Osteoarthritis and Cartilage

A new art to treating osteoarthritis pain?

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15th June 2023

Dear Prof. Malfait,

As per our email correspondence, please find enclosed my Editorial entitled, "*A new art to treating osteoarthritis pain?*" written in respect to the OAC manuscript OAC13131R1 "*Artemin sensitizes nociceptors that innervate the osteoarthritic joint to produce pain*".

I hope that aligns with expectations and would welcome any feedback.

Yours sincerely,

Prof. Ewan St. John Smith

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A new art to treating osteoarthritis pain?

Osteoarthritis (OA) pain involves peripheral and central neural pathways, as well as interactions between multiple cell types and soluble mediators[1]. Although therapeutic targeting of peripheral OA pain pathways might prove simpler and be associated with fewer side effects, the complexity of OA pain means that it will likely be the case that what works for one patient will be inefficacious for another. Consequently, it is important to further our understanding of OA pain mechanisms to develop new treatments and thus expand the clinician's therapeutic arsenal.

During OA, the impact of inflammation on pain is multifactorial. In addition to synovitis and the articular inflammatory soup, neuroinflammation occurs within the dorsal root ganglia (DRG, where sensory neuron cell bodies reside), the spinal cord (the first nerve-nerve communication in the pain pathway), and the brain[2]; two further sources of inflammation are those associated with metabolic syndrome and inflammageing, perhaps especially important considering that obesity and age are risk factors for OA.

Regarding local inflammation, magnetic resonance imaging (MRI) has shown that synovitis is associated with sensitization of knee OA pain[3], but what factors drive such sensitization? In individuals with OA, it has been demonstrated that synovial tissue from sites of patient-reported pain has a distinct phenotype with fibroblast-like synoviocytes (FLS) from such sites promoting inflammation[4], and, in a co-culture model, activated FLS can sensitize articular sensory neurons[5]. Considering the plethora of inflammatory mediators associated with OA, it is important to understand mechanistically how a given substance might cause OA pain to determine likely in vivo efficacy and predict potential side effects. Nerve growth factor (NGF) was first shown to produce thermal/mechanical hyperalgesia in rats[6] and a substantial body of work demonstrated a role of NGF in OA pain leading to the development of anti-NGF monoclonal antibodies. However, despite numerous promising clinical trials demonstrating analgesic efficacy of anti-NGF antibodies in treating OA pain[7], the occurrence of rapidly progressive OA resulted in one of these, tanezumab, failing to obtain Food and Drug Administration or European Medicines Agency regulatory approval. Does this suggest that targeting inflammatory mediators for the treatment of OA is a fruitless exercise? I would say, "no". Targeting pro-inflammatory substances or their signaling pathways in rheumatoid arthritis pain has proved efficaciou[8]s, thus demonstrating that such an approach can be beneficial. Although other options should be considered, such as viral-mediated inhibition of articular neuron activity[9] or mesenchymal stem cell-derived articular analgesia[10], more research is required to determine clinical implementation of such approaches. Therefore, if the door to anti-NGF antibodies for treating OA pain appears shut, which other inflammatory mediator shaped doors might be opening?

In this issue of *Osteoarthritis and Cartilage*, Morgan et al. use *in vitro* and *in vivo* approaches to provide novel insight into the role of the neurotrophic factor artemin in OA pain. Artemin is a member of the glial cell line-derived neurotrophic factor (GDNF) family and binds to GDNF family receptor α3 (GFRα3), which is mostly expressed by peptidergic, sensory neurons that co-express the receptor for NGF, tropomyosin receptor kinase A (TrkA), although TrkA shows broader expression overall.

In work leading up to the current publication, it has been shown that serum artemin concentration is increased in dogs with OA compared to healthy controls, with a similar trend observed in humans, and that there is more GFRα3 in DRG innervating OA vs. healthy joints in dogs[11]. Furthermore, in the monoiodoacetate (MIA) OA model in mice, an ipsilateral increase in DRG GFRα3 expression was observed and an anti-artemin sequestering antibody alleviated cold, heat and mechanical hypersensitivity, as well as limb asymmetry; hind paw artemin injection itself inducing cold, heat and mechanical hypersensitivity[12]. In rats, it has further been shown that bone marrow cavity artemin application sensitizes bone afferents and alters weight bearing, with weight bearing deficits induced by bone marrow complete Freund's adjuvant injection being ameliorated by anti-artemin antibody co-injection[13]; similar effects of an anti-artemin antibody on bone afferent activity were observed in a carrageenan inflammatory pain model[14]. However, a Phase 2 study, NCT03956550, investigating efficacy and safety of the anti-GFRα3 monoclonal antibody nadecnemab (REGN5069) in individuals with knee OA pain produced results that were less encouraging. Could then targeting of the ligand, rather than the receptor, prove more beneficial?

