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RESEARCH NOTE

Association between intermittent administration of parathyroid hormone 1-34 and ectopic calcification in rats [version 1; peer review: 1 approved, 1 not approved]

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Abstract

The present study was conducted to determine the association between parathyroid hormone 1-34 administration and ectopic calcification in rats with glucocorticoid induced osteoporosis. A total of 18 rats were used in the current study. Osteoporosis was induced in all rats via dexamethasone administration, then rats were randomly distributed into Control and Forteo groups and were sacrificed 4 weeks after initiation of drug administration. Hemi-mandibles were decalcified followed by routine histological analysis. Among the Forteo group rats, three rats displayed the presence of ectopic calcification. True pulp stone, intra-pulpal calcified structure with entrapped cells and intra periodontal bone-like calcified structure with entrapped cells were observed while no ectopic calcification was noticed in the control group.

Keywords

ectopic calcification, pulp stone, denticles, recombinant parathyroid hormone 1-34.

Open Peer Review

Approval Status

	1	2
version 1
26 Sep 2018	view	view

1. **Mohamed Shamel** , Modern Sciences and Arts University, Cairo, Egypt

2. **Suzan A. Kamel-ElSayed**, Oakland University William Beaumont School of Medicine (OUWB), Rochester, USA

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Radwan IA: Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; Sedky Korany N: Conceptualization, Supervision, Writing – Review & Editing; Adel Ezzat B: Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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Introduction

Ectopic calcification is pathologic deposition of minerals within soft tissues as dental pulp or periodontal ligaments (PDL)^{1,2}. Pulpal ectopic calcification may manifest as generalized, linear calcification, or as circumscribed calcification (also known as pulp stones or denticles). Pulp stones can be seen free within the pulp tissues, partially associated with dentin wall or completely embedded in dentin. They may manifest as false concentric calcification or true pulp stones³. The etiology of pulp calcification may be idiopathic⁴, although it may also be associated with pulp injury or degeneration⁵, orthodontic or physical forces^{6–8} or chemical stimuli^{9,10}. Its incidence tends to increase with age^{3,11}.

Parathyroid hormone (PTH) is a naturally occurring hormone, important for calcium homeostasis^{12,13}. Its level in the blood dictates its effect on the skeletal system, with bone catabolic effect upon chronic increase in PTH level associated with hyperparathyroidism^{13,14} and bone anabolic effect upon administration of small intermittent dosage^{15,16}. PTH secreted by the parathyroid gland (native PTH) is a polypeptide chain composed of 84 amino acids (PTH 1–84). While PTH 1–34, is a fragment of PTH molecule “synthesised through recombinant DNA technology using a strain of *Escherichia coli* bacteria”^{17,18}. Intermittent PTH 1–34 administration, owing to its bone anabolic effect, is successfully used for the management of osteoporosis^{19–22}. Its bone anabolic effect has been linked to elevated osteoblast differentiation, number and activity^{23–29}.

Methods

This research was conducted as a part of a study examining the effect of PTH 1–34 on microarchitecture of alveolar process of osteoporotic rats.

Animals

In the current research, 18 male Wistar rats of the species *Rattus norvegicus* weighing 175–200 gm, aged between 3 to 4 months were used. The animals were acquired from and maintained in the Animal House, Faculty of Medicine, Cairo University under the care of a specialized veterinarian. Each animal was kept in a separate cage. They were maintained under controlled temperature at 25±2°C with 12 h light/dark cycle and had *ad libitum* access to standard rats' chow and water. This study was approved by the Research Ethics committee faculty of Dentistry, Cairo University (approval number 151028).

Osteoporosis induction and treatment

Osteoporosis was induced in all experimental animals (n=18) by five weekly doses, of 7 mg/kg body weight dexamethasone sodium phosphate (Decadron® 4 mg/ml, Eipico Egypt), administered intramuscularly^{30,31}. The animals were then randomly distributed by random sequence generator program (randomizer.org) into two groups each including 9 animals, matching of the animals with the numbers was done blindly through the primary investigator. Animals received either a daily subcutaneous injection of 60 µg/kg body weight PTH 1–34 (Forteo®; Eli Lilly Pharmaceuticals) (n=9)³² or an equal volume of saline (control

group) (n=9). Drugs were administered in the early morning hours (8–9 am). The body weight of the animals was measured weekly, and drug dosages were adjusted accordingly. Animals were euthanized with an intra-cardiac overdose of sodium thiopental (80 mg/kg) 4 weeks after initiation of Forteo administration. Mandibles were dissected and separated into two halves, only one hemi-mandible from each rat was utilized for histological examination. The experimental unit was the hemimandible of rats. The primary investigator was blinded.

Hemi-mandibles (n=18) were fixed in 10% calcium formol solution for 48 hours. The specimens were then washed and soaked in 10% EDTA for 4–5 weeks for decalcification. After decalcification was completed, the specimens were dehydrated in ascending grades of alcohol, cleaned in xylol, and then embedded in paraffin blocks. Next, 6-µm-thick paraffin sections were cut and mounted on a clean glass slide, then stained with haematoxylin and eosin stain³¹. The specimens were examined using Leica DM300 light microscopic (Leica Microsystems, Inc., Switzerland). Histological examination was done through blinded primary investigator. Dental pulp and surrounding periodontal ligaments of all teeth within hemimandible of both experimental groups (n=18) were examined for the presence of ectopic calcification.

