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PEER-REVIEWED PUBLICATION SUPPORTS ARTHRITIC DISEASE MODIFYING EFFECTS OF PPS IN CHIKUNGUNYA VIRUS

KEY HIGHLIGHTS

- Preclinical study investigating the anti-inflammatory and arthritic disease modifying effects of pentosan polysulfate sodium (**PPS**) in the mouse model of the alphavirus chikungunga (**CHIKV**) induced arthralgia has been published online in the scientific peer-review journal, PLoS ONE.
- PPS showed significant functional joint improvement as measured by grip strength and anti-inflammatory effect by the reduction in hind limb foot swelling compared to infected control animals in the mouse model of CHIKV induced arthralgia.
- PPS treatment protected the joints from cartilage damage by reducing immune cell infiltration into the joints which were associated with statistically significant reduction of the chemokines CXCL1, MCP-1, MCP-3 and MCP-5 compared to untreated CHIKV infected animals.
- PPS actions were related to alterations in gene expression involved in growth factor signalling and lymphocyte activation locally within the inflamed arthritic joints of CHIKV infected mice.
- The demonstration that PPS can improve strength, swelling and Inflammation in the CHIKV preclinical model supports further clinical evaluation of PPS as a potential disease modifying therapy for CHIKV-induced arthralgia following the promising clinical outcomes of improved pain and function in subjects in Paradigm's phase 2A Ross River virus (**RRV**) pilot clinical trial.
- "As joint pain and loss of limb strength are common aspects of CHIKV induced disease, these critical findings provide confidence in PPS as a potential world first CHIKV therapy" *Dr Lara Herrero.*
- Currently, there are no specific treatments or vaccines available to treat CHIKV and RRV infections therefore advocating the need for the development of novel therapeutic strategies to treat alphavirus-induced arthralgia (CHIKV and RRV).

Paradigm Biopharmaceuticals Ltd (ASX: PAR) ("Paradigm" or "the Company") is pleased to announce the publication of "*Pentosan polysulfate sodium prevents functional decline in chikungunya infected mice by modulating growth factor signalling and lymphocyte activation*" in the peer-review scientific journal, PLoS ONE, which was published online on 8th September, 2021 (see link below for publication). The preclinical study was undertaken at Griffith University's, Institute for Glycomics by Principal Investigator and corresponding author of the publication, Dr Lara Herrero.

Paradigm reported on the 18th March[1] a peer revied publication of results from the phase 2A clinical trial undertaken in subjects with Ross River virus (RRV), an alphavirus. The pilot study demonstrated clinical evidence in a small population (n=18), that PPS treatment in

subjects with RRV induced arthralgia improved clinical outcomes in pain and function. The data also supported the anti-inflammatory and potential chondroprotective (cartilage) actions of PPS through the reduction in key inflammatory and cartilage biomarkers. The RRV clinical data provided the impetus to Investigate whether PPS was also capable of reducing the arthralgia associated with chikungunya virus (CHIKV), an alphavirus closely related with a considerably higher prevalence globally than RRV.

The company undertook a proof-of-concept animal study in CHIKV to investigate whether PPS demonstrated biological activity in the treatment of CHIKV induced arthralgia. The study design involved injection of the ankle joint with CHIKV to produce a local infection. The animals were grouped into CHIKV infected animals that were either a) treated with PPS or b) untreated, or control animals that were not infected that were either c) treated with PPS or d) untreated. PPS treatments consisted of injecting PPS daily till peak disease was achieved at 7 days post-infection or at 21 days when the model demonstrates self-resolution of the disease.

The data reported in this preclinical study showed objective measures of functional improvement as measured by grip strength, which was supported by reduction in inflammatory cells in the joints of animals treated with PPS. The chemokines, CXCL1, MCP-1, MCP-3 and MCP-5 involved in immune cell infiltration of the joint showed statistically significant reduction in serum levels because of PPS treatment. The anti-inflammatory effect of PPS was demonstrated by statistically significant reduction in the swelling of the foot joint compared to untreated CHIKV infected animals. The global gene expression analysis of the local joint demonstrated that the actions of PPS were related to the regulation of genes involved in growth factor signalling and lymphocyte activation.

Dr Ravi Krishnan, Paradigm's Chief Science Officer, commented: *"Our recent preclinical data in the CHIKV mouse model has provided scientific support for the potential of PPS to treat debilitating symptoms associated with long term CHIKV infection. Therefore, to establish the clinical efficacy and safety of PPS in alphavirus-induced arthralgia following Paradigm's completed phase 2A clinical trial in RRV-induced arthralgia, a larger clinical trial involving CHIKV infection which has a higher global prevalence is warranted".*

Results of the Preclinical Study:

PPS treatment of CHIKV in mice improves grip strength and foot swelling

Paradigm has previously reported that PPS is able to improve hand strength in patients suffering from RRV[1]. To further evaluate the capability of PPS to improve functional signs of CHIKV disease the mouse model of CHIKV infection was used for the assessment of grip strength. Grip strength of all limbs was measured daily with a validated computerised grip strength meter.

