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14. ABSTRACT

The following accomplishments with regard to the Aim 2 tasks (partnering-PI C. Price's responsibility) for grant PR120788P1 are noted. The animal surgery model (DMM) that constitutes the majority of the proposed research has been brought into the laboratory and we have completed ~30% of the projected surgeries and specimen collections with minimal complications. Currently, the entire cohort of Baseline, Age-Matched, and DMM-surgery controls have been collected and are undergoing analytical quantification. The cohort of Sham-surgery controls is currently in progress. Furthermore, we have established in-house the processing, embedding, microtomy, and staining techniques, as well as the micro-CT scanning and analysis techniques required for the studies in task 1. Specimen cohorts that have already been collected are undergoing these protocols and analytical procedures as proposed. Studies involving the treatment arm groups of the project will begin shortly and we foresee no difficulties in the performance of this portion of the project. Initial data regarding the natural time-course of joint structure and cellular biology changes in the DMM mouse model are being prepared for submission to the ORS Annual Meeting. The current data constitutes critical control data for the i.a. ZA treatment cohorts proposed in this study.

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INTRODUCTION: This grant focuses on the investigation of a pharmacological treatment for "post-traumatic osteoarthritis". Post-traumatic osteoarthritis (PTOA) is a disease of long-term cartilage degeneration that results from acute joint trauma (*e.g.*, meniscal or ligament tears), injuries that are common in military service. Currently, few preventative or curative treatments exist for PTOA, with typical outcomes being eventual loss of joint function and immobility, leading to joint replacement. In a proof-of-concept study we found that repeated systemic administration of the FDA-approved drug zoledronic acid (ZA), a bisphosphonate (BP) prescribed to treat bone loss, could suppress the development of PTOA in the DMM (destabilization of the medial meniscus) mouse model, a model recapitulating the altered joint loading associated with PTOA. However, the strong impact of BPs on bone remodeling limits their systemic use in the treatment of PTOA. In this grant, we test the efficacy of a more targeted BP delivery, the localized intra-articular (i.a.) injection of ZA, to prevent PTOA while minimizing adverse skeletal health effects. Furthermore, we will explore the cellular and molecular mechanisms underlying ZA's chondro-protection in order to develop therapeutic PTOA treatments that are more cartilage specific. This report details the portion (Aim 2: Determine the chondro-protective effect of locally delivered ZA using an animal model) of the grant being performed within the partnering-PI's lab (Price). This sub-project involves the pre-clinical establishment and testing of i.a. ZA injection for treating PTOA within a small-animal (murine) model of surgically induced PTOA (DMM model). The purpose of this sub-project is to evaluate the in vivo mechanisms by which PTOA progresses within the DMM model and to establish the efficacy and mechanisms of action of i.a. ZA in preventing PTOA. This sub-project will establish these relationships via the measurement of mechanical, structural, morphological, biochemical, molecular, and cellular properties of cartilage and bone in mice treated with i.a. ZA following injury via the DMM model. Since the in situ repair of degenerate cartilage is a challenging, and as-of-yet unrealized task, the prevention of PTOA through the innovative i.a. delivery of ZA may provide a simple, effective, and low-cost treatment for lessening the burden of this disease. Furthermore, the knowledge of ZA's chondro-protective mechanisms that may be elucidated by this project can provide additional molecular, cellular, and biochemical targets by which OA/PTOA may be treated in the future.

BODY: Within the Aim 2 sub-project of this grant "Determine the chondro-protective effect of locally delivered ZA using an animal model", of which the requisite work is being performed within the laboratory of the partnering-PI (C. Price), the following accomplishments are noted.

