Effect of inferior alveolar nerve transection on the inorganic component of bone of rat mandible

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Abstract

Objective: The aim of the study was to test the effect of transecting the inferior alveolar nerve on the inorganic bone component of the rat mandible. Methods: 7-9 weeks old, male Wistar rats were used for the study. The animals were divided in 3 groups: control, experimental (nerve was transected) and sham (nerve was only prepared but not transected). After 4 weeks, the animals were killed, their teeth were extracted, and the mandibular bone was divided in 4 parts. Inductively coupled plasma mass spectrometry was used to the levels of 7 elements in the bone. Results: The study results demonstrate that transection of the inferior alveolar nerve caused a decrease in calcium, iron, and strontium, and an increase of zinc. It caused the differences in potassium contents between the sides was significantly lower in the experimental group. The increase in the magnesium content, and decrease of sodium and potassium in the experimental group, as well as differences in the contents of: magnesium, sodium, potassium, iron and zinc between individual locations in the mandible are associated with the surgical approach. Conclusion: The results support our hypothesis - that sensory innervation has an impact on the inorganic component of the mandibular bone.

Keywords: Inferior Alveolar Nerve, Mandible, Rats, Elements, Nerve Transection

Introduction

Bone remodelling is a process that goes on throughout an individual’s lifetime. This process is slow and is regulated by several hormonal, paracrine/autocrine, mechanical, and transcription signals whose targets can be osteoclasts and osteoblasts at various stages of their lifespan1. A number of studies have been published demonstrating how the nervous system affects bone metabolism1-6. Aside from carrying sensory information, sensory neurons also produce various neuropeptides, which play an important role in maintaining bone homeostasis6. Bone is composed of 45% minerals, 30% organic matter, and 25% water7. It is richly innervated by sensory and sympathetic fibres. Nerve fibres are present in the periosteum, cortical bone, and bone marrow; no typical synapses are found between the nerves and bone cells. The direct contact of nerve fibres and bone cells indicates the importance of innervation for the function of these cells8. Nerve endings of inferior alveolar nerve (IAN) begin degeneration after injury that is not promptly treated, causing reduction in the release neuropeptides thus affecting the balance of bone metabolism. Therefore patients with IAN injury may suffer from not only paresthesia but also destruction of mandibular bone integrity8.

In our study we analysed the following elements: magnesium (Mg), sodium (Na), potassium (K), calcium (Ca), iron (Fe), zinc (Zn) and strontium (Sr).
Mg is osteoprotective through the attenuation of bone resorption and by improving bone quality. Similar to Cu and Zn, it is a co-factor of enzymes that regulate Ca metabolism.11 Na is the main extracellular element. Hyponatremia may stimulate osteoclastic proliferation and mobilise Na stored in bone.13 K is the main intracellular element, and along with Na it is responsible for maintaining the cell membrane potential. On the bone surface, there is a layer enriched by K.12 Ca is one of essential components of bones and teeth, where it is present as a part of hydroxyapatite. As indicated by an experimental study, high concentration of extracellular Ca stimulates osteoclast-like cell formation and bone resorption of mature osteoclasts, presumably via osteoblasts.14 Fe as an enzymatic co-factor is engaged in the synthesis of bone matrix (activation of lysyl hydroxylase) and in the synthesis of D-hormone (activation of 25-hydroxycholecalciferol hydroxylase). Intestinal absorption of calcium is stimulated by the D-hormone.11 Iron deficiency in rats has been shown to cause insufficient bone mineralisation.15 Zn acts as a co-factor enzyme, stimulating growth through activation of DNA and RNA synthesis, and enzymes involved in protein synthesis. In bone, Zn activates osteoblasts and increases their formation rate, it supports the synthesis of collagen, and attenuates osteoclastic resorption. At the same time, Zn increases bone strength.13 As reported by Yamauchi et al., Zn had a direct stimulatory effect on bone mineralisation in vitro, and bone protein synthesis was a necessary component of this response. Sr increases cartilage matrix secretion, osteoblastic proliferation, improves bone mineralisation, and it inhibits osteoclastic differentiation and resorption activity.13,17,18 The aim of the study was to test the effect of transecting the inferior alveolar nerve on the inorganic bone component of the rat mandible. Changes in the inorganic bone component caused by an innervation disorder could be reflected in altered bone properties e.g. changes in bone strength and healing.

