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A Case of Increased Density in Rabbit's Jaw Bone, Evaluated by Cone-Beam Tomography

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Abstract

Reduced mineral content affects the trabecular microstructure in the jawbones and skeletons of animals. The aim of this study was to ascertain the benefits in terms of increased bone density in the region of the lateral teeth in the upper and the lower rabbit jaws after application of a therapeutic solution of calcitonin and calcium.

The solution was injected to the right side of the maxilla and mandible on the lateral teeth, while the left side was the control. Statistically significant increases in density after application of the therapeutic solution were verified in the experimental regions, in front of the maxillary lateral teeth and around the first maxillary molar (Z=-3.824 and Z=-3.826; p=0.000). ANOVA testing has provided evidence on increasing bone density after the use of a therapeutic solution in the experimental regions of the bone (4.324; df1=7; df2=78.775; p=0.000). Cone beam computed technology (CBCT) proved very reliable for assessing the jawbone density in animals, locally applying the additionally therapeutic solution for acceleration of the increment of bone density.

Keywords: *bone remodelling, bone regeneration, bone biology, radiography, animal, mineralized tissue, maxilla.*

Introduction

The mineral content of bone implies the presence of important factors that enable regular metabolism of bone and the harmonic functioning of the orofacial system in mammals. Changes in the mineral structure occur due to aging or because of progressive pathologic processes such as osteoporosis, as well as in both aging and effects of bone diseases. Bellido et al. [1] reported that the reduced mineral density and osteoporosis affect the trabecular microstructure in the skeletal and jawbones of rabbits and mammals. The premolar or molar maxillary and mandibular regions could be of particular interest, because the catabolic changes that weaken the trabecular bone mineral density, along with the corresponding structural deteriorations, can affect chewing and other vital functions of the orofacial systems in animals.

Several radiographic techniques have been described in the literature with respect to screening the maxillary and mandibular bone structures in mammals. Cone beam computed tomography, CBCT, is a state of the art technology that provides 3D imaging in a 360° arc. The unique characteristics of CBCT allow image re-acquisition and display. Software packages generate numbers from these data; however, they do not correlate with Hounsfield units. Contrast resolution can also be limited by the interfaces and may blend between the different surfaces, resulting in volume artefacts. CBCT imaging provides information on the accessible bone characteristics and the internal anatomy of the alveolar ridge, particularly the corticated versus non-corticated areas [2].

The aim of this study was to ascertain the benefits in terms of increased bone density in the region of the lateral teeth in the upper and the lower rabbit jaws after application of a therapeutic solution of calcitonin and calcium.

Case report

Selected adult experimental female rabbit was anaesthetized (Xylasin 5.0 mg/kg and Zoletil 15 mg/kg). A therapeutic solution of calcitonin and ionized calcium was prepared in a percentage ratio of 3:1 in favor of the calcitonin. The therapeutic solution was injected locally, to the right side of the mandible in the region beside the lateral teeth, and also in the region around the lower molars, more to the right side of the upper jaw in front of the lateral teeth and in the region around the first upper molars. The solution was injected twice with a 2-week interval between

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treatments. The left side of each jaw was the control side.

To diagnose changes in the bone density around the premolars and molars in both the control and experimental sides, a 3D scanning roentgen apparatus (Scanora 3D, Soredex, Finland) was used. The conditions during radiography were 8 MA and 90 KVp; the tube rotated every 13 seconds with a total pulse exposure time of 3 seconds. Two series of recordings were done under the same conditions: the first was performed at baseline, before the therapy (Fig.1), and the second series was performed after the injection of the therapeutic solution (Fig.2).

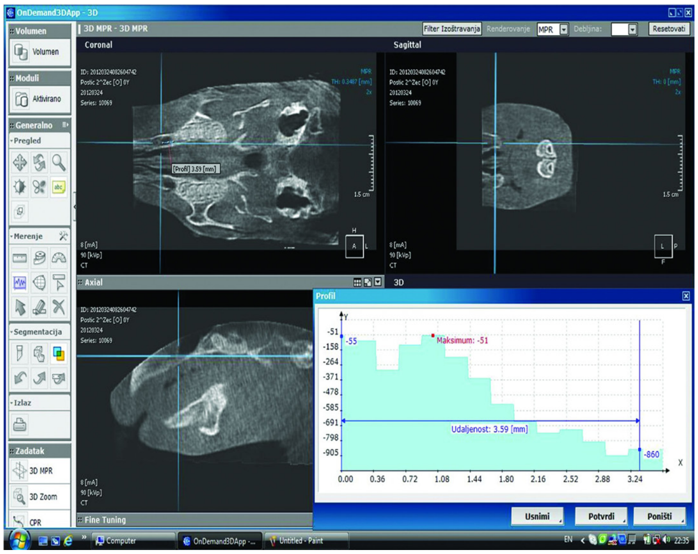


Figure 1.

