

# The potential use of Harmine in root resorption during orthodontic treatment

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*2022 Biomedical Research Awards (BRA)*

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# FollowUp Form

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## *Award Information*

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*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?*

### **Title of Project:\***

The potential use of Harmine in root resorption during orthodontic treatment

### **Award Type**

Biomedical Research Award (BRA)

### **Period of AAOF Support**

July 1, 2022 through June 30, 2023

### **Institution**

Orthodontics, The Ohio State University

### **Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)**

Toru Deguchi (principal advisor), Brian Foster (co-investigator), Do-Gyoon Kim (co-investigator), Jie Liu (researcher)

## Amount of Funding

\$29,425.00

## Abstract

(add specific directions for each type here)

### Abstract

A major undesirable side effect from orthodontic treatment is external apical root resorption (EARR). EARR is associated with inflammation and resorption of the tooth root<sup>1</sup>. Generally, EARR would be repaired by cellular cementum but may result in permanent loss of root structure depending on the severity of the resorption<sup>2</sup>. The etiologic factors are complex and multifactorial, including individual biologic variability, genetic predisposition, and mechanical factors.

Cementum is the thin layer of mineralized tissue covering the root surface and essential for tooth attachment. Cementoblasts, similar to osteoblasts, express the transcription factors including runt-related gene 2 (Runx2) and osterix to regulate type I collagen (COL1), alkaline phosphatase (ALP), and osteocalcin (OCN). While EARR is associated with heavy and sustained mechanical forces, it has also been associated with altered Wnt signaling and dysregulated receptor activator of nuclear factor  $\kappa$ B (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG) signaling<sup>3</sup>. During cementum repair, extracellular matrix proteins such as fibronectin, osteopontin (OPN), and osteocalcin contribute to the recruitment of cementoblast precursors to the root surface and to their subsequent adhesion, proliferation, and differentiation<sup>4</sup>. Local cytokines and growth factors including insulin-like growth factor (IGF-1), fibroblast growth factor (FGF), epidermal growth factor (EGF), bone morphogenetic proteins (BMPs), and transforming growth factor- $\beta$  (TGF- $\beta$ ) also play important roles in cementoblast precursor differentiation and proliferation<sup>4</sup>.

Accumulating evidence suggests harmine, a recently studied carboline alkaloid, inhibits osteoclast formation, differentiation, and function<sup>5</sup>. Harmine inhibited multinucleated osteoclast formation induced by RANKL in RAW264.7 cells. Furthermore, harmine prevented RANKL-induced bone resorption in vitro and in vivo. Additionally, harmine increased mRNA expression of TNAP and OCN, and enhanced mineralization by MC3T3-E1 osteoblast-like cells<sup>6</sup>. A recent study found that with harmine coated beads, ex vivo kidney capsule cultured mandibular molar bud successfully developed the whole structure of tooth root including dentin, cementum and periodontal ligament (PDL) after 3 weeks<sup>7</sup>. Therefore, harmine is a potential osteoinductive agent that can be a promising cue for preventing and/or recovering from EARR.

In pilot studies, we analyzed the effects of harmine in vitro on OCCM-30 cementoblast cells and optimized a mouse model of orthodontics-induced EARR. Harmine decreased OCCM-30 proliferation and had a dose-dependent effect to reduce migration in a scratch assay. RT-PCR and western blot revealed that harmine stimulates in OCCM-30 cells the expression of transcription factors and differentiation markers on both RNA and protein levels, including Runx2, Dlx5, Osterix, Msx2, Col1a1, Ibsp, and Spp1, and increased mineral deposition was shown by von Kossa and alizarin red staining. These collected data strongly support harmine as a novel factor that may prevent or repair EARR by accelerating differentiation and mineralization of cementoblasts. In our mouse model of orthodontic root resorption, 50 gm force at 28 days induced significant root resorption shown by microCT and SEM/EDS analysis, establishing a model for in vivo testing.

Based on these data, we propose the following experiments. Specific Aim 1: To test effects of harmine on cementoblasts and cementoclasts functions. Hypothesis 1: In co-culture in vitro, harmine promotes cementoblast activity and inhibits cementoclasts. Specific Aim 2: To determine efficacy of harmine to inhibit EARR. Hypothesis 2: Harmine reduces EARR during orthodontic tooth movement in mice in vivo. The long-term objective of this project is to provide insight about innovative approaches to prevent and repair EARR caused by orthodontic treatment. The short-term goal will be to test harmine as a novel agent, producing additional data to support an NIH application on the topic.

## *Respond to the following questions:*

### **Detailed results and inferences:\***

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".

OR

Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

I. *in vivo*:

1) Effect of root resorption: The percent reduction of root length after the tooth movement (28 days) resulted in 22.4±13.6%, 14.1±11.2%, 9.6±6.2%, in mesial, distal buccal, distal lingual root in the control, respectively. In the harmine group, it resulted in 10.2±9.1%, 7.83±6.95%, 7.44±3.7%, in mesial, distal buccal, distal lingual, respectively. There was no significant difference between 2 groups in mesial ( $p=0.13$ ), distal buccal ( $p=0.32$ ), and distal lingual ( $p=0.51$ ) roots.

2) Effect on the amount of tooth movement: After 28 days, the average amount of tooth movement was significantly greater in the harmine group (0.49±0.02mm) compared to the control (0.36±0.07mm) ( $p<0.01$ ).

3) Changes of root volume (mm<sup>3</sup>) and density (HA/cm<sup>3</sup>): Total average root volume (all three roots) resulted in 0.48±0.05, 0.43±0.12, 0.46±0.08, 0.51±0.14 in the control without tooth movement, control with tooth movement, harmine without tooth movement, harmine with tooth movement, respectively. There was significant difference between with and without tooth movement in both groups ( $p<0.05$ ). Root volume decreased in the control after tooth movement, however, in harmine group, it increased.

Total average root density resulted in 6986.8±700.8, 5547.4±376.4, 6129±263.7, 6495.4±305.1 in the control without tooth movement, control with tooth movement, harmine without tooth movement, harmine with tooth movement, respectively. In the control, significantly lower density was observed after tooth movement ( $p<0.01$ ). In the harmine group, there was no significant difference in the density after tooth movement. Lower density was observed in harmine group compared to the control before tooth movement ( $p<0.05$ ). Higher density was observed in harmine group compared to the control after tooth movement ( $p<0.01$ ).

II. *in vitro*:

1) The effect of Harmine in OCCM-30 Cementoblasts Cell Line: i) Harmine inhibited cell proliferation rate in OCCM-30 (The proliferation rate of OCCM-30 is inhibited by 10 µm harmine after 2 days and 3 days treatment. ii) Harmine inhibited cell migration rate in OCCM-30 (Scratch assay study shows a dose-dependent inhibition manner). Ten µm harmine decreases migration rate after 12 hours, 5 µm harmine slows down the migration after 24 hours and 1 µm of harmine treatment decreases the speed after 36 hours, compare to control group. iii) Real time PCR results indicated that 10 µm harmine stimulates the expression of transcription factors and differentiation markers in OCCM-30. Expression levels of Runx2, Dlx5 and Osterix are stimulated after 14 days treatment. The expressions of Msx2 of Col1a1, Ibsp and Spp1 increase on day 4. No significant expression change was found on Ctnnb1.

2) The effect of Harmine in RAW264.7 Cell Line: i) Effect of harmine in RANKL expression. We compared groups with a) non treatment (vehicle control), b) RANKL (0.1µg/ml), c) Harmine (5µm and 10µm), d) RANKL (0.1µg/ml) plus Harmine (10µM). There was no significant effect in RANKL (0.1µg/ml) with 10µm harmine in 4 and 7 days after. By comparing the effect of different volume of harmine (5µm and 10µm), there was still no significant effect. All groups resulted in decrease in mineralization from 4 to 7 days, however, there was a significant increase in only 5µm harmine treated samples. Thus, Administration of Harmine resulted in increased calcification from days 4 to 7 in spite of RANKL administration. ii) Effect of harmine in TRAP expression. Administration of RANKL enhanced differentiation into osteoclasts with significance. When comparing the two RANKL non-administered groups, there is a decrease in the number of TRAP-positive cells in the Harmine 10u group, although there is no significant difference. When comparing the two RANKL-administered groups, there is a decrease in the number of TRAP-positive cells in the Harmine 10u group, although there is no significant difference. Thus, these result indicates that Harmine could regulate the differentiation of osteoclast progenitor cell.

**Were the original, specific aims of the proposal realized?\***

Yes. Harimine as a novel factor that may prevent or repair EARR by accelerating differentiation and mineralization of cementoblast. Harime may also have a potential to accelerate tooth movement without interfering the bone remodeling/remodeling phenomenon.

**Were the results published?\***

No

**Have the results of this proposal been presented?\***

No

**To what extent have you used, or how do you intend to use, AAOF funding to further your career?\***

I would like to further use it to apply to NIH grants.

**Accounting: Were there any leftover funds?**

\$21,139.00

***Not Published***

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**Are there plans to publish? If not, why not?\***

Yes. In preparation.

***Not Presented***

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**Are there plans to present? If not, why not?\***

Yes. Will be plan to present in next AADR.

## *Internal Review*

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Reviewer comments

Reviewer Status\*

## File Attachment Summary

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### *Applicant File Uploads*

*No files were uploaded*