The study by Morgan et al. looked to further understand the contribution of artemin-GFRα3 signaling to OA pain using the rat MIA model. They firstly demonstrated that ~20% of rat knee afferents express GFRα3 and that these are small/medium sized neurons, i.e. putative nociceptors. Using an elegant *in vivo* electrophysiology model, the authors recorded from the medial articular nerve that innervates the knee joint capsule and observed that administration of artemin into the joint cavity reduced afferent activation threshold and increased discharge frequency compared to saline administration, thus the effects were most likely GFRα3 dependent, rather than due to increased volume/pressure.

Moving to the MIA model, increased levels of artemin were observed in the synovial fluid and synovial tissue during both the early phase (day 3) when inflammation is localized to the joint and joint, but not bone afferents are sensitized, and in the late phase (day 28) when damage occurs in the subchondral bone concomitant with recruitment of sensitized bone afferents[15]. These results align with the observation in dogs with OA of a positive association between synovial fluid artemin concentration and joint pain[11]. By contrast, whereas increased DRG GFRα3 expression occurs in dogs with OA[11], such an increase in DRG GFRα3 expression was not observed at early or late time points in the rat MIA model. In addition to sex/species differences, it is possible that an immunohistochemical analysis of GFRα3 expression in retrograde traced joint afferents could provide a higher signal to noise and might reveal a change in expression. Moreover, although the authors do not measure GFRα3 expression in synovial tissue samples, others have shown that GFRα3 is expressed by human FLS[4] and thus this opens the door to the effects of artemin on neuronal activity observed being due to direct stimulation of neurons and/or indirect stimulation following artemin-induced release of substances from FLS.

With artemin levels being elevated in early/late phases in the synovial fluid and late phase in the subchondral bone, the question is to what extent the raised artemin is involved in joint and bone afferent sensitivity? To measure this, the authors used their *in vivo* electrophysiology preparation to measure joint and bone afferent mechanical sensitivity in response to von Frey stimulation of the medial joint capsule and raising of intraosseous pressure in the tibial marrow cavity, respectively. Compared to saline treated animals, sensitization of both joint and bone afferents was observed in late phase MIA. In MIA treated rats, the sensitization of both joint and bone afferents in terms of activation threshold and discharge frequency had significantly resolved 15-minutes after administration of an artemin sequestering antibody, whereas no such effect was observed when an isotype control antibody was administered. Only animals with pain behavior underwent electrophysiological testing, and thus, these results strongly suggest that artemin is a key player in OA pain during the late phase.

Having previously observed that joint, but not bone afferents are sensitized during the early phase[15], and in the current paper that artemin expression in subchondral bone was only increased during the late phase, the authors finally investigated the effects of an artemin sequestering antibody on early phase joint afferent sensitization. Although MIA-induced joint afferent sensitization was observed in line with previous findings[15], measuring activity before and 15-minutes after administration of the artemin sequestering antibody showed that it had no discernable impact on afferent activity. This result is perhaps surprising considering that increased artemin was observed in the synovial fluid and synovial tissue of MIA rats in early and late phases. However, whereas the change in synovial fluid artemin concentration was similar at both early and late phases, a ~2-fold increase in synovial tissue artemin concentration at day 3 increases to a ~3-fold increase at day 28, thus the contribution of artemin to OA pain could increase with disease progression, which might explain the lack of effect of artemin sequestration in the early phase. Alternatively, as the authors speculate, it could be that one or more of the myriad of other sensitizing factors identified in OA[1,2] play a more significant role during the early phase, such that artemin sequestration alone has no effect.

Overall, considering the compelling evidence from mouse[12], dog[11] and, as presented in the current paper, rat, for a role of artemin-GRFα3 signaling in OA pain, it would seem sensible to pursue this as a line of further investigation towards developing novel treatments of OA pain in humans. This is especially the case considering that the analgesic efficacy of anti-NGF antibodies in OA is undisputed and thus the targeting of atermin-GRFα3 signaling, which is largely confined to a subset of TrkA expressing afferents, might result in analgesic efficacy without the associated side effects of anti-NGF antibodies.

Conflict of interest statement
The author has no relevant conflicts of interest to declare.

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AUTHORS' DISCLOSURE

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Authorship
All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted. By signing below each author also verifies that he (she) confirms that neither this manuscript, nor one with substantially similar content, has been submitted, accepted or published elsewhere (except as an abstract). Each manuscript must be accompanied by a declaration of contributions relating to sections (1), (2) and (3) above. This declaration should also name one or more authors who take responsibility for the integrity of the work as a whole, from inception to finished article. These declarations will be included in the published manuscript.

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