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

Emily Rumpf, Sonali Govande, Kennedy McKendall, Deepa S. Kurpad, Theresa A. Freeman, Ryan E. Tomlinson
Thomas Jefferson University, Philadelphia, PA
emily.rumpf@jefferson.edu

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 pain1. In previous work, LPA was shown to activate LPA1 receptors to induce nerve demyelination following injury to generate neuropathic pain2. Moreover, loss of the LPA1 receptor protected against the development of arthritis in mice3. 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 patients4, their role in OA-related joint degradation and pain is poorly understood. Furthermore, previous work has shown that LPA and LPC directly affect mesenchymal cell differentiation5. 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 confirmed 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 morphogenetic protein 2 (BMP2) alone, or mixed with either LPC (5 mM), LPA (25 uM), or LPC and LPA (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 Hematoxylin, Eosin, and Alcan 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 chondrocyte 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 a 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.

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 LPA+LPC 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 LPP and LPA treated masses as compared to other groups by Alcan blue staining. Finally, the innervation and vascularization of the Matrigel mass was significantly diminished in LPA and LPA+LPC groups as compared to control and BMP2 alone using CD31 immunohistochemistry on frozen sections from Thy1-YFP mice. In particular, thick nerve bundles could be seen entering the BMP2 and LPA masses (Fig. 1F-G), but this innervation pattern was missing in LPA and LPA+LPC masses (Fig 1H-I). In mesenchymal cells differentiated under chondrogenic conditions, we found by qRT-PCR that LAC, LPC, 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 LPA did not further diminish ossification. This result suggests that the action of LPA 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 LPA+LPC, but not LPA 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: