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TITLE: Development of Intra-Articular Drug Delivery to Alter Progression of Arthritis Following Joint Injury

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14. ABSTRACT

DESIGN: A novel injectable and in situ forming drug depot based on thermally-responsive elastin-like polypeptide (ELP) will be used to deliver an anti-inflammatory drug to an injured joint and provide slow release over time.
METHOD(S): Loaded ELP depots were incubated at 37ºC with or without 10% serum to quantify in vitro drug release over 1 week. Drug release was quantified by UV-Vis spectroscopy for serum-free conditions and by commercially available ELISA kits (R&D Systems) in the presence of serum. Bioactivity of released sTNFRII was evaluated via the murine L929 cytoprotection assay, an industry standard. DATA ANALYSIS: Means and SEM (n=4) of released drug were calculated at each time point (1, 2, 3, 24, 48, 72, 96, and 168 hours), and data were fit to a nonlinear model of one dimensional steady-state diffusion.
FINDINGS: Over 7 days, 80% of IL1Ra was released from the ELP depot in serum-free and serum-containing conditions. For sTNFRII, 8% of loaded drug was released from the ELP depot in serum-free conditions, compared to 79% in the presence of serum. These findings confirm the mechanism that drug is released from the degrading drug depot. Released sTNFRII was found to retain activity against TNFα at each time point.

15. SUBJECT TERMS
ELP - Elastin-like polypeptide, Drug Depot – technology allowing sustained release of biologically active agent , Active agents used include IL1Ra (anakinra, 150mg/ml), or sTNFRII (etanercept, 25 mg/ml).

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RE: Contract: W81XWH-10-1-0890
Title: Development of Intra-Articular Drug Delivery to Alter Progression Of Arthritis Following Joint Injury
PI: Steven Olson, MD

Introduction

We are writing to provide the annual report for the project entitled "Development of Intra-Articular Drug Delivery to Alter Progression Of Arthritis Following Joint Injury". We have recently been granted a no cost extension for this award – approved 05/04/2012. With the no cost extension approval the final report date was moved to April 14, 2013. The current annual report was due April 14, 2012. I apologize for the delay in submission of this annual report. This report was submitted with an included abstract. The abstract has been removed and an appendix has been included.

In brief this protocol explores the use of elastin like polypeptide (ELP) as a sustained delivery vehicle for intra-articular therapy following intra-articular fractures. The ELP used contained IL-1Ra (kineret) or sTNFRII (etanercept) alone or both drugs in combination as active agents.

Body

To date we have accomplished the successful production of sterile ELP depots. The ELP depots were successfully loaded with the drugs IL-1Ra (kineret) and sTNFRII (etanercept). The drug release profiles of IL-1Ra and sTNFRII from the ELP depots were successfully quantified. Closed articular fractures of the tibial plateau were successfully created with an 83% success rate in all mice. Following fracture, intra-articular injections of the ELP depots with IL-1Ra, sTNFRII, IL-1Ra+sTNFRII, and PBS were successfully administered in all mice with no complications or adverse events. All animals were sacrificed at 4 and 8 weeks post-fracture. Serum, synovial fluid and hind limbs were harvested from all animals. Hind limbs from all mice have been formalin fixed. Hind limbs from ELP-IL-Ra, ELP-PBS and age-matched controls have been scanned by microCT, bone morphological analysis is complete, and limbs have been processed and embedded in paraffin. MicroCT scanning and bone morphological analysis of hind limbs from ELP-sTNFRII, ELP-IL-1Ra+sTNFRII and fracture with no treatment controls is in progress.

We are requested the no cost extension in order to section and stain histologic sections of hind limbs from ELP-IL-Ra, ELP-PBS, and age-matched control mice; to complete microCT scanning and bone morphological analysis of hind limbs from ELP-sTNFRII, ELP-IL-1Ra+sTNFRII, and fracture with no treatment control mice, followed by histologic processing, paraffin embedding, sectioning, and staining. The outcome from the histologic and bone morphological analyses will be used to assist in selection of biomarkers and cytokines to be assayed immediately following.

The delay in completing these analyses was due to issues in the initial quantification of the drug release profiles from ELP. We wanted to confirm drug release and to develop a reliable protocol for quantifying drug release both in vitro and in vivo. We overcame these technical challenges but quantification of the drug release profile for IL-1Ra from the ELP drug depots was completed in April 2011 and quantification of the drug release profile for sTNFRII from the ELP drug depots was completed by August 2011, later than we had anticipated. Since this initial delay, all other aspects of the project have proceeded as expected, and the scope of the work has not changed. We are progressing with the analysis of the limbs from all mice. Currently Micro-CT imaging is complete. Histologic sectioning is underway for these specimens. Preliminary work to begin biomarker assessment is also in progress.

