**BENEFICIAL EFFECTS OF LIVER X RECEPTOR (LXR) MODULATION ON MATRIX METABOLISM AND PROSTAGLANDIN E2 (PGE2) PRODUCTION IN CARTILAGE**

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**Introduction**

Osteoarthritis (OA), the most common arthritic condition in humans, is characterized by the progressive degeneration of articular cartilage accompanied by chronic joint pain. Inflammatory mediators, such as cytokines and prostaglandin E2 (PGE2), which are elevated in OA joints, play important roles in the progression of cartilage degradation and pain-associated nociceptor sensitivity. LXR6 possess potent anti-inflammatory properties, possibly through antagonism of the NFκB signaling pathway. This study was undertaken to evaluate the function of LXR6 in cartilage, particularly their capability to inhibit degradation of matrix proteins such as aggrecan, and production of inflammatory and pain mediators such as PGE2.

**Methods**

Quantitative real-time PCR was performed to compare expression levels of LXRα/β, ABCG1, apolipoproteins D and E in human OA and normal cartilage. RNA was isolated from human osteoarthritic articular cartilage samples obtained from patients (n=18, mean age = 66.2 years, range 49-84 years) undergoing total knee replacement surgery (New England Baptist Hospital), or from non-osteoarthritic cartilage obtained from above-knee amputations (n=10, mean age = 71.6 years, range 43-100) (Clinomics).

Mouse cartilage explant culture (wild type and Lxrβ-/-): Hip cartilage from 3-week-old wild type (wt) C57BL/6 and Lxrβ-/- mice were collected and cultured in serum-free medium with or without IL-1β (1 ng/ml) to study the role of Lxrβ in regulating inflammation (PGE2 production) and cartilage matrix metabolism (proteoglycan degradation/GAG release). Culture medium pooled from wt or Lxrβ-explant cultures was also analyzed for aggrecanase-generated AGEG aggrecan neoepitope.

Human cartilage explant study: Cartilage explants from human OA joints were treated with/without IL-1β (1 ng/ml) + Oncostatin M (5 ng/ml) and with/without co-treatment with LXR agonist GW3965. The treatments lasted for 10 days, with medium changed every 2 days. The effects of LXR activation on proteoglycan degradation (cumulative percent GAG release) and PGE2 production were measured. Culture medium was also analyzed for aggrecanase-generated AGEG aggrecan neoepitope.

TLDA (Taqman Low Density Array) analysis: A TLDA was designed to include a list of selected genes known to be important in cartilage metabolism and inflammation. Cartilage RNA from 5 human cartilage samples treated with/without IL-1β/Oncostatin M +/- GW3965 was analyzed to determine the effects of GW3965 treatment on the expression of genes such as ADAMTS4, mPGES, TIPPM3, MMP-1 and MMP-13.

**Results**

Both LXRα/β were found to be expressed at significantly lower levels in OA cartilage, as were LXR target genes such as ABCG1 and apolipoproteins D and E, suggesting a defect in LXR signaling in human OA. LXRβ appears to be the predominant isoform in cartilage. Genetic disruption of Lxrβ gene expression in mice resulted in significantly increased proteoglycan (aggrecan) degradation (GAG release) and PGE2 production in articular cartilage explant culture treated with IL-1β, indicating a protective role of LXRβ in mouse cartilage (Fig 1). Increased GAG release from Lxrβ-/- explants after IL-1β treatment is mediated by aggrecanases since higher amount of aggrecanase-generated AGEG fragment is detected in the media of Lxrβ-/- explants than wt explants. These results imply that LXRβ, in particular LXRβ, play a protective role in cartilage.

LXR activation using GW3965 dramatically diminished basal level as well as cytokine-induced PGE2 production by human osteoarthritic cartilage (Fig 2). Moreover, activation of LXRs by GW3965 significantly reduced cytokine-induced degradation and loss of aggrecan from the tissue. Cytokine treatment strongly increased AGEG neoepitope levels in the culture medium, which is diminished by GW3965 co-treatment, suggesting that LXR activation lead to a decrease in aggrecanase activity (not shown). TLDA analysis show that GW3965 significantly represses the expression of microsomal prostaglandin E synthase-1, a rate-limiting enzyme in the PGE2 synthesis pathway, and ADAMS4, a physiological cartilage aggrecanase (not shown).

**Discussion**

In summary, we demonstrate that the deletion of Lxrβ gene expression in mice strongly amplifies cytokine-induced aggrecan degradation and PGE2 production in cartilage. In addition, we show that the activation of LXRs using LXR specific agonists significantly reduce cytokine-induced degredation of aggrecan from human cartilage explants, consequently increasing the total aggrecan retained in the tissue. This appeared to be achieved at least partly by repressing the expression of ADAMTS4. LXR activation also dramatically diminished basal level as well as cytokine-induced PGE2 production by cartilage explants from OA donors via inhibition of cytokine-mediated induction of mPGES-1. Therefore, modulation of LXR signaling in cartilage using small molecule agonists may have great potential for OA therapy, not only to treat symptoms such as pain by blocking PGE2 synthesis, but also to prevent and possibly reverse disease progression by blocking cartilage matrix degradation.