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AAO Foundation Final Report Form
(a/o 1/3/2018)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Please prepare a report that addresses the following:

Type of Award, e.g., Orthodontic Faculty Development Fellowship Award, Postdoctoral Fellowship Award, Biomedical Research Award, Center Award, Educational Innovation Award, Program Award, Research Aid Award

Name(s) of Principal Investigator(s) Sumit Yadav

Title of Project: Mechanism of Anti-TGF beta for the treatment of the mandibular condylar cartilage in osteogenesis imperfecta

Period of AAOF Support (e.g. 07-01-18 to 06-30-19):07-01-2018 to 12-31-2019

Amount of Funding: 30,000

Summary/Abstract

INTRODUCTION: Osteogenesis Imperfecta (OI) is characterized by low bone mass, which predisposes the patients to spontaneous fractures. Mutations in the structural protein genes Col1A1 and Col1A2 results in the dominant form of OI. The Temporomandibular Joint (TMJ) is a unique diarthroidal, bilateral joint formed by the articulation of the Mandibular Condylar Cartilage (MCC), the TMJ disc, and the cartilage of the glenoid fossa. The MCC, the TMJ disc and the cartilage of the glenoid fossa are fibrocartilaginous and predominantly consist of chondrocytes which expresses type 1 collagen. Furthermore, osteoblasts in the subchondral bone express type 1 collagen. In spite of the well-known effects of OI on bone, other musculoskeletal tissues containing type I collagen (mandibular condylar cartilage and the subchondral bone of the temporomandibular joint) have not been studied. The objectives of this project are to gain insight into the structure of the MCC and the subchondral bone in OI and the effects of alendronate treatment on the load response of the osteochondral tissues of the TMJ.
METHODS: All procedures were approved by the UConn Health Institutional Animal Care and Use

Committee and performed in an AAALAC accredited facility. We used genetically modified OI mice, which have a spontaneous mutation in the pro-alpha2 chain of Col1A1. The animals used in the research were generated by crossing homozygous animals and genotypes were determined by PCR. The mice were randomly allocated to receive either alendronate or vehicle (saline). Body weight of the animals was not significantly different between the groups. Six-week old animals were treated with alendronate (dose:) and saline for 2 weeks. The alendronate and saline were injected every alternate day. The animals were injected with 5-ethynyl- 2'-deoxyuridine (EdU) at two-days and one-day prior to euthanization.The mice were also injected with alizarin complexone three-days prior to sacrifice and then injected with calcein one-day prior to sacrifice. Mice were sacrificed one day after the last injection of the alendronate/saline. Five animals from each group (alendronate and saline) group were loaded for 1 hour each day, for 5 days, using a preformed spring applying 50cN of force on the cartilage of the TMJ. Mice were euthanized by CO_2 inhalation and the TMJ was harvested and stored at -4 °C in 10% formalin for microCT and histological evaluation. *microCT Testing:* One mandible each from the alendronate treated or saline treated animals was dissected, cleaned and fixed in 4% paraformaldehyde. The mandible was washed through a series of solution and processed up to 70% alcohol. The samples (n = 5 per group) were scanned in 70% alcohol and serial tomographic projections were acquired at 55 kV and 145μA, with a voxel size of 6 μm and 1000 projections per rotation were collected at 300,000μs. The region of interest was the mushroom shaped head of the condyle which includes the MCC and the subchondral bone. Bone volume fraction (BVF (%)), trabecular thickness (Tb.Th (μm)) and trabecular spacing (Tb.Sp (μm). All statistical analysis was conducted Graph Pad software. *Histological Evaluation:* The MCC along with the subchondral bone were fixed for 2 days in 4 % P\paraformaldehyde, and then placed in 30% sucrose overnight and embedded in cryomedium. Serial sagittal sections of MCC were stained with Toluidine Blue to evaluate overall histology and cartilage matrix and proteoglycan content. Tartrate Resistant Alkaline Phosphatase (TRAP) staining was used to detect osteoclasts. **RESULTS**: *microCT:* We observed significant increase ($p<0.001$) in bone volume fraction and tissue density in the alendronate treated group when compared to the saline treated group. Similarly, there was significant increase in trabecular thickness ($p<0.05$) in the alendronate treated group. *Histological Evaluation:* Examination of the cellular pathophysiology of the MCC and the subchondral bone of the TMJ indicated that in the OI treated with alendronate had more Col10a1 positive cells when compared to the saline group. Furthermore, loading of the cartilage of the TMJ resulted in more Col10a1 positive cells in the alendronate group when compared to the saline group. There was a significant increase ($p<0.002$) in the area of mineralization in the alendronate treated mice when compared to the saline treated group. Loading of the cartilage leads to significant increase ($p<0.001$) in the cartilage thickness in the alendronate group when compared to the saline group. Additionally, loading of the cartilage leads to loss of subchondral bone in the saline group. Furthermore, we noticed significantly increased ($p<0.001$) area of proteoglycan secretion in the alendronate group when compared to the saline group. **DISCUSSION**: TMJ is the most commonly used joint in the human body and yet none of the previous research has characterized the osteochondral tissues of the TMJ in the OI mice. This study demonstrates that commonly used treatment for OI individuals (bisphosphonates) improves the joint load bearing capacity in the mice model. Furthermore, we observed overall beneficial effects of alendronate on the cartilage health of the TMJ. **SIGNIFICANCE/CLINICAL RELEVANCE**: Osteochondral tissues of TMJ are connective tissue and susceptible to degeneration in an OI individual. Our group is the first one to characterize the osteochondral tissues of the TMJ in the mice and we have also shown the load response of the osteochondral tissues of the TMJ treated with bisohosphonates (alendronate). Bispjosphonates are commonly used to treat individuals with OI.
ACKNOWLEDGEMENTS: This work was supported by grant awarded to Sumit Yadav by NIDCR/NIH and American Association of Orthodontic Foundation.

Response to the following questions:

1. Were the original, specific aims of the proposal realized? Yes
2. Were the results published? Manuscript in preparation
    a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers
    b. Was AAOF support acknowledged?
    c. If not, are there plans to publish? If not, why not?
3. Have the results of this proposal been presented? The partial results have been presented in IADR and Orthopedic Research Society Meeting. AAOF support was acknowledged.
    a. If so, list titles, author or co-authors of these presentation/s, year and locations

    Title: Alendronate Treatment of the Osteogenesis Imperfecta Mouse Improves Load Response of the Cartilage of TMJ

    Co-Authors: Po-Jung Chen, M. Heather O'Brien, Sumit Yadav

    Orthopedic Research Society, Austin 2019
    b. Was AAOF support acknowledged? Yes
    c. If not, are there plans to do so? If not, why not?
4. To what extent have you used, or how do you intend to use, AAOF funding to further your career? **Based on the data from this grant, I was successful in obtaining a Center Grant through UCONN Health. We plan to submit an RO1 in year 2020.**