Key Research Accomplishments

To date the progress with the project is summarized below

Task	Description	Original Timeline from SOW	Status
Task 1	Animal protocol review and approval.	Months 1-4	Completed
Task 2	Produce ELP constructs.	Months 1-2	Completed
Task 3	Create closed articular fractures in the left knee of mice in 64 animals.	Months 3-5	Completed
Task 4	Sacrifice mice and harvest samples at 4 weeks post-trauma for 64 mice.	Months 6	Completed
Task 5	Sacrifice mice and harvest samples at 8 weeks post-trauma for 64 mice.	Months 7	Completed
Task 6	Perform analyses on hind limbs from first 64 mice.	Months 8-9	In progress
6a.	MicroCT analysis	Months 8-9	Completed
6b.	Histology processing and paraffin embedding completed, limbs to be sectioned and stained.	Months 8-9	In progress
Task 7	Create closed intra-articular fracture of the left knee of remaining mice.	Months 10-12	Completed
Task 8	Sacrifice mice and harvest samples at 4 weeks post-trauma for remaining mice.	Months 13	Completed
Task 9	Sacrifice mice and harvest samples at 8 weeks post-trauma for remaining mice.	Months 14	Completed
Task 10	Perform analyses on hind limbs from remaining mice.	Months 14-15	In progress
10a.	MicroCT analysis	Months 14-15	Completed
10b.	Histology processing and paraffin embedding completed, limbs to be sectioned and stained.	Months 14-15	In progress
Task 11	Perform assays to assess levels of biomarkers	Month 16.	In progress
Task 12	Perform immunohistochemistry on paraffin sections for all hind limbs	Month 17-18	Not yet initiated

Reportable Outcomes

Over 7 days, 80% of IL1Ra was released from the ELP depot in serum-free and serum-containing conditions. For sTNFRII, 8% of loaded drug was released from the ELP depot in serum-free conditions, compared to 79% in the presence of serum. These findings confirm the mechanism that drug is released from the degrading drug depot. Released sTNFRII was found to retain activity against TNFα at each time point.

The IL1-RA delivered intra-articularly via ELP significantly reduced synovitis after intra-articular fracture and had modified Mankin scores that were equal to the non-fracture controls. The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue. However, the inhibition of TNF-α resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue. This study shows a novel reduction in post-trauma inflammation that has potential for clinical applications, and provides evidence for an alternate delivery method of anti-inflammatories for joint trauma.

Conclusions

We anticipate completion of the project within the additional year provided through the no cost extension.

Sincerely,

Steven A. Olson, M.D.
Principle Investigator

We appreciate the support from the Department of Defense for this work in Post-Traumatic Arthritis.

Appendix

Prolonged Local Delivery of IL-1Ra Prevents Post-traumatic Arthritis in Mice

Kimmerling, KA[1]; Furman, BD[1]; Mangiapani, DS[1]; Moverman, MA[2]; Sinclair, SM[2]; Setton, LA[1]; Guilak, F[1]; Olson, SA[1].
[1]Department of Orthopaedic Surgery, Duke University Medical Center, Durham, NC
[2]Department of Biomedical Engineering, Duke University, Durham, NC

PURPOSE: Post-traumatic arthritis (PTA) is a form of osteoarthritis (OA) that results from joint trauma. Pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) have been implicated in OA and are reported to be upregulated following joint trauma. Presently, surgical restoration is the only treatment for articular fractures. We hypothesize that local sustained inhibition of IL-1, TNF-α, or IL-1 and TNF-α prevents the development of post-traumatic arthritis following articular fracture. Our objectives were to locally inhibit early intra-articular inflammation following articular fracture in the mouse knee and assess the severity of arthritic changes in cartilage and joint tissues long-term.

METHODS: All animal procedures were performed in accordance with an IACUC-approved protocol. Male C57BL/6 mice (n=77) were subjected to an articular fracture at 16 weeks of age using an established model. Five groups were established (n=12-16 per group). One group received no treatment after fracture. The remaining groups received intra-articular injections of PBS, IL-1 Receptor antagonist (IL-1Ra; anakinra; Kineret®), soluble TNF receptor II (sTNFRII; entanercept; Enbrel®) or both IL-1Ra and sTNFRII in combination. The treatments were encapsulated in elastin-like polypeptide (ELP) drug depots which slowly disaggregate for prolonged release. Mice (n=6-8 per group) were sacrificed at 4 and 8 weeks. The left (fractured) limb and right (non-fractured) limb were harvested and fixed. Micro-computed tomography (microCT) of both limbs was performed to assess bone morphology. Joints were processed for standard histology, and sections were assessed by 3 independent blinded graders for cartilage degeneration using a modified Mankin score and synovial inflammation using a modified synovitis score with semi-quantitative scales. Non-parametric statistical analyses were performed for histological assessment, and parametric analyses were performed for bone morphological measures.

RESULTS: The groups that were administered sTNFRII and sTNFRII+IL-1Ra showed a detrimental effect in bone morphology changes, cartilage degeneration, and synovial inflammation. However, the IL-1Ra group did not have a detrimental effect on bone morphology changes and, more importantly, reduced cartilage degeneration at 4 and 8 weeks. IL-1Ra was the only group in which the Mankin score of the fractured limb was not significantly different from the control limb (Figure 1). Additionally, IL-1Ra significantly reduced synovial inflammation at 8 weeks. IL-1Ra was the only group in which synovitis scores of fractured limbs were not significantly different than control limbs (Figure 2).

