Summary of Peer Reviewed Publications supporting PPS Mechanism of Action for the treatment of Osteoarthritis.

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Osteoarthritis (OA) Background

- Osteoarthritis is a degenerative disease involving the whole joint.
- The development of osteoarthritis has been characterised through the assessment of disease markers.
- These markers have included:
    - X-ray and MRI to identify structural changes within the joint that indicate OA development and progression.
      AND
    - Biomarkers that have demonstrated changes in blood, urine, and synovial joint fluid.

Pentosan Polysulfate Sodium (PPS)

- PPS is a semi-synthetic polysaccharide derived from Beechwood.
- PPS has been widely used and proven to be beneficial for the treatment of interstitial cystitis and bladder pain syndrome in Europe and the US for over 20 years.
- PPS has also been used in Veterinary practice for over 20 years for the treatment of osteoarthritis in dogs and horses.

List of Abbreviations

ADAMTS-5	Adamalysin with thrombospondin motifs 5
ADL	Activities of daily living
BML	Bone marrow lesion
C2C	Type II collagen cleavage product
HIF-1α	Hypoxia inducible factor 1 alpha
IL-1β	Interleukin 1 beta
iNOS	Inducible nitric oxide species
MMP-3	Matrix metalloproteinase 3
mRNA	Messenger ribonucleic acid
NF-κB	Nuclear factor kappa B
NGF	Nerve growth factor
NO	Nitric oxide
OA	Osteoarthritis
PPS	Pentosan Polysulfate sodium
ROM	Range of movement
TIMP-3	Tissue inhibitor of metalloproteinases 3
TNF-α	Tumour necrosis factor alpha
VAS	Visual analogue scale

Summaries of nonclinical and clinical publications relevant to the mechanism of action of PPS.

Nonclinical Studies

Reference	Summary of Findings
Sunaga T, Oh N, Hosoya K, Takagi S, Okumura M. Inhibitory effects of pentosan polysulfate sodium on MAP-kinase pathway and NF-κB nuclear translocation in canine chondrocytes in vitro. J Vet Med Sci. 2012 Jun;74(6):707-11. Epub 2011 Dec 28. PMID: 22214865. https://doi.org/10.1292/jvms.11-0511	***Top line findings: PPS inhibits activation of the transcription factor, NF-κB, which reduces production of pro-inflammatory cytokines and cartilage degrading enzymes.*** This study describes the anti-inflammatory effects of PPS treatment in a cell culture model of cartilage cells (chondrocytes). The data demonstrate that PPS treatment of inflamed chondrocytes inhibits inflammation by blocking the transcription factor, NF-κB in the cytoplasm of the cell. This blockade prevents the translocation of NF-κB to the nucleus. The effect of PPS on NF-κB blockade is such that it inhibits the gene transcription of several inflammatory molecules. Inflammation was induced by exposing chondrocytes to the pro-inflammatory cytokine, IL-1β. Chondrocytes under the stimulation of IL-1β demonstrated increased translocation of NF-κB into the nucleus by fluorescence microscopy. PPS treatment in IL-1β stimulated cells demonstrated the retention of NF-κB within the cytoplasm. In addition, the inhibition of NF-κB translocation by PPS significantly reduced the expression of the cartilage degrading enzyme, matrix metalloproteinase-3 (MMP-3), signifying a role in reducing cartilage degradation. ***Relevance to OA :*** **PPS may have a role in reducing inflammation and preventing cartilage degradation in OA joints by acting as an inhibitor of NF-κB.** ***Pro-inflammatory cytokines :*** Molecules that mediate inflammation. ***IL-1β :*** Interleukin-1β is a pro-inflammatory cytokine that stimulates NF-κB activation, which contributes to inflammation observed in OA. ***NF-κB :*** Nuclear factor kappa B is a transcription factor that is inactive in the cytoplasm of the cell and when activated, translocates to the cell nucleus where it transcribes mRNA from DNA to produce many copies of inflammatory mediators such as IL-1β. ***MMP-3 :*** Matrix metalloproteinase-3 is a cartilage degrading enzyme that contributes to cartilage breakdown observed in OA, and its function can be inhibited by TIMP-3.
Troeberg L, Mulloy B, Ghosh P, Lee MH, Murphy G, Nagase H. Pentosan polysulfate increases affinity between ADAMTS-5 and TIMP-3 through formation of an electrostatically driven trimolecular complex. Biochem J. 2012 Apr 1;443(1):307-15. https://doi.org/10.1042/bj20112159	***Top line findings: PPS is an inhibitor of the enzyme ADAMTS-5, which breaks down the proteoglycan, aggrecan, a major component of cartilage.*** In this study, the mechanism of how PPS inhibits the enzyme ADAMTS-5 is described. There is a naturally occurring inhibitor to ADAMTS-5 called TIMP-3. PPS was shown to form a complex with TIMP-3 and ADAMTS-5. The enzyme inhibition mediated by PPS was attributed to the increase in the binding affinity between the cartilage degrading enzyme, ADAMTS-5 and its natural inhibitor TIMP-3. ***Relevance to OA :*** **PPS reduces the activity of cartilage degrading enzymes, indicating a potential for PPS to prevent cartilage degradation.** **ADAMTS-5 :** Adamalysin with TS (thrombospondin) motifs 5 is an enzyme that breaks down the extracellular protein called aggrecan and is found in high levels in articular cartilage in OA. The actions of ADAMTS-5 can be inhibited by TIMP-3. **TIMP-3 :** Tissue inhibitor of metalloproteinases 3 is a protein that is protective against cartilage degrading enzymes (ADAMTS and MMPs).