Within Task 1 "*Evaluate the in vivo chondro-protective efficacy of immediate and delayed targeted local administration of zoledronic acid (ZA) to prevent and post-traumatic osteoarthritis (PTOA) in a murine model of PTOA development"* we have completed the collection of experimental specimens for the entire cohort of baseline, age-matched, and DMM + no treatment control groups ranging from post-surgery day 0 to day 112. This included a total of 75 surgeries performed on 89 animals for a total collection of 178 joints. All of the control-group DMM surgeries were performed without incident and initial findings indicate the long-term outcomes of the surgeries (i.e. development of PTOA by 8-12 weeks) occurred as expected. Of these specimens approximately 1/3rd were reserved for micro-computed tomography analysis, followed by micro-indentation, and histomorphometric processing. The remaining 2/3rd were reserved for histological processing and histological and immunohistochemical evaluation. Within these control cohorts all the specimens in the histological arm have been processed, in house, for paraffin embedding and subsequently embedded in paraffin for microtomy. Proceeding in a staggered order, which permits sequentially sampling from all of the collected experimental groups, approximately 80% of these "control" samples have been sectioned and undergone safranin-O/fast green/Weigert's Iron Hematonxylin staining to permit the quantification of cartilage degeneration due to the DMM surgery. The OARSI-describe PTOA scoring system was brought into the lab and 4 individuals have been trained on it. Currently, the safranin-O stained samples are undergoing three-scorer, blinded, semi-quantitative scoring using this system to establish the natural course of PTOA development in our model prior i.a. ZA administration. Additionally, we have begun the development of specimen-specific immunohistochemical staining techniques to quantify the degree of chondrocyte proliferation (Ki-67), apoptosis (activated caspase-3), hypertrophy (collagen type X), anabolism, catabolism, and inflammation within our DMM and treatment model (currently developed techniques in parentheses). Lastly, our UD DRI Cytomechanics Core facility (Wang) received its micro-computed tomography (u-CT) specimen scanner (May 2014). Using this piece of equipment we have optimized the scanning and analysis protocols in order to allow us to perform u-CT analysis on our experimental specimen joints. To date we obtained u-CT scans for approximately 25% of our experimental specimens and analysis of subchondral bone and trabecular bone changes associated with the DMM surgery is ongoing. Currently, the Sham and Vehicle Injection cohorts of our experimental study are underway and the associated specimens will be collected between August and October 2014. The PI notes that the initiation of the study was delayed due to the internal-transfer of the Partnering-PI (C. Price) to the Biomedical Engineering Program at the University of Delaware and a degree of delay associated with the initiation and development of the PIs personal laboratory. In this case the laboratory was able to hire a research technician to begin the studies in September of 2013 and the first graduate students joined the lab in November/December 2013. However, at the present, all of the sub-tasks associated with Task #1 are either on or ahead of the schedule proposed in the grant submission and statement of work.

Within Task 2: "*Analysis of data from three aims; Preparation of final report, publication and grant proposal based on our findings"* we are continuously performing the analysis of study data for experimental planning and publication. Presently, we have published and presented initial information regarding this study at the University of Delaware Center for Biomechanical Engineering Research 11th Annual Biomechanics Research Symposium and are preparing to submit at least one abstract to the Orthopaedic Research Societies Annual Meeting for 2015.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

- Animals and Surgery (ahead of schedule)
  - DMM surgery training and technique import into P-PI laboratory complete.
  - Baseline, DMM-only, and age-matched cohort groups completed for D0 through D112 time points.
  - Sham and Vehicle-Injection Control group surgeries performed and awaiting time-point completion.
- Micro-CT Imaging of Bone (ahead of schedule)
  - Micro-CT system acquired by UD DRI Cytomechanics Core laboratory (Wang) enabling in house micro-CT performance and analysis.
  - Micro-CT scanning protocol optimized for murine knee joints.
  - Micro-CT image/data analysis protocol developed for knee joint scans from Core Facility micro-CT.
  - ~25% of Baseline, DMM-only, and age-matched cohort group specimens underwent successful micro-CT scanning.
  - Micro-CT image/data analysis ongoing.
- Histological PTOA Assessment (ahead of schedule)
  - In house specimen processing, embedding, and microtoming capabilities established.
  - 100% of Baseline, DMM-only, and age-matched cohort group specimens processed for paraffin-embedded thin-section histology.
  - ~80% of Baseline, DMM-only, and age-matched cohort group specimens sectioned via microtomy for histology and immunohistochemistry.
  - ~ 80% of Baseline, DMM-only, and age-matched cohort group specimens stained with safranin-O/fast green/Weigert's Iron Hematoxylin for semi-quantitative scoring of cartilage degeneration and PTOA development.
  - OARSI-described PTOA scoring system was brought into the laboratory and 4 researchers were trained on its implementation.
  - ~40% of the Baseline, DMM-only, and age-matched cohort group specimens have been scored by three separate, blinded scorers using the OARSI-described PTOA scoring system.
- Immunohistochemical (IHC) Evaluation of Cartilage Response (on schedule)
  - IHC antigen retrieval techniques for cartilage and bone were validated within the lab for our specimens.
  - Immunofluorescent and immunohistochemical staining techniques for proliferative markers (Ki-67) and apoptotic markers (activate Caspase-3) have been developed in the lab and are being applied to the currently available specimens.
  - Immunohistochemical staining techniques for hypertrophy (collagen type X), anabolism, catabolism, and inflammation are currently being developed within the lab.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- Abstracts:
  - David, M., Price, C., Tissue, cellular, and molecular effects in early post-traumatic osteoarthritis; implications for pharmacological treatment. 11th Annual Biomechanics Research Symposium. Center for Biomechanical Engineering Research. April 21st, 2014. Newark, DE
- Infomatics:
  - The destabilized medial meniscus (DMM) surgical model was taught to three researchers in the lab (PI, Research technician, and graduate student) for use within he laboratory. The surgical model was perfected and is being routinely performed by the research technician and graduate student). Approximately 75 successful surgeries have been performed to date with a complication rate of less that 2%.
  - In house specimen processing, embedding, and microtomy capabilities established and optimized.
  - Micro-CT scanning and analysis protocols were established and optimized for murine knee joints on the Scanco u-35 system in house.
  - The OARSI-described PTOA scoring system was implemented within the laboratory with multiple researchers being trained on its use (P-PI, Research Technician, Graduate Student, several Undergraduate Students)
- Grants:
  - Awarded: Delaware Rehabilitation Institute Cytomechanics Core Equipment User Grant. Title "Micro-CT analysis of local and global skeletal changes following the targeted treatment of post-traumatic osteoarthritis via intra-articular injection of bisphosphonates." $3000 equipment use grant to supplement additional micro-CT scanning cost associated with current project.

