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# Lysophosphatidic acid provides a missing link between osteoarthritis and joint neuropathic pain

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SUMMARY

*Objective:* Emerging evidence suggests that osteoarthritis (OA) has a neuropathic component; however, the identity of the molecules responsible for this peripheral neuropathy is unknown. The aim of this study was to determine the contribution of the bioactive lipid lysophosphatidic acid (LPA) to joint neuropathy and pain.
*Design:* Male Lewis rats received an intra-articular injection of 50 μg of LPA into the knee and allowed to recover for up to 21 days. Saphenous nerve myelination was assessed by g-ratio calculation from electron micrographs and afferent nerve damage visualised by activation transcription factor-3 (ATF-3) expression. Nerve conduction velocity was measured electrophysiologically and joint pain was determined by hindlimb incapacitance. The effect of the LPA antagonist Ki-16425 was also evaluated. Experiments were repeated in the sodium monoiodoacetate (MIA) model of OA.
*Results:* LPA caused joint nerve demyelination which resulted in a drop in nerve conduction velocity. Sensory neurones were ATF-3 positive and animals exhibited joint pain and knee joint damage. MIA-treated rats also showed signs of demyelination and joint neuropathy with concomitant pain. Nerve damage and pain could be ameliorated by Ki-16425 pre-treatment.
*Conclusion:* Intra-articular injection of LPA caused knee joint neuropathy, joint damage and pain. Pharmacological blockade of LPA receptors inhibited joint nerve damage and hindlimb incapacitance. Thus, LPA is a candidate molecule for the development of OA nerve damage and the origin of joint neuropathic pain.
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## Introduction

The number one concern of people living with osteoarthritis (OA) is the chronic intractable joint pain that patients suffer from. Pharmacological treatment of OA pain is primarily accomplished by the use of non-steroidal anti-inflammatory drugs (NSAIDs); however, chronic use of NSAIDs can lead to a loss in efficacy and increased risk from harmful side-effects such as gastrointestinal ulceration or kidney failure. Anti-inflammatories tend to be less effective in severe OA patients and more effective in those individuals with synovitis and intermittent flares[1]. In addition, evidence suggests that OA has a neuropathic component in which the pain arises due to direct damage to the peripheral nervous system itself[2]. This neuropathic component of OA pain is unlikely to be affected by intervention with NSAIDs, thus there are significant sub-populations of OA patients who are poorly served by current standards of care for treating the pain and symptoms of OA.

Preclinical evidence of neuropathic damage includes studies which showed that the morphology of sensory nerves innervating injured joints was abnormal and consistent with a peripheral neuropathy[3,4]. Later studies using the sodium monoiodoacetate (MIA) model of OA revealed that joint sensory nerves express the

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neuronal damage biomarker, activating transcription factor-3 (ATF-3)[5,6]. Further preclinical evidence of a neuropathic component of OA pain comes from the observation that nociceptors innervating OA joints fire spontaneously, indicative of sensory nerve damage[7]. Finally, drugs typically used to treat neuropathic pain (e.g., the anti-convulsant gabapentin) have been shown to be effective in alleviating joint nociception and pain in various arthritis models[5,8–10]. Clinical evidence is also emerging which indicates that a subset of OA patients suffer from joint neuropathic pain[11]. Using a modified painDETECT questionnaire in a cohort of knee OA patients, Hochman *et al.* discovered that 28% of participants displayed signs of neuropathic pain[12] although other studies put the number somewhat lower at around 15% of OA patients[13]. The selective serotonin/norepinephrine reuptake inhibitor duloxetine is typically used to treat diabetic neuropathic pain, but has also been shown to be effective in alleviating the symptoms of OA in some patients[14], again corroborating a neuropathic component of OA pain. The chemical mediators responsible for joint neuropathy in chronic OA have yet to be determined.

A common observation in animal models of neuropathic pain is demyelination of damaged sensory nerves with resultant ectopic nerve activity[15–17]. The lipid mediator lysophosphatidic acid (LPA) has emerged as a prominent candidate in initiating afferent demyelination and generating neuropathic pain[18,19]. Biosynthesis of LPA occurs via two distinct pathways. The first involves hydrolysis of phospholipids by phospholipase D into phosphatidic acid which is then converted into LPA by the action of phospholipase $A_1$ or phospholipase $A_2$[20]. The second biosynthetic pathway involves the formation of lysophospholipids from phospholipid precursors which are further converted into LPA by the enzyme autotaxin. There are currently six known LPA receptors ($LPA_{1-6}$) which are all G protein-coupled and heterogeneously expressed in different tissues. In the nervous system, LPA is involved in neurodevelopment as it promotes neurogenesis, neuronal migration and growth cone guidance[21,22]. Intrathecal injection of LPA has been shown to cause demyelination of dorsal root nerves within 24 h and this nerve damage was accompanied by neuropathic-like pain behaviour[23,24]. In $LPA_1$-receptor knockout animals, these morphological and behavioural responses were absent following LPA treatment as well as in animals subjected to sciatic nerve injury. Thus, LPA is a major biochemical moiety that links nerve damage to the development of neuropathic pain.

