achieved at reduced drug doses. Although previous reports indicated that Brd4 and CDK9 control the expression of primary response genes by regulating a common checkpoint, namely transcriptional elongation, microarray analysis showed that there were also inflammatory genes that were only affected by each drug individually. This suggests that Brd4 and Cdk9 regulate the transcription of primary response inflammatory genes not only through common mechanisms, but also independent mechanisms. In vivo study also demonstrated the combination of lower dose of the drugs has similar intensity of effect. This indicates that using both drugs together may be able to suppress inflammation after trauma with preventing side effects induced by overdose, leading to novel treatment for PTOA.

Significance: We are currently exploring the possibilities in the context of cartilage biology, to reduce the transcription of inflammatory genes upon joint injury and in osteoarthritis. This represents a novel therapeutic possibility for treatment of inflammatory conditions in osteoarthritis.
Figure 1

IL-1β: + + + + -
Inhibitor: - J F F+J -

Genes induced >1.5-fold by IL-1β (873 total)

log2 ratios

Figure 2

day 6

GAG released (µg/mg cartilage)

Ctrl | IL1b | J1200 | F250 | J250F60

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Figure 3

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