The Role of LPC and LPA in Osteoarthritis-Related Ossification and Pain

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INTRODUCTION:

Recent reports have shown that lysophosphatidic acid (LPA) plays a critical role in the development of osteoarthritis (OA) and associated pain (McDougall et al. 2016). In addition to the traditional inflammatory discomfort of OA, an estimated 15-25% of patients suffer from neuropathic pain, which is thought to be caused by random stimulation of afferent nerves due to demyelination. In fact, intra-articular LPA has been shown to directly correlate with the severity of neuropathic pain[1]. In previous work, LPA was shown to activate LPA1 receptors to induce nerve demyelination following injury to generate neuropathic pain[2]. Moreover, loss of the LPA1 receptor protected against the development of arthritis in mice[3]. These results imply that nerve demyelination by LPA interferes with joint innervation, and may lead to OA-related joint pathologies, including subchondral bone loss, cartilage fragmentation, and osteophyte formation. In general, LPA is generated by the conversion of lysophosphatidylcholine (LPC) by the ubiquitously expressed enzyme Autotaxin (ENPP1). Although levels of LPC and LPA are known to be increased in diabetic and dyslipidemic patients[4, 5], their role in OA-related joint degradation and pain is poorly understood. Furthermore, previous work has shown that LPC and LPA directly affect mesenchymal cell differentiation[6]. Therefore, our overall hypothesis was that LPC and/or LPA contribute to OA progression by inducing nerve demyelination, inhibiting angiogenesis, and altering osteogenic and chondrogenic cell differentiation. We directly tested these hypotheses using an *in vivo* model of endochondral ossification as well as *in vitro* studies of mesenchymal cell differentiation.

METHODS:

We utilized an *in vivo* mouse model of heterotopic ossification to examine the effects of LPC and LPA on bone formation. Briefly, 8 to 10 week old C57BL/6J mice were injected with 100 uL of Matrigel containing 2 ug of bone morphogenic protein 2 (BMP2) alone, or mixed with either LPC (5 mM), LPA (25 uM), or LPA and LPC combined (n = 5 per group). The Matrigel masses were carefully removed after 14 days for analysis using microCT (Scanco μCT, Basserdorf, Switzerland), then processed for histological analyses (n = 3). Multiple sections from defined regions of the masses were stained with Alizarin Red to identify mineral deposition. Additional sections were stained using Hemotoxylin, Eosin, and Alcian Blue to identify chondrocytes via proteoglycan deposits. Separately, a group of Thy1-YFP mice, in which all nerves robustly express YFP fluorescence, were injected with Matrigel masses for the analysis of innervation and vascularization. To evaluate the roles of LPC and LPA on chondrocyte differentiation, C3H/10T1/2 cells (ATCC (CCL-226), Manassas, VA) were cultured in DMEM with 5% fetal bovine serum (FBS), 5% fetal calf serum (FCS), 100 units/ml penicillin, and 100 μg/ml streptomycin. To induce chondrogenic differentiation, 0.1mM TGF-β1 and 0.2mM ascorbic acid was added to media for 7 or 21 days. Western blot and qRT-PCR was performed to quantify chondrogenic differentiation markers. Statistical analysis was performed using one-way analysis of variance. Differences with a p-value of less than 0.05 were considered significant. Representative data is presented as mean ± standard error of the mean using three or more independent analyses.

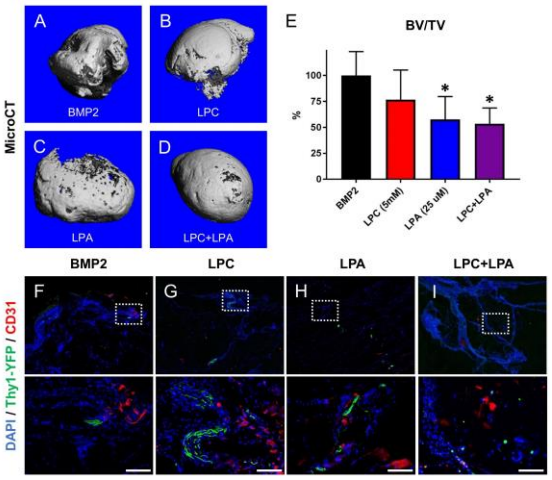

Figure 1. A-D) 3D reconstructions of Matrigel masses by microCT with E) quantification of bone volume fraction for each group, normalized to control. F-I) Frozen sections from masses implanted in Thy1-YFP mice with immunohistochemistry against CD31. * $p < 0.05$ vs. Control.

RESULTS:

From microCT analysis, we observed that the addition of LPA, LPC, or the combination to Matrigel masses containing BMP2 tended to decrease the mineralization of each sample as compared to control. By bone volume fraction, we observed no significant difference between BMP2 control (Fig. 1A) and the LPC mass (Fig. 1B), but the LPA mass (Fig. 1C) and LPC+LPA mass (Fig. 1D) were both significantly decreased by -43% and -48%, respectively (Fig. 1E). Consistent with these findings, we observed significantly diminished calcium deposition in all treatment groups as compared to BMP2 control by Alizarin Red staining. Furthermore, chondrocytes were more prominent in LPC and LPA treated masses as compared to other groups by Alcian blue staining. Finally, the innervation and vascularization of the Matrigel mass was significantly diminished in LPA and LPC+LPA groups as compared to control and LPC alone using CD31 immunohistochemistry on frozen sections from Thy1-YFP mice. In particular, thick nerve bundles could be seen entering the BMP2 and LPC masses (Fig. 1F-G), but this innervation pattern was missing in LPA and LPC+LPA masses (Fig 1H-I). In mesenchymal cells differentiated under chondrogenic conditions, we found by qRT-PCR that LPC, LPA, and the combination led to significant upregulation of the chondrogenic markers Col2, Runx2, and Sox9. However, there were no significant changes in the gene expression of ColX or MMP13.

DISCUSSION:

The results from these experiments were consistent with our overall hypothesis that LPA inhibits endochondral bone formation by decreasing innervation and vascularization as well as aberrantly promoting chondrocyte proliferation. In our analyses of endochondral bone formation, the Matrigel masses with LPA had the greatest decrease of bone volume fraction, and the addition of LPC did not further diminish ossification. This result suggests that the action of LPC on endochondral bone formation is primarily due to its conversion to LPA, presumably through the enzymatic action of Autotaxin. Furthermore, the main mechanism of action appeared to be diminished chondrocyte maturation, as illustrated by histology and *in vitro* studies. However, the addition of LPA or LPC+LPA, but not LPC alone, significantly impaired innervation and vascularization, which may affect mesenchymal cell fate and/or mineralization separately. As a result, future work will examine the synergy of these effects on OA-related pain and joint degradation.

SIGNIFICANCE:

This project assessed the role of a universally expressed phospholipid which may influence the progression and symptoms of osteoarthritis. Understanding the role of this protein represents a crucial step for uncovering novel therapies to treat OA-related pain and progression.

REFERENCES:

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