Fibroblast-like synoviocytes cultured from the joints of rheumatoid arthritis patients are known to express $LPA_{1-3}$ receptors and stimulation of these receptors with exogenous LPA leads to the release of pro-inflammatory cytokines and prostaglandins[25,26]. The severity of collagen-induced arthritis in mice can be ameliorated by treatment with an $LPA_1$ receptor antagonist while arthritic mice lacking the $LPA_1$ receptor showed no sign of joint disease at all[27]. These data suggest that LPA and its receptors are strongly involved in the development of inflammatory joint disease; however, their role in OA has yet to be determined.

The present study examined the effect of LPA on joint nerve myelination and neuronal integrity. Electrophysiological recording from joint primary afferents was used to see if LPA altered neuronal excitability while behavioural tests provided a readout of joint pain. The $LPA_1$ receptor antagonist Ki-16425 was used to block LPA responses and its effectiveness in reducing demyelination and pain behaviour in a model of OA was assessed.

## Materials & methods

### *Human LPA analysis*

A total of 57 patients (44–87 years old; 28 female, 29 male) presenting with knee OA were graded for disease severity based on cartilage pathology using a point scaling system[28]. Disease severity was determined at arthroscopy to be either mild ($n = 11$), moderate ($n = 13$), or severe ($n = 21$). The remaining 12 subjects were non-arthritic normal controls accessed post mortem (Indiana Organ Procurement Organization), whose joints were similarly graded for joint pathology[28]. Samples were analysed by a previously published protocol[29]. Synovial fluid samples (25 μL) were added to a 96-well plate and an internal standard mixture was added to the samples. After centrifuging the plate for 10 min at 5°C and 4000 rpm, 100 μL of supernatant was transferred to a clean 2-mL 96-well plate for liquid chromatographic mass spectrometry (LCMS) analysis.

The quantification of LPA in synovial fluid was accomplished using liquid chromatography electrospray ionization (ESI) tandem mass spectrometry using a TSQ Quantum Ultra-Triple quadrupole mass spectrometer (ThermoFisher, San Jose CA, USA) equipped with an ESI probe and interfaced with an LC system. The lipid extracts were separated with a Gemini C18, 2.1 × 50 mm, 3.5 μm (Waters, Milford MA, USA). Mass spectrometric analyses were performed online using ESI tandem mass spectrometry in the negative multiple reaction monitoring (MRM) mode. LPA levels were quantified by the ratio of analyte and internal standard and calibration curves obtained by serial dilution of LPA.

### *Animals*

All experimental procedures and protocols adhered to the guidelines set out by the Canadian Council on Animal Care and Dalhousie University regulations for the use of laboratory animals, or the Eli Lilly Institutional Animal Care and Use Committee. Male Lewis rats (180–350 g) were obtained from either Charles River (Canada, Quebec) or Harlan (Indianapolis, IN, USA). Animals were housed at 22 ± 2°C on a 12:12 h light:dark cycle (light-on from 7:00–19:00) with free access to food and water.

### *LPA model of joint neuropathy*

Rats received an intra-articular injection (50 μL) into the right knee joint of either vehicle (5% ethanol in 0.9% saline), or 50 μg of lysophosphatidic acid (LPA; Sigma Aldrich Corporation). While other studies have found subcutaneous injection of lower doses of LPA to be effective at sensitizing spinal neurones[30], pilot dose-response analyses indicated that articular concentrations needed to be higher to elicit a robust pain response. A separate cohort of animals was treated with either the LPA receptor antagonist Ki-16425 (30 mg/kg s.c.) or vehicle (20% hydroxypropyl-β-cyclodextrin in distilled water). Ki-16425 is a competitive inhibitor of LPA receptors in order of potency $LPA_1 \geq LPA_3 >> LPA_2$[31]. A loading dose was given 24 h prior to LPA injection and then on 5 consecutive days. After 3 weeks the rats were anesthetized with isoflurane (2–4% in 100% $O_2$; 1 L/min) and subsequently euthanized by an intra-cardiac (1 ml) injection of euthansol (340 mg/ml pentobarbital sodium; CDMV, Quebec).

### *Saphenous nerve isolation and preparation*

The saphenous nerve was isolated and immediately placed into 4% paraformaldehyde fixative at room temperature for at least 24 h. The nerve samples were then removed from the fixative and rinsed three times with 0.1 M sodium cacodylate buffer. The samples were fixed in 1% osmium tetroxide for 2 h, rinsed with distilled water, and then placed in 0.25% uranyl acetate (4°C) overnight. The samples were then dehydrated in a graduated series of acetone (50%, 70%, 95%, and finally 100%). The samples were then dried in 100% acetone for 10 min. Epon araldite resin was utilized to infiltrate the samples in a series of

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