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

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INTRODUCTION

The acute response to joint trauma increases transcription of pro-inflammatory cytokines and proteinases such as matrix metalloproteinases (MMPs), which trigger the onset of OA changes. Bromodomain protein 4 (Brd4) and cyclin-dependent kinase 9 (CDK9) control the rate-limiting step of the transcription of primary response genes, including most pro-inflammatory genes, by positively regulating mRNA elongation through phosphorylating and activation of RNA polymerase II. The purpose 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 a mouse posttraumatic osteoarthritis (PTOA) model. Moreover we investigated the effect of these inhibitors under various inflammatory stimuli in ex vivo and in vitro cultures of bovine cartilage tissue and human chondrocytes.

METHODS

Treatment of human chondrocytes

Human chondrocytes were cultured 5 hours with pro-inflammatory cytokines (either 10ng/ml IL1b, 10ng/ml TNFa, or 100ng/ml IL6 and 60ng/ml IL6 receptor), with or without inhibitors. Treatment conditions were: 1) vehicle only (Ctrl), 2) no inhibitors 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 real time RT-PCR and microarray.

PTOA animal model

The right knees of mice were injured with a mechanical compression, to induce rupture of the anterior cruciate ligament leading to PTOA. Immediately after injury, mice were treated daily with JQ1 and/or Flavopiridol. 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. The mRNA expression, MMP activities, inflammatory features, histological OA change, and bone change in knee joints after the injuries were assessed at specific time points.

RESULTS

The effects of the inhibitors on mRNA expression in chondrocytes under inflammatory stimuli

IL1b, IL6 and TNF induced the mRNA expression levels of pro-inflammatory genes (iNOS, Cox2) and catabolic genes (MMP-1, -3, -9, and -13, and ADAMTS4) and this induction was suppressed by all 3 inhibitor treatments, either of JQ1 or Flavopiridol, and both. The combination of JQ1 and Flavopiridol at lower doses showed similar or stronger effect to suppress those gene expressions than single high doses of each individual inhibitor.

Distribution of genes induced by IL1b and suppressed by the inhibitors

Microarray analysis showed that expression, 873 genes were induced >1.5-fold by IL1b compared to baseline. IL1b treatment in the presence of either JQ1 or Flavopiridol alone prevented the induction of many genes. However, a combination of low dose both inhibitors prevented the induction of most IL1b response genes.

Suppression of pro-inflammatory gene expression, MMP activity, and synovitis in early phase after trauma by inhibitors

In the PTOA mouse model, knee injury caused significant increase 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 (Figure 1A). MMP activities in injured knees assessed with MMPSense750, in vivo imaging agent, were suppressed by all three inhibitor treatments similarly at 24 and 48 hours after the injuries. (Figure 1B) Histological grading suggested that synovitis could be significantly induced by the injuries in 3 days and suppressed by treatment with the combination of the inhibitors. (Figure 2A, B)

Histological OA progression following the knee injury with or without inhibitor treatment

Compared with knees of naïve mice without OA feature, knees 4 weeks after injuries without inhibitor treatment showed significant OA progression evaluated with OA scoring. On the other hand, the scoring of mice knees treated with inhibitors for 4 weeks after the injuries was significantly lower than that of untreated mice and there was no significant difference between the naïve mice and the mice treated with the inhibitors. (~~Figure 2~~C, D)

Bone change following the knee injury

The knees were scanned by μCT and analysis of trabecular bone in the distal femoral epiphysis was performed to quantify ~~tra~~becular bone volume per total bone volume (BV/TV). BV/TV of only uninjured knees of drug combination group was significantly greater than naïve knees at 14 and 28 days after the injury, but that of injured knees of drug combination group was significantly lower than the other groups. (Figure 3)

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 inhibitors showed a synergistic interaction, with similar or better repression achieved at reduced 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 inhibitors individually. This suggests that Brd4 and CDK9 regulate the transcription of primary response inflammatory genes not only through common mechanisms, but also independent mechanisms. Our in vivo study also demonstrated the combination of lower doses of the inhibitors has similar intensity of effect to repress inflammatory and catabolic mRNA expression, and MMP activity on knee joints following trauma. The effect of the combination against inflammation was found in synovitis grading in vivo also. It is supposed that the inhibitors might modulate OA progression accelerated by the injury via suppression of post-traumatic inflammation and catabolism, and could be novel treatment for PTOA. The results of BV/TV analysis could be interpreted that the drugs worked to upregulate bone formation against bone resorption induced by the injury but that kind of rebound effect happened after 28 days post-injury to show significant bone resorption, which implies the necessity to investigate better schedule of drug administration.

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 post-traumatic osteoarthritis through modulating inflammatory conditions.

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