Analysis of normalised grip strength [force (g)/body weight (g)] at baseline (day 0) and peak disease (day 6) did not show any significant changes in the uninfected control groups, or the CHIKV infected PPS-treated groups compared to baseline. However, the CHIKV-infected untreated group showed a significant reduction ($P < 0.0002$) in normalised grip strength at peak disease (6.5 ± 0.4; mean ± SEM) compared to baseline values (8.2 ± 0.3). This equated to an overall 19.8% ± 5.1 reduction in grip strength in the CHIKV-infected untreated group between 0- and 6-days post-infection (d.p.i.). In the CHIKV-infected PPS-treated mice, grip strength was unchanged between days 0 and 6 post-infection (reduction of 0.5% ± 3.6, mean ± SEM). When CHIKV-infected untreated and CHIKV-infected PPS-treated groups were compared, a significant difference in the percentage change between 0 and 6 d.p.i. was shown ($P < 0.02$).

To assess the anti-inflammatory activity of PPS on disease severity, hind foot swelling was assessed daily in both peak disease (6–7 d.p.i.) and disease resolution (21 d.p.i.). CHIKV-infected untreated mice had an increase from baseline of 99.7% ± 5.6; mean ± SEM (6 d.p.i.); and 88.6% ± 4.0 (7 d.p.i.). CHIKV-infected PPS-treated animals only showed an increase of 45.4% ± 4.3 (6 d.p.i.) and 51.3% ± 4.3 (7 d.p.i.). This represented a significant reduction in swelling between CHIKV-infected untreated and CHIKV-infected PPS-treated mice ($P < 0.0001$).

PPS reduces the number of infiltrates in the hind limbs at peak infection

Histological analysis was conducted to assess the activity of PPS on local inflammation following CHIKV infection. Abundant infiltrates characteristic of immune cells (monocytes and neutrophils) were seen in the inflamed ankle joint (calcaneal region, surrounding muscle, metatarsal bones, and bone marrow) in the CHIKV-infected untreated group. In contrast, CHIKV infected PPS-treated mice displayed a visible reduction in the overall number of infiltrates in these structures of the hind limbs indicating that PPS protected muscle fibres from damage. Furthermore, PPS treatment appeared to accelerate the inflammatory repair processes with evidence of an increase in the number of regenerating myocytes.

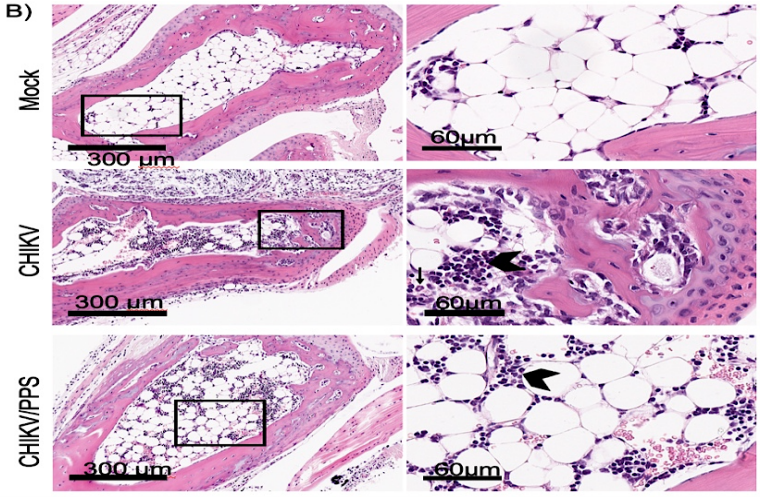

Fig 1: CHIKV-infected PPS-treated mice displayed a visible reduction in the overall number of infiltrates in these structures of the hind limbs.

PPS treatment reduces joint destruction

Cartilage integrity was determined semi-quantitatively by the amount of proteoglycan content in cartilage determined by Saf-O staining and by cartilage shrinkage on samples with masked identity. CHIKV-infected untreated mice showed a marked depletion of proteoglycans with corresponding cartilage shrinkage, which was significantly improved with PPS treatment ($P = 0.0125$). CHIKV infected untreated mice had a score of 2.2 ± 0.4 (mean ± SEM) on day 7 p.i. and 1.4 ± 0.4 on day 21 post-infection. In comparison, CHIKV-infected PPS-treated mice had less severe cartilage changes 1.0 ± 0.002 on day 7 p.i. and 0.8 ± 0.2 on day 21 post-infection. Control uninfected animals did not show changes in cartilage proteoglycans. While bone integrity was assessed histologically and scored based on osteoclast/osteoblast activity, bone necrosis and vascular changes there were no significant changes observed with PPS treatment.