Bwalya EC, Kim S, Fang J, Wijekoon HMS, Hosoya K, et al.
Pentosan polysulfate inhibits IL-1β-induced iNOS, c-Jun and HIF-1α upregulation in canine articular chondrocytes.
PLOS ONE. 2017 12(5): e0177144. https://doi.org/10.1371/journal.pone.0177144

Top line findings: PPS prevents entry of NF-κB into the cell nucleus reducing the gene expression of pro-inflammatory mediators.

OA is a degenerative joint disease that causes loss of joint function affecting the entire joint including articular cartilage, subchondral bone, ligaments, capsule, synovial membrane and menisci. Osteoarthritic chondrocytes in affected joints have been shown to produce increased levels of inflammatory cytokines. Particularly, OA chondrocytes express inducible nitric oxide synthase (iNOS) and produce high concentrations of nitric oxide (NO), especially upon stimulation by proinflammatory cytokines. This pathologically increased NO production plays an important catabolic role in OA cartilage degradation. The excess production of NO combined with an upregulated IL-1 receptor level has been shown to be an additional enhancer of the catabolic effects of IL-1β in OA. Therefore, the selective inhibition of pathologically enhanced NO synthesis has been identified as a promising novel therapeutic target for the prevention and treatment of inflammatory joint diseases. The present study demonstrates for the first time that PPS is a novel inhibitor of IL-1β-induced iNOS, c-Jun and HIF-1α mRNA upregulation. The mechanism of action of PPS as demonstrated by immunocytochemistry analysis show that PPS colocalizes with NF-κB and c-Jun and inhibits their nuclear translocation and localization. This observation potentially supports the inhibitory effects of PPS on OA by direct interaction with NF-κB and c-Jun, consequently repressing the downstream target genes like iNOS and MMP-13 which are implicated in the progression and perpetuation of OA.

***Relevance to OA :* PPS may have a role in reducing inflammation in OA by inhibiting the production of pro-inflammatory mediators.**

iNOS : Inducible nitric oxide species are produced by chondrocytes in response to stress and inflammation and contribute to cartilage damage.
c-Jun : c-Jun is a transcription factor involved in the inflammatory processes observed in OA.
HIF-1α : Hypoxia-inducible factor-1α is a transcription factor that is dysregulated in OA. `

Stapledon CJM, Tsangari H, Solomon LB, et al. Human osteocyte expression of Nerve Growth Factor: The effect of Pentosan Polysulphate Sodium (PPS) and implications for pain associated with knee osteoarthritis.
PLoS One. 2019;14(9):e0222602. https://doi.org/10.1371/journal.pone.0222602

Top line findings: PPS reduces the expression of nerve growth factor, a mediator of pain in osteoarthritis.

As bone pain is a considerable clinical symptom in knee OA, it was hypothesised that bone cells (osteocytes) derived from patients with knee OA could produce the pain mediator, nerve growth factor (NGF) locally within subchondral bone. The induction of high levels of NGF by proinflammatory cytokines in OA has been proposed to sensitise peripheral sensory neurons to pain. The seminal data reported in this study demonstrated that freshly isolated osteocytes from the subchondral bone samples of OA patients undergoing total knee replacement could produce NGF. Of important relevance to OA was that the pro-inflammatory cytokine tumour necrosis factor alpha (TNF-α) when added to osteocytes produced high levels of NGF mRNA and protein. When osteocytes in the presence of TNF-α were treated with PPS, there was a significant reduction in the expression of NGF (mRNA and protein) albeit to normalised baseline levels, when compared to control cells (without PPS treatment). These findings indicate that high levels of NGF are normalised to low baseline levels by PPS acting at the transcriptional level and consequently affecting NGF protein expression and secretion. Therefore, in an inflammatory milieu depicted in the OA joint, PPS treatment would be expected to normalise NGF levels and consequently reduce sensitisation to pain.

Relevance to OA: PPS may have a role in reducing the pain observed in OA by reducing expression of the pain mediator NGF.

NGF : Nerve growth factor is a major pain mediator involved in the pain response in several chronic diseases including OA.
TNF-α : Tumour necrosis alpha is a pro-inflammatory cytokine, which activates NF-κB and is also produced upon NF-κB activation. TNF-α expression is increased in OA, reflecting inflammation that occurs in the joint.