CONCLUSION: At the end of the first reporting period for the current grant we report that the progress on the major tasks and sub-tasks associate with the P-PI's Aim 2 responsibility are either on or ahead of schedule. Following a slight delay, of approximately three months, in the initiation of the research following the award granting we are pleased to report that we have caught up with ad in some cases surpassed the accomplishment schedule set forth in the originally proposed statement of work. The initial delay that we

note was the result of two factors; First, the transitioning of the P-PI's appointment within the University of Delaware from the Mechanical Engineering Department to the Biomedical Engineering Program, along with the P-PI's initiation of a new personal lab, and second, an initial difficulty in recruiting laboratory staff to assist in the immediate performance of the proposed research. We are please to report that with the successful start of the P-PI's lab and the recruitment of one research technician, one graduate student, and three undergraduate researchers we have been able to realign our progress with the time line proposed. In the completed research we have completed the collection of the control animal/surgery data and are progressing toward the completion of the analysis of these cohorts. Studies involving the treatment arm groups of the project will begin shortly and we do not foresee any difficulties in the performance of this portion of the project. Currently we are planning to submit research study abstracts regarding the natural time-course of joint structure and cellular biology changes in the DMM mouse model to the Orthopaedic Research Society Annual Meeting (deadline Aug 25th, 2014). This data will provide the key baseline and control data for our treatment cohorts as well as increase our understanding of the natural biology of PTOA initiation and progression with the murine DMM model. Such information is critical to the implementation of our pre-clinical i.a. ZA treatment study, as well as the development of potentially more cartilage/chondrocyte focused PTOA prevention/treatment regimens.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine*, etc.).

- **N.A.**

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, study questionnaires, and surveys, etc.

David, M., Price, C., Tissue, cellular, and molecular effects in early post-traumatic osteoarthritis; implications for pharmacological treatment. 11th Annual Biomechanics Research Symposium. Center for Biomechanical Engineering Research. April 21st, 2014. Newark, DE

26 TISSUE, CELLULAR, AND MOLECULAR EFFECTS IN EARLY POST-TRAUMATIC OSTEOARTHRITIS; IMPLICATIONS FOR PHARMACOLOGICAL TREATMENT