PPS treatment modifies the serum levels of chemokines and cytokines in CHIKV-induced inflammation

Serum chemokine and cytokine levels of all groups were quantified at 7 d.p.i. (peak disease) to assess the anti-inflammatory activity of PPS. Compared to CHIKV-infected untreated mice, CHIKV-infected PPS-treated mice showed significant reductions in serum biomarkers for the chemokines (CXCL-1; P = 0.0331), MCP-1 (P = 0.058), MCP-3 (P= 0.0047) and MCP-5 (P =0.0007). Other inflammatory chemokines and cytokines, which were not significantly altered but were trending towards a reduction in expression after treatment with PPS included interlukin-2 (IL-2), interleukin-6 (IL-6), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory 1 protein-1 beta (MIP-1β; CCL4), TNF-α, CX3CL1 (fractalkine), and interferon-inducible T-cell alpha chemoattractant (CXCL11; I-TAC).

PPS modulates specific pathways in both joint and muscle tissues during CHIKV infection

Gene expression analysis of RNA isolated from joint tissue and muscle at peak disease was performed using the commercially available NanoString™ nCounter1mouse Myeloid Innate Immunity gene expression panel. Bioinformatic analysis of the tissues demonstrated that among the most significantly modulated genes were of particular interest due to their clinical relevance for arthritis.

Of relevance to the mechanism of PPS, three pathways were seen to be associated with arthropathies. These include pathways in cytokine-cytokine receptor interactions, pathways in cancer and PI13-AKT signalling. When examining the critical functional groups, PPS was shown to exert effect on growth factor signalling, lymphocyte activation and pathogen response, all of which likely contribute to the improvement of CHIKV-induced arthritic disease.

Please refer to the following link for the full article:
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0255125

Dr Lara Herrero, Principal Investigator and corresponding author, commented*:*

"Our foundational preclinical studies demonstrate that PPS not only improves the inflammation/arthritis that ensues after infection, but also improves limb function with mice showing improvements in grip strength. As joint pain and loss of limb strength are common aspects of CHIKV induced disease, these critical findings provide confidence in PPS as a potential world first CHIKV therapy. With no vaccines or specific therapeutics to prevent/treat alpha viral-induced arthralgia, the results of our study gives hope to millions suffering with alpha-viral arthritis globally".

About Chikungunya Virus (CHIKV):

CHIKV is closely related to the Ross River virus (both of the alphavirus genera) and causes similar debilitating symptoms of joint pain, fever, headache, conjunctivitis, and rash. The disease course is divided into an acute stage, lasting approximately one week, and a chronic stage, also known as the persistent stage, which can last from months to years. Acute fever and polyarthralgia are highly indicative of an infection, with arthralgia (joint pain) appearing in 30–90% of cases. This joint pain is often bilateral, symmetric, and debilitating. There are occasional ophthalmic, neurological, and cardiac symptoms[1].

CHIKV outbreaks have been reported in parts of Africa, Europe, Southeast Asia, and islands in the Indian and Pacific Oceans. In 2013, CHIKV was found for the first time in the Americas and has spread to the Caribbean as well as South and Central America[3]. The lack of anti-viral agents, vaccines or effective pharmaceutical agents to treat the debilitating effects of CHIKV make iPPS a potentially valuable product for this unmet medical need.

About Paradigm Biopharmaceuticals

Paradigm Biopharmaceuticals LTD (ASX: PAR) is a late-stage drug development company with the mission to develop and commercialise pentosan polysulfate sodium for the treatment of pain associated with musculoskeletal disorders driven by injury, inflammation, ageing, degenerative disease, infection or genetic predisposition.

Forward Looking Statements

This Company announcement contains forward-looking statements, including statements regarding anticipated commencement dates or completion dates of preclinical or clinical trials, regulatory developments and regulatory approval. These forward-looking statements are not guarantees or predictions of future performance, and involve known and unknown risks, uncertainties and other factors, many of which are beyond our control, and which may cause actual results to differ materially from those expressed in the statements contained in this presentation. Readers are cautioned not to put undue reliance on forward-looking statements.

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[1]ASX Release 18th March 2021: Phase 2 RRV data published in Peer-Reviewed Journal
[2] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6348186/
[3] https://onlinelibrary.wiley.com/doi/pdf/10.1002/acr.22392
[4] https://www.ncbi.nlm.nih.gov/pubmed/18793006

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