Material and methods

Experimental animals

26 male Wistar rats, aged 7-9 weeks, with body weights 320-405 g were used for the study. The animals were obtained from the breeding station of the Institute of Physiology of the 1st Faculty of Medicine, Charles University in Prague. The experiment was performed in line with the applicable directives governing the use of laboratory animals - EU Council Directive 86/609EEC. The animals were placed in boxes at 20-23°C, using the standard 12h light-dark cycle. The animals received normal food (ST-1: www.velaz.cz/product/st-1) and had water available ad libitum. The rats were divided in three groups: a control group (intact nerve) (n=6), an experimental group (IAN on left side was transected) (n=12), and the sham group (IAN was prepared but not transected) (n=8).

Inferior alveolar nerve transection

The surgical procedure was carried out under general anaesthesia, produced by intraperitoneal application of thiopental 4 mg/100 g rat weight. A microsurgical technique (using the microscope: Carl Zeiss OPTON S4, Germany) was used to approach and excise the nerve. An arch-shaped, 12 mm long skin incision was made on the left part of the face, from the mouth corner to the lower edge of the external acoustic meatus. The midpoint of the incision was found between the corner of the mouth and the lower edge of the external acoustic meatus. We exposed the masseter muscle fascia, which was then cut in the direction of muscle fascicles, between the dorsally oriented facial nerve and the ventrally...
located parotid duct. After blunt preparation of the muscles, the lateral part of the mandible was reached at the place of its prominence. The bone crest was identified in the direction from the prominence to the condylar process. Caudally from the prominence, a round (1.2 mm) dental milling cutter (500.104.001.001.012, Medin, a.s., Nové Město na Moravě) was used to remove a portion of the bone. We exposed 3mm of the neurovascular bundle, which was prepared using a microsurgical technique to ensure the blood vessel were not injured during the excision of the nerve. The nerve was partially pulled out of the mandibular canal, and a 3mm section was excised. The wound was rinsed with 1 ml saline. The muscle edges were adapted using a non-absorbable suture (Prolene 4/0). The same non-absorbable material was used to close the skin.

**Tissue preparation**

The rats were weighed after 4 weeks, and then killed with intraperitoneally administered thiopental. The left and right parts of the mandible were prepared. Incisor and molars were extracted (they were not included in this study). The mandibular bone was divided in 4 locations. Osteotomy was performed vertically, mesially from the extracted first molar, and distally from the extracted third molar. In addition, a sample was taken from the middle part of the mandibular branch, from the entry point of the mandibular nerve into the canal, and where the mandible widens and passes into the articular projection. In total, 4 bone samples were taken from each side, and labelled according to location: mesial (1), central (2), distal (3) and ridge (4) (Figure 1).

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**Figure 2.** The relationships between concentrations of Mg (ppm) in groups, location of sampling sites, and side of sampling were evaluated using ANOVA model. The model consisted of Subject factor explaining inter-individual variability, between-subject factor Group (control (C), experimental (E), sham group (S)), within-subject factors Location (four sites were investigated in animal such as mesial (1), central (2), distal (3), and ridge (4)) and factor Side (right (R) vs. left (L)), and all corresponding interaction between the factors except of the subject factor. $F$ represents the Fisher's statistic and $p$ designates statistical significance for the factors and interaction. The symbols with error bars represent re-transformed means with their 95% confidence intervals (triangles, circles, and squares symbolize $C$, $E$, and $S$ group, respectively). The full and empty symbols represent right and left side of sampling, respectively. The 95% confidence intervals are computed using the least significant difference multiple comparisons ($p<0.05$). The confidence intervals, which do not overlap each other, denote significant difference between the respective subgroup means. Statistical software Statgraphics Centurion, version 18 from Statgraphics Technologies, Inc. (The Plains, Virginia, USA) was used for the statistical analysis.

All - Group: $F=111.3$, $p<0.001$ (Panel A); Location: $F=67.1$, $p<0.001$ (Panel B); Side: $F=0.3$, $p=0.571$ (Panel C); Group × Location: $F=18.8$, $p<0.001$ (Panel D); Group × Side: $F=0.2$, $p=0.812$ (Panel E); Location × Side: $F=0.1$, $p=0.987$ (Panel F); Group × Location × Side: $F=0.9$, $p=0.5$; Subj(Group): $F=39.7$, $p<0.001$

Group C - Location: $F=95.8$, $p<0.001$; Side: $F=0$, $p=0.887$; Location × Side: $F=0.3$, $p=0.862$ (Panel G); Subj: $F=22.9$, $p<0.001$