Michael David, BS, and Christopher Price, PhD

Biomedical Engineering

Post-traumatic osteoarthritis (PTOA), an accelerated form of osteoarthritis (OA), is an insidious disease of progressive diarthoridal joint articular cartilage degeneration that results directly from traumatic joint injury, e.g., meniscus or ligament tears, and is common in athletes and military service members. Approximately 50% of those who experience a ligament tear develop OA within 15 years. Unlike other causes of idiopathic OA, such as chronic joint loading or ageing, with PTOA the initial timing of the disease is known. Interestingly, previous work at UD using systemic administration of Zolendronic Acid (ZA), a FDA-approved bisphosphonate used to treat bone loss, demonstrated histologically the long-term prevention of cartilage damage and PTOA. However, in that study, and many like it, the early cellular and molecular mechanisms involved in PTOA-related cartilage damage and drug-mediated protection remain unknown. We hypothesize that immediately following injury changes in chondrocyte metabolism and fate determination precipitate the vicious cycle of cartilage degeneration in PTOA. By targeting these early changes pharmacologically with intra-articular injection (i.a.) of ZA, the development of PTOA can be prevented or hindered. To address this hypothesis, a murine joint instability injury model of PTOA (destabilization of the medial meniscus) is being utilized to study the early structural, compositional, cellular, and molecular changes associated with trauma-induced PTOA. In this study we will present histological and immunohistochemical analyses of non-treated, control and injured knees across a wide range of post-surgical time points at 3-, 7-, 14-, 56- 84- and 112-days post-injury. Ultimately, this study will identify early mechanisms in injury-related cartilage degeneration and determine the efficacy of targeted drug delivery systems, such as i.a. ZA, to prevent the onset of PTOA.

SUPPORTING DATA: All figures and/or tables shall include legends and be clearly marked with figure/table numbers.

Table #1: Progress of animal surgery, treatment, and specimen collection tasks for Aim 2 (P-PI C. Price) in grant number PR120788P1. Table breaks out the experimental groupings along with the projected number of animals required for each group and the current collections status for each cohort. All of the animals for which complete experimental groupings have been collected are indicated via the checkmark; a diamond indicates experiment groupings currently in progress.

Table 1 — **Post-Operative Collection Schedule**

Experimental Groups	Surgery @ 12wks	i.a. ZA Treatment @	0d		3d		7d		14d		3wk		8wk		12wk		16wk	
			Hist.	*u-CT*	*Hist.*	*u-CT*	*Hist.*	*u-CT*	*Hist.*	*u-CT*	*Hist.*	*u-CT*	*Hist.*	*u-CT*	*Hist.*	*u-CT*	*Hist.*	*u-CT*
Baseline and Age-Matched Controls	N.A.	N.A.	***5✓***	***5✓***	-	-	-	-	-	-	-	-	***5✓***	***5✓***	***5✓***	***5✓***	***5✓***	***5✓***
Sham Controls	Sham	N.A.	-	-	3◆	2◆	3◆	2◆	3◆	2◆	-	-	3◆	2◆	3◆	2◆	3◆	2◆
DMM Controls	DMM	N.A.	-	-	***5✓***	***5✓***	***5✓***	***5✓***	***5✓***	***5✓***	-	-	***10✓***	***5✓***	***10✓***	***5✓***	***10✓***	***5✓***
DMM + Peri-Operative Tx	DMM	Day 0	-	-	5	5	5	5	-	-	-	-	10	5	10	5	10	5
DMM + Post-Operative Tx	DMM	Day 7	-	-	-	-	-	-	5	5	-	-	10	5	10	5	10	5
DMM + Post-Operative Tx	DMM	Day 14	-	-	-	-	-	-	-	-	5	5	10	5	10	5	10	5

✓ – sub-experiment specimen collection complete and specimens undergoing analytical protocls
◆ – sub-experiment currently in progress

Table #2: Progress of analytical task for Aim 2 (P-PI C. Price) in grant number PR120788P1. Table indicated the current progress of the analytical tasks proposed within the grant. The number of collected joints, and the degree of completed tasks are indicated as a function of total projected specimens as well as of currently collected specimens. Tasks are divided based upon the two major specimen handling arms of the study, namely the Histology and micro-CT arms. Note: Some task can only be accomplished following completion of the analytical tasks that precede them; they are noted as such.

Table 2

Analysis Arm/Grouping	Collected Joints		Projected Joints	Analytical Task	Number Completed	% of Collected	% of Projected
Histology/IHC Arm	**105**	**of**	**366**	***Processing & Embedding***	*105*	*100%*	*29%*
				Microtomy	*85*	*81%*	*23%*
				Histological Staining	*85*	*81%*	*23%*
				OA Scoring	*50*	*48%*	*14%*
				IHC Staining and Analysis	*Currently In Development*		
Micro-CT Arm	73	of	234	***u-CT Scanning and Analysis***	*42*	*40%*	*11%*
				Micro-indentation Testing	*Awaiting Completion of Prior Procedure*		
				Histomorphometric Processing	"		
				Histomorphometry Analysis	"		