Results

Upon histological examination of the Forteo group specimens, six rats showed normal pulp and periodontal ligaments with no ectopic calcifications, while ectopic calcifications were detected in three specimens (Dataset 1)³³. Where true pulp stone with pre-dentin and dentin surrounding a central cavity lined by cells was detected in one specimen (Figure 1a). Another specimen showed the presence of intra-pulpal calcified structure with entrapped cells (Figure 1b). Meanwhile, one specimen displayed the presence of intra-periodontal bone-like calcified structure with entrapped cells (Figure 1c). On the other hand, no ectopic calcification was perceived in the control group specimens (Dataset 1)³³, which showed normal pulp and periodontal ligaments (n=9) (Figure 1d).

Dataset 1. Images captured from each mouse in each group not shown in Figure 1

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Discussion

Despite the fact that PTH 1–34 can successfully lower blood calcium level, and prevent vascular calcification³⁴, in the current work, PTH 1–34 was associated with ectopic calcifications within the pulp and PDL, while none was observed in the control group specimens.

Guimaraes *et al.* observed increased dentin deposition rate and elevated level of serum alkaline phosphatase in PTH 1–34 treated rats³⁵. In a subsequent research, Guimaraes *et al.* elucidated that PTH 1–34 can regulate odontoblast like cells via protein kinase A- and protein kinase C-dependent pathways, with

Figure 1. Light microscope images of the Forteo-treated group. (a) A true pulp stone with dentin, pre dentin and central cavity lined by cells (original magnification, x100 (left) and x400 (right)). (b) Intrapulpal calcification with entrapped cells (original magnification, x100 (left) and x400 (right)). (c) Intra-periodontal ectopic calcification with entrapped cells surrounded with disorganized periodontal ligaments (original magnification, x400). (d) Light microscope image of the control group showing normal pulp and periodontal ligaments with no ectopic calcification (original magnification x 100).

increases in odontoblast-like cells proliferation upon short PTH exposure and increases in their apoptosis upon longer exposure³⁶.

Wang *et al.* demonstrated the ability of PTH to induce human PDL stem cells to differentiate into osteoblasts, which was associated with increased alkaline phosphatase activity and increased mineralization capacity³⁷. Moreover, Li *et al.* described the ability of PTH 1–34 to induce the formation of calcified nodule in cementoblast cell line, which was attributed to the ability of the drug to increase cementoblast activity, alkaline phosphatase level and subsequently calcification³⁸.

The stimulatory effect of PTH 1–34 on odontoblast, cementoblast and osteoblasts function can help explain the findings of the current research.

Recommendations

Further research studying the effect of different dosage schemes of PTH 1–34, administered for different time periods, on dental pulp and odontoblast cells is recommended.

Data availability

Dataset 1. Images captured from each mouse in each group not shown in **Figure 1**. DOI: <https://doi.org/10.5256/f1000research.16298.d218523>³³.

Grant information

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Suzan A. Kamel-ElSayed

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The manuscript describes the histological findings of the dental pulp and periodontal ligaments after intermittent administration of PTH (1-34) to induced osteoporotic male rats. I think the manuscript requires additional data to be acceptable. The followings are my comments:

1. The title should include the word microarchitecture or histology to reflect the manuscript's content.
2. Data for ALP level or bone density measurement or ash value is required to confirm that osteoporosis was induced in all male rats.
3. The results should include images from both Forteo treated group and saline treated group. Although it is stated that the dataset included images captured from each mouse in each group, I did not find any image from control group. The names of the images included the word "Forteo" and thus I assumed that all images are captured from the treated group only. In addition, figure 1 and the supplemented images should include arrows that describe different parts e.g. dentin, pulp, periodontal, different calcifications (intrapulpal, intraperiodontal ...etc).
4. The discussion should include a possible explanation of why only 3 out of 9 rats developed ectopic calcification following PTH (1-34) intermittent injection and how the authors excluded the possibility of a prior existence of ectopic calcification.
5. Did the treatment improve the microarchitecture of the mandible of all osteoporotic treated rats? (please see the additional citation¹)
6. Results of the submitted manuscript should not be included as a reference (# 33).

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Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bone Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 01 October 2018

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Mohamed Shamel

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The current study performed by the authors is an interesting one which aims to determine the association between parathyroid hormone 1-34 administration and ectopic calcification formation in pulp and periodontal ligaments of osteoporotic rats.

Results revealed that rats receiving PTH 1–34 showed ectopic calcifications in their pulp and periodontal ligaments.

The study is well organized and the authors well documented their work in particular the images captured from rats of each group.

However I have some minor remarks as follows:

Aim:

I suggest that the aim should include that the investigations were carried on dental structures (Pulp and periodontal ligaments)

Results:

I suggest that the histology of the calcified areas to be thoroughly examined to determine if its histology resembles dentin, bone or cementum in each area.

Discussion:

More information is needed to determine the mechanism by which the PTH 1–34 is involved in stimulation of odontoblast, cementoblast and osteoblasts functions.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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