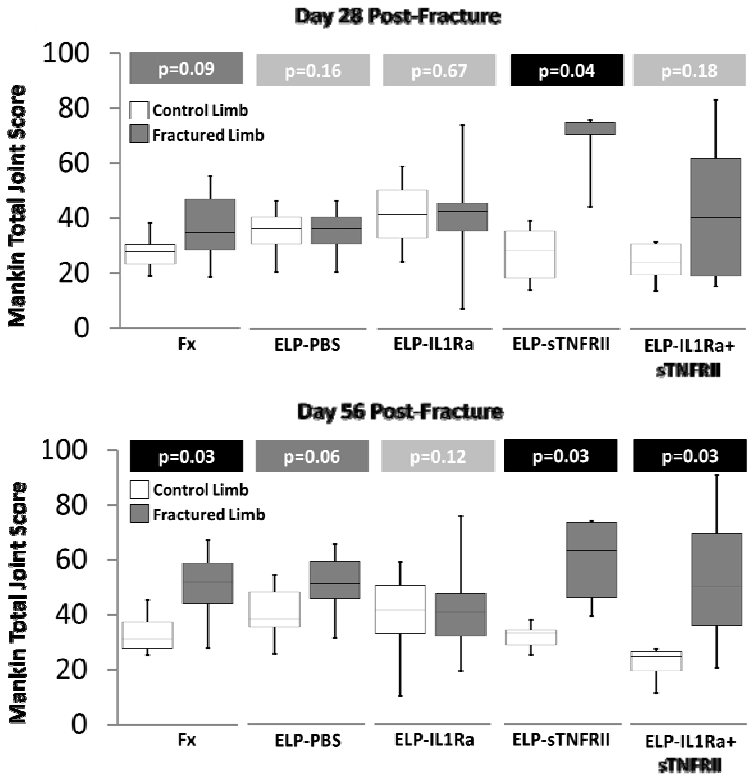

Figure 1. Mankin total joint scores at (A) 28 days post-fracture and (B) 56 days post-fracture for all treatment groups. P values denote significance between fractured (L) and control limb (R) using Wilcoxon Matched Pairs test.

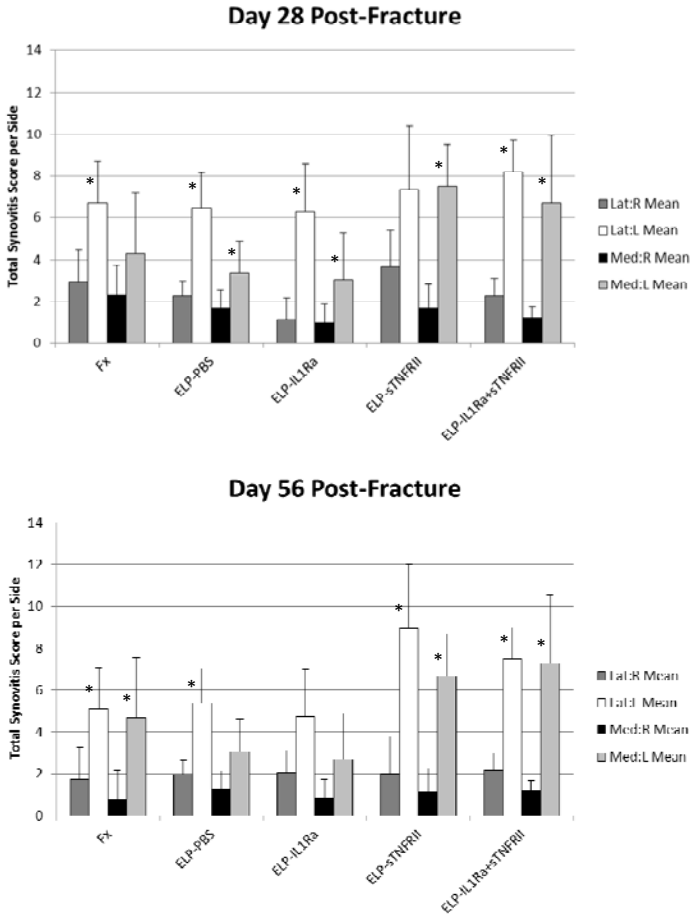

Figure 2. Synovitis scores of medial and lateral regions of knee joint capsule for fractured (L) and non-fractured control (R) limbs at (A) 28 days post-fracture and (B) 56 days post-fracture for all treatment groups. * denotes significance ($p<0.05$) between fractured limb and control limb using Wilcoxon Matched Pairs test.

CONCLUSIONS: The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue. However, the inhibition of TNF-α resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue. This study shows a novel reduction in post-trauma inflammation that has potential for clinical applications, and provides evidence for an alternate delivery method of anti-inflammatories for joint trauma.