Bone site in the experimental region on the right side of the maxilla in front of the upper lateral teeth at baseline at the time of the first application of the therapeutic solution of calcitonin and calcium.

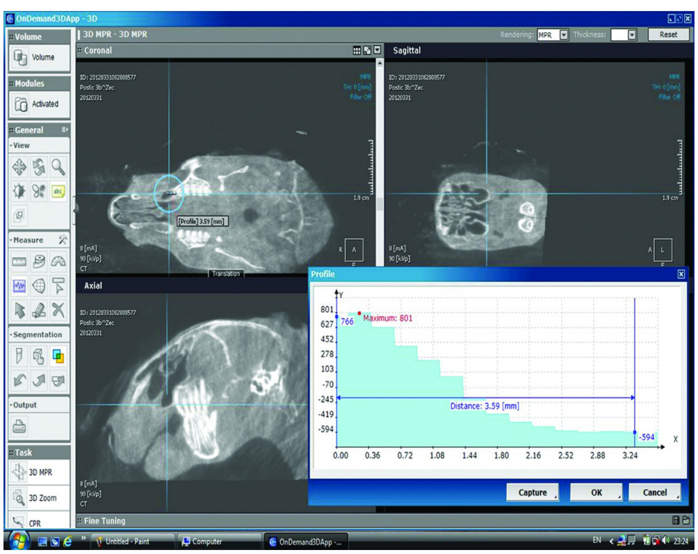


Figure 2.

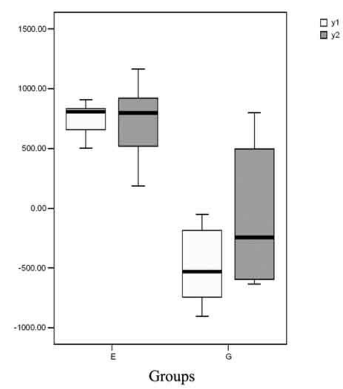
Bone site in the experimental region on the right side of the maxilla in front of the upper lateral teeth after repeated application of the therapeutic solution of calcitonin and calcium.

The regions of interest were determined on each radiographic plane: region “A”- region of the front surface of the lateral teeth on the left side of the mandible; region “B”- around the first lower molar on the left side of the mandible; region “C”- on the right side of the mandible in front of the lateral teeth; region “D”- on the right side of the mandible around the first lower molar; region “E”- on the left side of the maxilla in front of the lateral teeth; region “F”- region on the left side of the maxillary surface beside and in front of the first upper molar; region “G”- on the right side of the maxilla in front of the upper lateral teeth (Fig.1); and region “H”- on the right side of the maxilla beside the first maxillary molar.

The regions “A”, “B”, “E” and “F” were the control regions, whereas regions “C”, “D”, “G” and “H” were the experimental regions. The therapeutic solution was applied to the experimental regions at baseline, and again after two weeks.

Bone densities were measured through the intersections of each bone area and graphically represented with numerical values of bone density in the regions of interest, shown radiographically on 3D radiographs using computer programs OnDemand 3D Application,Cybermed. The numerical values of the densities were assigned as y1 for the first series of measurement, and y2 for the second series of measurement. Ten different density values were measured in the bone intersections in each group.

Comparisons of the bone density before and after the application of a therapeutic solution of calcitonin and calcium were calculated, using the Mann-Whitney U test (MedCalc statistical software, Version11.4 Software, Mariakerke, Belgium) (Tables 1-3 and Figures 3 and 4).



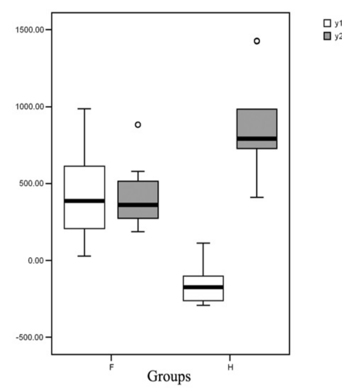


Figure 3.

The results of Mann-Whitney U test on density values between groups E and G.

Figure 4.

The results of Mann-Whitney U test on density values between groups F and H.

