Blocking Acute Neutrophil Recruitment with Poly Salicylic Acid Particles Mitigates Pain and Inflammation in PTOA

Lindsey Lammlin[1, 2], Michael LaRue Felder[2], Adrienne A. Giannone[1, 2], Omolola Eniola-Adefeso[3], Tristan Maerz[1, 2]
[1]ETH Zürich, Zürich, Switzerland, [2]University of Michigan, Ann Arbor, MI, United States, [3]University of Illinois Chicago, Chicago, IL, United States.
lindsey.lammlin@hest.ethz.ch

Disclosures: TM is a paid consultant for RelationRx.

INTRODUCTION: Neutrophils are among the first responders that home to the joint following injury to initiate the inflammatory response. We propose that targeting neutrophils represents a promising strategy for early intervention against inflammation and pain development in post-traumatic osteoarthritis (PTOA). Previously, systemic treatment with poly salicyclic acid (PolySA) particles has been shown to target activated neutrophils, rerouting them away from the site of injury in murine models of acute lung injury and acute respiratory distress syndrome. Here, we tested the therapeutic efficacy of acute, neutrophil-rerouting intervention via systemically administered PolySA particles in a murine model of PTOA.

METHODS: With IACUC approval, mice underwent Sham procedure (anaesthesia/analgesia only) or non-invasive anterior cruciate ligament rupture (ACLR) to induce PTOA. A summary of experimental cohorts is specific in **Table 1**. Knee synovia were collected 0 to 48hrs post-ACLR to evaluate early neutrophil influx by flow cytometry. Additional mice underwent ACLR and retro-orbital treatment 12hrs post-ACLR with vehicle or neutrophil-targeting poly-salicylic acid particles (PolySA, low dose = $2*10^8$ particles, high dose = $4*10^8$ particles). Synovia were harvested 24hrs post-ACLR to quantify neutrophils and monocytes by flow cytometry. To evaluate the effect of PolySA at early and late stages of PTOA development, mice received retro-orbital vehicle or PolySA (high dose) treatment at 12hrs and 48hrs post-ACLR, followed by PTOA phenotyping at 7d (early stage) or 28d post-ACLR (established disease). Pain was evaluated by knee hyperalgesia testing. Intra-articular broad inflammation was assessed by ProSense 680, a near-infrared (NIR), cathepsin-activated probe. Micro-computed tomography (microCT) was performed at 28d only to quantify osteophyte (OP) formation. In a separate cohort, synovia were harvested at 7d post-ACLR, live cells were sorted by flow cytometry, and bulk RNA sequencing was performed. Differential gene expression and pathway analysis (PantherDB, GO) compared vehicle vs PolySA treatment. Male and female mice were used unless otherwise specified. As an analgesic effect was only observed in females at 7d post-ACLR, the follow-up 7d bulk RNA-seq and 28d phenotyping were performed in only females. When possible, to reduce the overall number of animals, bilateral ACLRs were employed (3R).

RESULTS: Endogenous neutrophil influx into synovium peaked 24hrs post-ACLR (**Fig. 1A**). Systemic PolySA treatment at 12hrs post-ACLR (inflammation onset) decreased the number of neutrophils (low and high dose) and monocytes (high dose only) in the synovium at 24hrs post-ACLR (**Fig. 1B**). Two acute doses of PolySA (12hrs and 48hrs post-ACLR) resulted in increased cathepsin activity in only females at 7d post-ACLR (**Fig. 1C**). Females treated with PolySA also had increased knee withdrawal threshold, indicative of lesser injury-induced pain, in the ACLR limb at 7d and 28d post-ACLR relative to vehicle (**Fig. 1D**). No treatment effect was observed in males at 7d post-ACLR for cathepsin activity or knee hyperalgesia. At 28d post-ACLR PolySA-treated females had lower normalized OP volume relative to the vehicle treated group (**Fig. 1E**). Bulk RNAseq analysis of female synovia at 7d post-ACLR revealed upregulation of genes related to Wnt/βcatenin inhibition (*Spink5*), chondrogenesis (*Sox9, Mmp9*), and anti-fibrosis (*Fgf7, Fgfr2*) and downregulation of immune and inflammation related genes (*Csf1r, Ccl4, Lyz2, Cx3cr1, Cd4*), consistent with mitigated immune cell recruitment (**Fig. 1F**). Gene ontology pathway analysis of female synovia at 7d shows strong stromal activation and anti-inflammatory effects in the PolySA group (**Fig. 1F**).