Group E - Location: $F=16.4$, $p<0.001$; Side: $F=0$, $p=0.981$; Location × Side: $F=1.5$, $p=0.228$ (Panel H); Subj: $F=61.9$, $p<0.001$

Group S - Location: $F=10.1$, $p<0.001$; Side: $F=0.5$, $p=0.474$; Location × Side: $F=0.3$, $p=0.826$ (Panel I); Subj: $F=20.8$, $p<0.001$
Chemical analysis

The weighted amount of 10-130 mg of the dried bone samples were inserted into 10 ml volumetric flasks; a value of 0.5 ml of concentrated HNO₃ was added; subsequently, the samples were dissolved by careful heating of the glass on the heating plate at approx. 100°C. After cooling, deionized water was added to the mark of the volumetric flask. A blank samples were prepared for every series of 20 samples. The measurement quality was tested by analyzing the standard reference material (SRM 1400, Bone Ash, National Institute of Standards and Technology, Gaithersburg, MD). Differences between the measured and certified values were lower than the 10% RSD (relative standard deviation). All the acids used in the dissolution procedure were reagent grade (Merck, Darmstadt, Germany). Deionized water from MilliQPlus (Millipore, Billerica, MA) were used to prepare the solutions. The contents of Mg, Na, K, Ca, Fe, Zn and Sr in the solutions were determined using inductively coupled plasma mass spectrometry (ICP MS, X Series II, Fisher Scientific, Gmbh, Bremen, Germany) under the following conditions: ICP 1350W, "peak jump" measurement mode, measurement time 3 x 50 s, ion optics parameters optimized with Ge, Rh and Re 20 µg l⁻¹ solutions (Aastasol, Analytika, Czech Republic), gas flows 13.5 l/min (cooling), 0.7 l/min (auxiliary), 0.65 l/min (nebulizer). Measured isotopes of ⁷²Ge, ¹⁰³Rh, ¹⁸⁵Re, were used as internal standards.

Statistical analysis

1. The mean weight increase of the rats between the groups after 4 weeks were compared using the t-test. GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA) was used for the statistical analysis. A statistical significance level of 5% was used.
2. Respecting the skewed distribution and non-constant variance in most dependent variables, these were transformed by power transformations to data symmetry and homoscedasticity prior further processing. The homogeneity and distribution of the transformed data, and residuals, were checked by residual analysis as described elsewhere. The model consisted of Subject factor explaining inter-individual variability, between-

Figure 3. The relationships between concentrations of Na (ppm) in groups, location of sampling sites, and side of sampling were evaluated using ANOVA model. The drawings and symbols are the same as for Figure 2.

All - Group: F=212.7, p<0.001 (Panel A); Location: F=23.7, p<0.001 (Panel B); Side: F=6.6, p=0.011 (Panel C); Group × Location: F=18, p<0.001 (Panel D); Group × Side: F=0.6, p=0.579 (Panel E); Location × Side: F=0.2, p=0.92 (Panel F); Group × Location × Side: F=0.3, p=0.926; Subj(Group): F=55.7, p<0.001
Group C - Location: F=95.2, p<0.001; Side: F=0.6, p=0.458; Location × Side: F=0.4, p=0.784 (Panel G); Subj: F=164.8, p<0.001
Group E - Location: F=8.8, p<0.001; Side: F=4.3, p=0.041; Location × Side: F=0.4, p=0.747 (Panel H); Subj: F=42.2, p<0.001
Group S - Location: F=4.4, p=0.008; Side: F=3.7, p=0.062; Location × Side: F=0.3, p=0.83 (Panel I); Subj: F=49.4, p<0.001
subject factor Group (control (C), experimental (E), sham (S)), within-subject factors Location (four bone sites were investigated: mesial (1), central (2), distal (3), and ridge (4)) and factor Side (right (R) vs. left (L)), and all corresponding interaction between the factors, except of the subject factor. For instance, significant Group × Location interaction indicates that the Group factor significantly influences the differences between location of sampling sites. Statistical software Statgraphics Centurion, version 18 from Statgraphic Technologies, Inc. (The Plains, Virginia, USA) was used for the statistical analysis.

The null hypotheses for all factors and all possible inter-factor interactions were tested. However, the primary questions were associated with null hypotheses for the interactions as follows: factor difference between groups, side x group, side x location and side x group x location.

