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AAO Foundation Final Report Form
(a/o 1/3/2020)

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., Biomedical Research Award

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

Title of Project: Role of PTH in Mandibular Chondrocytes De-differentiation

Period of AAOF Support (e.g. 07-01-19 to 06-30-20): 07-01-2020 to 12-31-2020

Amount of Funding: 30,000

Summary/Abstract

INTRODUCTION: Temporomandibular Joint Osteoarthritis (TMJ-OA) is a degenerative joint disease characterized by cartilage loss and sclerosis of the subchondral bone, causing pain and disability. Clinical management of TMJ-OA is largely palliative and there is an urgent need for effective disease modifying treatments. Therefore, it is critical to develop new therapeutic agents that are anabolic for the TMJ cartilage and subchondral bone. However, given the severity of this condition and the unique nature of the osteochondral tissues of the TMJ, it is not likely that a single therapeutic agent will be able, on its own, to promote or restore the anabolic responses in TMJ-OA. Accordingly, identifying signals that can synergize to produce additive anabolic functions in the cartilage and subchondral bone are essential for the development of clinically feasible treatments for TMJ-OA. To best of our knowledge, there is no study comparing the individual and synergistic effects of the I-PTH and alendronate on the cartilage and the subchondral bone of the TMJ. The purpose of this project was to study whether the use of I-PTH and alendronate together would provide a therapeutic advantage by combining different mechanism and have an anabolic effect on the cartilage and the subchondral bone. Our objective was to determine the effects of I-PTH and alendronate treatment on the microarchitecture of the cartilage and the subchondral bone of the TMJ. Additionally, we also studied the effects of independent and simultaneous treatment with I-PTH

and alendronate.

METHODS: All the experimental protocol used in this research involving triple transgenic reporter mice were reviewed and approved by the Institutional Animal Care Committee of the University of Connecticut Health Center. Our study followed the ARRIVE guidelines for *in vivo* experiments using these mice.
Ninety-six, 4 to 5-week-old male and female triple transgenic reporter mice (Col1a1 X Col2a1 X Col10a1) on a CD-1 background were used for this study. The GFP reporter transgenes used in this study have been described previously. Transgenic mice with each reporter were crossed to generate the triple transgenic Col3.6-green X Col2-blue X Cola10-red mice used in this study. All mice received subcutaneous injection of saline or PTH [1–34] (60μg/kg body weight, Prospec, East Brunswick, NJ, USA) or/and Alendronate (ALN, alendronate sodium trihydrate, 50μg/kg, Sigma Aldrich, St. Louis, MO).
Mice were divided into 6 groups and equal number of males and female mice were used in each group (n = 8 male and 8 female per group). Groups were divided as described below:
(1) Control group: saline was injected daily for 14 days;
(2) PTH group: PTH was injected daily for 14 days;
(3) Alendronate (ALN) group: Alendronate was injected every alternate day for 14 days;
(4) Combined PTH and ALN group: PTH was injected daily combined with ALN injected every alternate days for 14 days;
(5) PTH then ALN group: PTH only was injected daily for 14 days followed by ALN only injections in alternate days for another 14 day;
(6) PTH wait ALN group: PTH only was injected daily for 14 days. There was a waiting period of a one week before initiation of ALN only injections in alternate days for another 14 day.
Mice were injected with EdU (5-ethnyl-2'-deoxyuridine, Life Technologies, Grand Island, NY, USA) (30 mg/kg body weight) 48 hours and 24 hours prior to euthanization. All animals in the control and experimental groups were healthy and gained weight during the entire duration of the study. All mice were euthanized 24 hours after the last injection of EdU.

RESULTS: *Chondrocyte proliferation:* Edu proliferation assay revealed that PTH administration alone induced an increased chondrocyte proliferation in comparison to saline or when PTH in combined with Alendronate in female mice (PTH vs saline: $p = 0$.PTH vs PTH + Alend: $p < 0.001$; PTH vs PTH then Alend: $p < 0.001$; PTH vs PTH wait Alend: $p < 0.05$). However, no statistical difference was observed between PTH vs Alend or between all other groups in female mice. The effects of different treatment in chondrocyte proliferation in males was comparable to females; PTH led to increased proliferation in relation to combined treatment and saline (PTH vs saline: $p < 0.001$; PTH vs Alend: $p = 0.001$; PTH then Alend: $p < 0.05$; PTH wait Alend: $p < 0.05$). Although there was no statistical difference between PTH vs Alend, chondrocyte proliferation in the Alend group was significantly increased in comparison to Saline (Alend vs saline: $p < 0.05$).
Bone remodeling: We studied bone remodeling by fluorescent TRAP staining which revealed that Alend administration led to a significant reduction in TRAP activity when Alend was combined with PTH in females (Saline vs PTH + Alend: $p = 0.001$; Saline vs PTH then Alend: $p < 0.01$; Saline vs PTH wait Alend: $p < 0.05$; PTH vs PTH + Alend: $p = 0.001$; PTH vs PTH then Alend: $p = 0.01$; PTH vs PTH wait Alend: $p < 0.05$). However, there was no statistical difference between Alend injection alone and saline or PTH alone groups. We observed a similar trend in males; Alend injection inhibited TRAP activity in most groups in which Alend was administrated (Alend vs PTH + Alend: p , 0.05; Alend vs PTH wait Alend: $p = 0.01$; PTH vs Alend: $p < 0.005$; PTH vs PTH + Alend: $p < 0.0001$; PTH vs PTH then Alend: $p < 0.000$; PTH vs PTH wait Alend: p 0.01). On the other hand, PTH alone led to a significant increase in TRAP activity in relation to saline group in males (PTH vs Saline: $p = 0.01$).
Cartilage Thickness: Mandibular condyle cartilage thickness was evaluated in toluidine blue stained sections. In female mice, PTH or Alend alone caused a substantial increase in cartilage thickness (PTH vs Saline: $p = 0.01$; Alend vs Saline: $p < 0.001$). Nevertheless, when these two drugs were combined in different scenarios, the individual beneficial effects were lost and a decrease in cartilage thickness was observed in females (PTH vs PTH then Alend: $p < 0.0001$; PTH vs PTH wait Alend: $p < 0.0001$; Alend vs

PTH + Alend: $p = 0.01$; Alend vs PTH the Alend: $p < 0.0001$; Alend vs PTH wait Alend: $p < 0.0001$). In males, PTH alone led to a significant increase in cartilage thickness (PTH vs Saline: $p < 0.0001$; PTH vs Alend: $p < 0.01$; PTH vs PTH then Alend: $p < 0.0001$; PTH vs PTH wait Alend: $p < 0.0001$;). Surprisingly, Alend alone did not induce a significant change in cartilage thickness, but the combination of PTH + Alend led a greater increase than any other groups in which Alend was injected (PTH + Alend vs Saline: $p < 0.001$; PTH + Alend vs Alend: $p < 0.05$; PTH + Alend vs PTH then Alend: $p < 0.0001$; PTH + Alend vs PTH wait Alend: $p < 0.0001$).

SIGNIFICANCE/CLINICAL RELEVANCE: Osteochondral tissues of TMJ are connective tissue and susceptible to degeneration. Our group is the first one to characterize the effects of different therapeutics on the osteochondral tissues of the TMJ in the mice. We have shown the beneficial effects of PTH and Alendronate on the osteochondral tissues of the TMJ.

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? Due to COVID19 most of the meetings were cancelled.
    a. If so, list titles, author or co-authors of these presentation/s, year and locations
    b. Was AAOF support acknowledged?
    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, my resident submitted his K99R00 grant and will revise and resubmit in March of 2021