Clinical Studies

Reference	Summary of Findings
Ghosh P, Edelman J, March L, Smith M. Effects of pentosan polysulfate in osteoarthritis of the knee: A randomized, double-blind, placebo-controlled pilot study. Curr Ther Res Clin Exp. 2005;66(6):552-571. https://doi.org/10.1016/j.curtheres.2005.12.012	***Top line findings: Once weekly PPS injections for 4 weeks are effective in reducing pain and improving function in knee osteoarthritis.*** In this randomised, double-blind, placebo-controlled pilot study of 114 knee OA subjects, the effects of PPS treatment on pain, function and stiffness as assessed by activities of daily living (ADL) and visual analogue 10-point scale (VAS) were explored. Subjects received weekly 3 mg/kg intramuscular PPS injections for 4 weeks and the effects of PPS were assessed during the treatment period and post-treatment at weeks 8, 12, 16 and 24 post-initial injection. PPS treatment in these subjects resulted in significant improvements in a number of pain and function related outcomes. More specifically, joint stiffness and pain at rest were significantly improved at 4-, 12- and 20-weeks post treatment in PPS-treated subjects. Subjects receiving PPS also had significantly improved activities of daily living (ADL) function scores at weeks 8 and 12 post treatment, indicative of improvement in their ability to carry out everyday tasks. ***Relevance to OA:*** **This study directly demonstrates the potential beneficial effects of PPS in human knee OA.** **ADL :** Activities of daily living is a widely recognised questionnaire designed to measure effects of an intervention on quality of life. **VAS :** Visual analogue scale is a measurement instrument used to measure pain intensity across a range of continuum values in patients with arthritis.
Kumagai K, Shirabe S, Miyata N, et al. Sodium pentosan polysulfate resulted in cartilage improvement in knee osteoarthritis--an open clinical trial. BMC Clin Pharmacol. 2010;10:7. https://doi.org/10.1186/1472-6904-10-7	***Top line findings: PPS treatment reduced pain, improved function, and reduced the cartilage degradation biomarker C2C with durability of response for up to 52 weeks in knee osteoarthritis subjects.*** Twenty symptomatic primary knee OA subjects were enrolled into a single centre, open label trial which aimed to investigate the efficacy, safety, and subject satisfaction following weekly 2 mg/kg subcutaneous PPS over a 6-week period. Subjects underwent pain, function, biomarker, and radiographic assessment at baseline and several timepoints up to one year to determine improvements in their condition. Pain at rest and walking, pain with range of movement (ROM) exercises, walking up and down stairs, 50 m walking and at 5 mins rest after exercises were assessed routinely as was the serum biomarker of cartilage degradation (C2C). PPS treatment resulted in significant improvements in functional outcomes (flexion angle of the knee), pain (pain score post-stair climbing) up to 1 year following study initiation and a significant reduction in serum C2C levels at 8- and 24-weeks post study initiation. ***Relevance to OA:*** **This study directly demonstrates the potential beneficial and long-lasting effects of PPS in human knee OA.** **C2C:** Serum cartilage type II collagen is a biomarker of collagen degradation and serum levels correlate with osteoarthritis severity.

Sampson MJ, Kabbani M, Krishnan R, Nganga M, Theodoulou A, Krishnan J. Improved clinical outcome measures of knee pain and function with concurrent resolution of subchondral Bone Marrow Edema Lesion and joint effusion in an osteoarthritic patient following Pentosan Polysulphate Sodium treatment: a case report.
BMC Musculoskelet Disord. 2017 Sep 12;18(1):396.
https://doi.org/10.1186/s12891-017-1754-3

Top line findings: PPS treatment reduced bone marrow lesion size and associated pain in a case study of a 70-year-old chronic OA patient.

In this case study of an OA patient presenting with high levels of pain, reduced function and a large BML at the time of diagnosis, intramuscular PPS at a dose of 2 mg/kg given twice weekly for 3 weeks was investigated. At baseline, the patient reported a high pain score of 8 using the Numerical Rating Scale (0 = no pain & 10 = the worst pain imaginable), reduced functional capacity measured by the Lysholm Knee Score was reported as poor with a score of 37 out of 100 at baseline. In addition, a large subchondral BML was also diagnosed by MRI at baseline in this patient. At 4 weeks following the final PPS injection, the patient reported a robust reduction in pain (NRS = 0) and a marked improvement in function (Lysholm Knee Score = 65). Finally, at 5 weeks following the first PPS injection a complete resolution of the large subchondral BML was reported, indicating that PPS may act as an effective therapy for the treatment of pain and BML in knee OA.

***Relevance to OA:* PPS treatment may be effective at reducing BML size and improving BML-associated pain through reducing inflammation.**

BML : Bone marrow lesions are areas of capillary leakage and extracellular fluid accumulation present in the subchondral bone in knee OA and are a major source of pain and discomfort.

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