DISCUSSION: This study demonstrated that early systemic PolySA treatment, which reroutes neutrophils away from the site of injury, mitigates synovial immune cell influx, and in females induces anti-inflammatory effects in synovium, prevents osteophyte development, and alleviates pain. Interestingly, female synovial bulk RNAseq at 7d post-ACLR showed a transcriptomic signature indicative of anti-inflammatory effects in PolySA mice; however, PolySA mice at 7d exhibited greater cathepsin activity by intravital imaging. Cathepsins have pro- (cathepsin S, G) and anti-inflammatory (cathepsin K) roles, therefore, further experiments are necessary to determine which cathepsins are driving the measured increase in cathepsin activity. Importantly, even though male and female mice exhibited decreased synovial immune cell influx with PolySA treatment, pain alleviation at 7d post-ACLR was only observed in females. Additional experiments are required to address this critical sex-specific analgesic effect of PolySA. Early PolySA treatment in females led to sustained alleviation of pain – the primary symptom of PTOA – likely due to blocking synovial inflammation and OP development – both of which are clinical correlates to OA-pain. Taken together this study demonstrated in a clinically relevant timeline that affecting synovial cell crosstalk with systemic hematopoietic niches by early intervention with PolySA is a highly promising therapeutic for preventing PTOA development.

SIGNIFICANCE: No disease-modifying treatments exist for OA/PTOA. We employed a clinically-relevant design whereby early systemic administration of a neutrophil-targeting therapeutic (PolySA) shortly after injury exerted lasting analgesic, anti-inflammatory, and osteophyte-blocking effects.

Table 1. Methods summary including timepoint, injury, sex, sample pooling, and sample size for each experiment. M – male. F – female.

Assessment	Timepoint & Injury	Sex	Sample Pooling Strategy	Sample Size
Neutrophil influx time course – synovial flow cytometry	Sham or 0-48hr biACLR	M and F	1 sample = 1 synovium	n=2-4 per sex/timepoint
Low vs high dose PolySA – synovial flow cytometry	24hr biACLR	M and F	1 sample = bilateral pooling of 2 synovia	n=4 per sex/treatment
Early PTOA – knee hyperalgesia, ProSense NIR imaging	7d uniACLR	M and F	No pooling (hindlimb harvests)	n=4 per sex/timepoint
Early PTOA – synovial bulk RNAseq	7d biACLR	F only	1 sample = bilateral pooling of 2 synovia	n=3 per treatment
Late PTOA – knee hyperalgesia, ProSense NIR imaging, microCT	28d uniACLR	F only	No pooling (hindlimb harvests)	n=8 per treatment

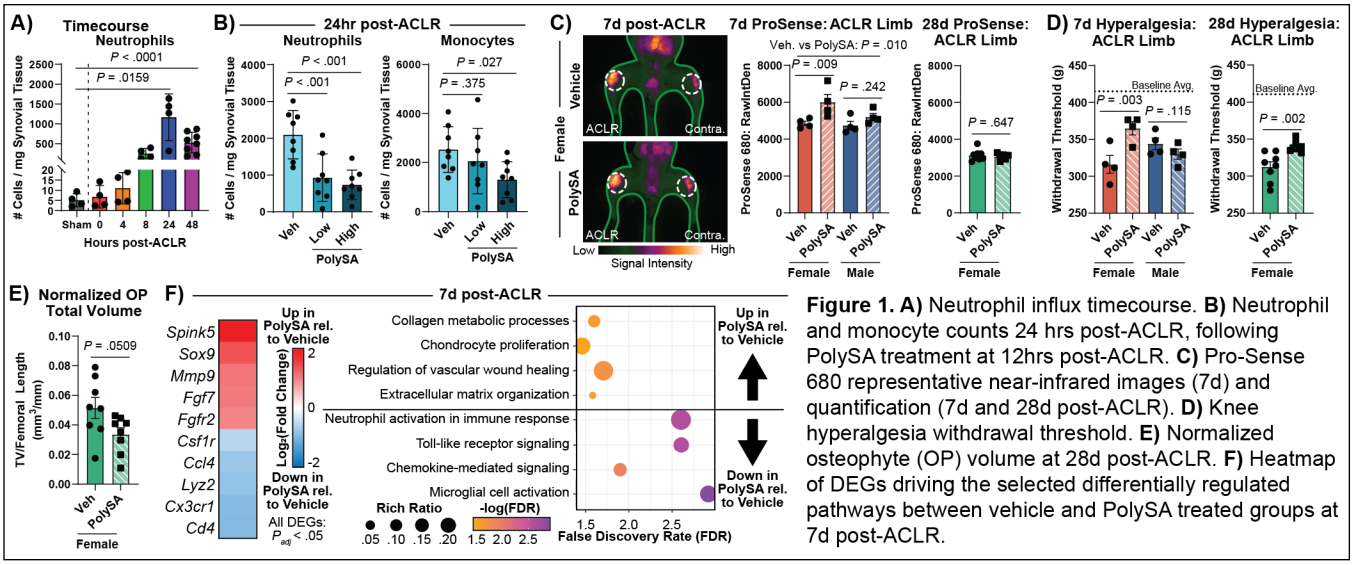

Figure 1. A) Neutrophil influx timecourse. **B)** Neutrophil and monocyte counts 24 hrs post-ACLR, following PolySA treatment at 12hrs post-ACLR. **C)** Pro-Sense 680 representative near-infrared images (7d) and quantification (7d and 28d post-ACLR). **D)** Knee hyperalgesia withdrawal threshold. **E)** Normalized osteophyte (OP) volume at 28d post-ACLR. **F)** Heatmap of DEGs driving the selected differentially regulated pathways between vehicle and PolySA treated groups at 7d post-ACLR.