Results

1. We found no statistically significant differences in mean weight gains between individual groups during the observation period.

2. The contents of 7 elements: Mg, Na, K, Ca, Fe, Zn and Sr were determined in all three groups.

   Mg contents in the control group was lower compared to the experimental and sham groups. The content in the central and distal locations were considerably lower compared to the mesial and ridge locations in the control group. There was a difference between the mesial location and distal location in the experimental and sham group (Figure 2).

   Na content showed an obvious decrease in the experimental and sham groups compared to the control group. No essential differences were found between the experimental and sham groups. In the control group, a lower content of Na was found in the central and distal locations compared to the mesial location and ridge. This difference was not seen in the experimental and sham groups (Figure 3).

   The K content is lower in the experimental and sham groups than in the control group. Furthermore, there is a clear statistically significant difference between the left and right side in the control group and in the sham group. The difference between the sides was significantly lower in the experimental group. In the control group, the K content between the locations differed from the differences between the locations.
Regarding Ca, a decrease of its content was found in the experimental group compared to the control group and sham group (Figure 5).

A decrease Fe content was observed in the experimental group compared to the control group and sham group. In the experimental group and sham group, there was a difference between the distal and ridge locations, while in control group no difference was demonstrated (Figure 6).

The content of Zn was higher in the experimental group compared to the control group; at the same time, a lower content was observed in this group compared to the sham group. We demonstrated of reduction in the difference between locations in the experimental and sham groups compared to the control group (Figure 7).

A statistically significant decrease in Sr content was found in the experimental group compared to the control group and sham group (Figure 8).

**Discussion**

Determining the role nerves play on bone remodelling is of vital importance. Kunc documented the disappearance of neuralgia after a surgery on the contralateral side. As found by this author, the contralateral second neuron, whose path occurs not far from the nucleus caudalis hilum after the crossing point, was also affected by a vertical nucleotomy. This fact explains the observed changes in the element contents on the contralateral side i.e. although unilateral transection of IAN was performed, the contralateral nerve termination was also affected.

As noted by Travers, the spinal trigeminal nucleus affects afferentation of the ipsilateral motor trigeminal nucleus, and via connections over the reticular formation, hypothalamus and cortex, it affects the contralateral area supplied by the trigeminal nerve.

These innervation patterns may exert an effect on mastication and thus on the load of the mandible. As reported by various authors, the mandible load has an impact on bone mineralisation. As follows from the literature, bone mineralisation differs in individual parts of the mandible. Therefore we divided the mandible into 4 parts (Figure 1).

We assume these changes impact the element content in the mandibular bone, changes visible concurrently on both sides of the mandible as indicated by the results of our study. Based on literature, we used male rats, aged 7-9 weeks.
old in our study. In the case of females, the results would be influenced by hormonal changes of the animals in connection with the oestrous cycle. At the same time, as regards the males and as reported e.g. by Wang et al.29, rat is suitable for the study of osteoporosis in men because bone remodelling in the rat is similar to that in man. The authors recommended animals older than 9 months. We excluded this effect in animals used in our study29.

Surgical options causing only a sensory lesion to a nerve were considerably limited due to many peripheral nerves also containing a motor component. From this point of view, an experimental model with IAN lesion was appropriate; the IAN is a sensory nerve that runs through the mandibular canal and contains no motor nerves30.

For example, previous studies using the described model have demonstrated the effect of nerve transaction on bone remodelling during an experimental movement of the teeth31, or the consequences of IAN transaction on healing of a periodontal defect and CGRP expression28. The effect of IAN transaction on chemical elements in bone has not been previously described in available literature.

Various authors have studied the processes involved in bone remodelling8,27-35. As reported by Wu et al.8, the role of IAN in maintaining homeostasis is closely related to the anabolic effect of CGRP, which suppresses the number of osteoclasts through OPG/RANKL ratio and control of growth factors expression. Offley et al.27 showed that application of capsaicin to adult rats causes a reduction in trabecular bone integrity, bone mineral density (BMD) and bone strength. These findings indicate an effect of sensory innervation on bone mineralisation27. A reduction in BMD is characteristic for osteoporosis, where an overall worsening of the quality of bone tissue is observed32. The BMD of the jaws is gaining importance in contemporary dental practice as mandibular BMD may have an important role in the treatment planning, management, and prognosis of dental procedures such as osseointegrated implants, periodontal disease, and grafting33. Sample et al.34 described that right ulna loading induces adaptive responses in other bones in both thoracic limbs and that brachial plexus anaesthesia during loading abrogated bone formation in the loaded ulna and other thoracic limb bones. As reported by these authors, functional adaptation to loading of a single bone in young rapidly growing rats is neuronally regulated and involves
multiple bones\textsuperscript{34}. As reported by Růžičková\textsuperscript{35}, differentiation and activation of osteoblasts and osteoclasts is regulated by transcription factors, cytokines and growth factors products locally, by the bone cells themselves, and by systemic factors. The role of osteoblasts consists of the synthesis of new bone mass and osteoid, allowing for subsequent mineralisation. Osteoclasts are responsible for bone resorption\textsuperscript{35}.