Statistically very significant differences in bone density were calculated between groups “E” and “G” where the arithmetic mean of differences was 450.448 (p= .000). Differences were defined as significant if $p < 0.05$). Statistically significant changes in the bone density after the application of the therapeutic solution of calcitonin and calcium were verified in groups “F” and “H” where the arithmetic mean of differences was 974.57 (p= .000). In the “F” group the average value at the baseline was 435.42 ± 317.29 and the median=388; by the end of treatment with the therapeutic solution of calcitonin and calcium, the average value was 412.11 ± 206.95 and median=362. In the “H” group the average value at the baseline was 139.11 ± 137.59 and the median= -173, by the end of treatment with the therapeutic solution of calcitonin and calcium, the average value was 812.15 ± 715.80 and median= 411. In other groups, the differences in the numerical values for bone density were enhanced or reduced, but they were not statistically significant (Table 1, Table 3).

Table 1.

Values of bone densities at the time of initial application of the therapeutic solution and after repeated application of the therapeutic solution.

	Group	N	Average	Median	Minimum	Maximum	Range	SD
y1	A	19	154.2632	-33.0000	-999.00	2095.00	3094.00	732.48063
	B	19	623.4737	617.0000	-478.00	1712.00	2190.00	678.12465
	C	19	316.3158	-80.0000	-400.00	2110.00	2510.00	668.48685
	D	19	680.4737	704.0000	-231.00	1640.00	1871.00	698.37059
	E	19	754.8421	808.0000	504.00	909.00	405.00	129.54161
	F	19	435.4211	388.0000	28.00	987.00	959.00	317.29154
	G	19	-470.5789	-531.0000	-905.00	-51.00	854.00	318.78054
	H	19	-139.1053	-173.0000	-291.00	114.00	405.00	137.58952
	Total	152	294.3882	204.0000	-999.00	2110.00	3109.00	648.01646
y2	A	19	202.5263	36.0000	-999.00	3145.00	4144.00	968.91694
	B	19	737.6316	700.0000	-341.00	2301.00	2642.00	590.12072
	C	19	248.0000	-62.0000	-750.00	2532.00	3282.00	907.65155
	D	19	910.0000	1175.0000	-320.00	2339.00	2659.00	872.10652
	E	19	708.6316	799.0000	188.00	1166.00	978.00	321.72732
	F	19	412.1053	362.0000	188.00	883.00	695.00	206.95273
	G	19	-66.3421	-245.0000	-634.00	801.00	1435.00	549.51772
	H	19	812.1579	792.0000	411.00	1428.00	1017.00	292.63444
	Total	152	495.5888	411.0000	-999.00	3145.00	4144.00	715.80099

Note: *Y1=values at the baseline; Y2=values of bone densities after repeated application of therapeutic solution only to experimental sites.*

Table 2.

Results of statistical tests for repeated measurementsfor areas of bone densities between groups

Groups		y2 - y1
A	Z	-.196
	P=	.845
B	Z	-.282
	P=	.778
C	Z	-.201
	P=	.841
D	Z	-.966
	P=	.334
E	Z	-.564
	P=	.573
F	Z	-.524
	P=	.600
G	Z	-3.824
	P=	.000
H	Z	-3.826
	P=	.000

Table 3.

ANOVA testing of statistical significance of differences in bone densities for inhomogeneous variances

d

Brown-Forsythe

Statistic (a)	df1	df2	P=
4.324	7	78.775	.000

Statistically significant increments in the bone density prior and post application of the therapeutic solution of calcitonin and calcium were determined in groups “G” (Z=-3.824 and P= .000) and “H” (Z=-3.826 and P=.000) with respect to other groups (Table 2).

Based on ANOVA testing of significance of differences for inhomogeneous variances it was determined that highly statistically significant differences in bone densities 4.324, p=0.000 existed between the groups at the baseline, and post treatment with the therapeutic solution of calcitonin and calcium (Table 3).

Discussion

The problems inherent in the assessment of the mineral content and densities of the mammalian jawbones have not been comprehensively studied in the literature. Not many reports are available in the literature on the techniques of measuring bone density. Furthermore, descriptions of the radiographic techniques used to show and measure mineral content and density in the jawbones in animals have been moderate and restricted until recently. Only very few investigations have been conducted on experimental animals, in which the decrements in bone density have been tentatively induced [1].

CBCT is a state of the art method for the assessment of bone and other structures of the stomatognathic system. Several similarities exist between the CBCT and conventional CT; both are computer-generated imaging techniques offering multiplanar reformatting and allowing data export in DICOM (digital imaging and communications in medicine) as the standard format. The CT involves a finely calibrated fan-shaped x-ray beam that can be single, spiral or multislice. The CBCT has a much higher image quality, producing 3D pictures, although the scan time is longer and substantial scatter and signal to noise ratio may be present. In addition, significant beam hardening and poor soft tissue contrast may be found, but there are also generally fewer metal artefact images. For the orofacial measurements, CBCT is as accurate as the conventional CT [3]. The CBCT analysis appeared to correlate with the markers of bone metabolism [4].