No statistically significant changes in the animal weights were demonstrated during the 4 week observation period. As a result, we presume no effect of food intake on the chemical element content. We also expect no effect on the gut microbiota, which was previously described by Sjögren et al.\textsuperscript{36} as an important regulator of bone mass.

The effect of transection of the nerve on the chemical elements is indicated by statistically significant differences between the control group and experimental group, and by the difference between the experimental group and sham group. If there is no difference between the experimental and sham groups, but there is a difference between these two groups and the control group, the results would represent a consequence of the surgical procedure.

From our results, IAN transection caused a decrease in the contents of Ca, Fe, and Sr in the mandible (experimental group). We assume that the transection of the nerve may also have caused the Zn content to increase in this group. The higher content of Zn in the sham group compared to the control group and experimental group remains unclear. Furthermore, transection of the nerve caused differences in K contents between the sides, which was significantly lower in the experimental group. The difference in K between the sides in the control group is difficult to explain. Smrčka\textsuperscript{12} reported different K levels between the left and right femur in a 21 year old human cadaver. This difference has not been shown in cadavers of higher age, and the author attributed the differences to growth\textsuperscript{12}.

We also noted a decrease of K and Na contents in the experimental group. Considering that the findings in the sham group were identical, it is presumed that the surgical approach to the nerve had an effect. The increased content of Mg in the experimental group is also believed to be a consequence of the surgical approach. Similarly, the changes in the distribution of Mg, Na, K, Fe and Zn at the various locations in the mandible is also thought to be a result of the approach (Figures 2-8).

Figure 7. The relationships between concentrations of Zn (ppm) in groups, location of sampling sites, and side of sampling were evaluated using ANOVA model. The drawings and symbols are the same as for Figure 2.

All - Group: F=35.2, p<0.001 (Panel A); Location: F=57.3, p<0.001 (Panel B); Side: F=7.6, p=0.007 (Panel C); Group × Location: F=7.2, p<0.001 (Panel D); Group × Side: F=1.6, p=0.215 (Panel E); Location × Side: F=0.4, p=0.771 (Panel F); Group × Location × Side: F=2, p=0.07; Subj(Group): F=71.4, p<0.001

Group C - Location: F=45.4, p<0.001; Side: F=0, p=0.937; Location × Side: F=1.4, p=0.258 (Panel G); Subj: F=2.4, p=0.056

Group E - Location: F=20.1, p<0.001; Side: F=7.8, p=0.007; Location × Side: F=3.2, p=0.028 (Panel H); Subj: F=76, p<0.001

Group S - Location: F=13.8, p<0.001; Side: F=5.2, p=0.028; Location × Side: F=0.1, p=0.974 (Panel I); Subj: F=148.2, p<0.001
The statistically significant difference between the groups indicate that IAN transection has an impact on the contents of some elements in the mandible. At the same time, the results indicate changes in the inorganic component of the mandible due to the surgery.

Considering the speed at which these changes occurred, we expect that the elements move in the bone as ions. These facts are also supported by the changes in Na and K contents in the experimental and sham groups compared to the control group. These elements maintain the electrochemical gradient across the cell membrane, which will be damaged by the surgical procedure.

As follows from the study, the experimental rat mandible model can be used to test changes in chemical elements in bone tissue after transection of the inferior alveolar nerve. This fact can be used in further research on how sensory innervation affects some other elements in bone tissue, and also to determine any effects on mandibular teeth.

**Conclusion**

The results support our original hypothesis, i.e. that sensory innervation has an impact on the inorganic component of the mandibular bone. The results make it possible to expect changes in elements in the human mandible caused by IAN transection and thus to assume that the nervous system has an effect on these changes. The study shows that these changes affect both of the jaw.

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