Several radiological methods are used for bone mineral density (BMD) measurements yielding close relationships, such as dual-X-ray absorptiometry (DXA) scanning and Hounsfield unit scale (HUs) from computed tomography imaging [5]. Texture analysis has been applied in the micro-CT [6], while the Hounsfield units have been used in spiral CT as a measure related to jaw BMD [7]. The CBCT is a more recent development than the spiral CT and its clinical application in the field of dentomaxillofacial radiology is gaining importance [2,8].

However, CBCT does not necessarily allow for reliable and accurate bone quality assessment when focusing on the inherent radiographic density information that is otherwise expressed as Hounsfield Units [9]. Conversely, a recent study reported a strong positive correlation between the radiographic bone density, assessed by CBCT, and bone volumetric fraction assessed by micro-CT at the site of the dental implants in the maxillary bones [10].

Cancellous bone with reduced mineral content and osteoporotic cancellous bone are characterized by low bone mass, as well as a deterioration of the microarchitecture. The microarchitecture of the cancellous bone has been largely attributed to the density-mechanical property relationship. Bone density approximates the amount of bone tissue within a cancellous bone specimen but does not quantify the inherent microarchitecture. Together with the bone density readings, a quantitative measurement of the microarchitectural parameters may improve the ability to estimate bone strength [2,11]. On the contrary, other authors have reported success with the CBCT for assessment of increases in the bone density and described how the volumes of the entire defect, areas of osseous consolidation (density values >2350) and nonmineralized areas (density values <2350) of the defects were all determined, as well as the application of a visual semi-quantitative CBCT score to assess bone defect healing. In this research, a significant positive correlation between the histologically visible newly-formed bone and the extent of bone regeneration on CBCT volumetry was established, and the visual score matched with the volumetric results in 75% of the cases [12]. Moreover, in another animal study, the structural parameters and bone mineral density were determined by microcomputed tomography and conventional computed tomography, respectively. Findings in this study implied that the regional anatomic domains must be considered in the planning of pre-clinical studies, taking osteoporotic changes into account [13].

In a remarkable study, problems due to the performance of the CBCT in the bone density analysis in animal jaws were resolved using a highly selective 3D software to analyze the Hounsfield unit scores. Data from this study showed that the CBCT is a sensitive method to detect jaw osteonecrosis in the treatment of reduced jawbone density [14]. In another study on humans and jawbone density, the CBCT results were presented in Hounsfield units. Bone density measurements using preoperative CBCT were determined to be helpful as a diagnostic tool, providing the objective assessment of bone quality [15,16].

The reliability of computed tomography has also been proven in animal studies on selected rats. The quantitative assessment of periodontal osseous structures demonstrated the reliability of 3-D CT measurements of the alveolar bone. Quantification of the alveolar bone provided adequate criteria and reproducibility to measure the volumetric osseous parameters using the CT method [17].

The results of the present study confirm that cone-beam tomography can be successfully applied in diagnostics, and specifically to the assessment of jawbone density in animals. Total bone widths in the segments facing the lateral teeth, viz., the premolars and molars, and the density around the first upper and first lower molars were measured as representative of the local bone metabolism in functions of mastication and deglutition. This is the reason for including only the lateral segments of the jawbones in the measurements in the study.

The results of this study are difficult to compare with other studies, because experiments of this nature using a therapeutic solution to increase the mineral content in animal jawbones have not yet been conducted. The only relevant and comparable study on the use of calcitonin was performed for the transdermal iontophoresis of salmon calcitonin sCt, which elicited a decrease in the serum calcium levels in rabbits [18]. Using a reservoir prepared from a drug solution, anodal iontophoresis at pH 4.2 was more effective than at pH 7.4, probably due to a higher sCt net positive charge. The reservoir prepared from a solid drug deposit concentrated sCt next to the skin. In the results of this study, however, the effects of calcitonin on the rabbit bone were not discussed.

It appears that the results of the present study could be very interesting, with respect to the statistically significant differences in the increased density in the jawbone that were detected only in the regions of the first upper molars, despite the fact that increases in the mineral content and increments in the bone density were measured in each experimental region in the upper and lower jaws of the experimental rabbits. The reason for this fact is probably related to the evidence that the upper jaw consists mainly of spongy parts while, by contrast, the lower jaw is mainly structured with cortical parts, resulting in a more intensive and accelerated influx of the therapeutic solution into the spongy parts of the upper jaw. The initially higher bone density values measured around the first upper molar, immediately after the first application of the therapeutic solution at baseline, showed that the solution diffused quickly through the spongy trabecular bone.

Conclusion

Thus, the CBCT technology could be a very reliable tool used for the assessment of jawbone density in animals, applying additionally the therapeutic solution for acceleration of increment of the jaw-bone density.

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