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Comparison of Dentoalveolar Morphology in WT and P2X7R KO Mice for the Development of Biomechanical Orthodontic Models

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Abstract

It has been suggested that the absence of the P2X7 receptor affects long bone morphology, and that one of the cytokines dependent on its activation may also affect tooth morphology. *P2X7R* KO (knockout) were compared with C57B/6 WT mice (background strain) to identify differences in a maxillary molar and surrounding bone. Nineteen WT and 12 KO mouse maxillae were scanned and 3D-reconstructed using microCT. Tooth dimensions were measured and 3D bone morphometry was conducted. A finite element model was constructed based on the results. No statistically significant differences were found in dentoalveolar characteristics between the two mouse types. A single finite element model of the tooth can be used to mechanically represent both strains. *P2X7R* does not have a major effect on alveolar bone or tooth morphology. The *P2X7R* effects are site-specific.

Keywords

orthodontic biomechanics; P2X7R; mouse knockout; bone morphometry; tooth morphology

The purpose of this study was to evaluate the role of the P2X7 receptor (P2X7R) in maxillary dental and trabecular bone morphology. P2X7R is an ATP-gated ionotropic channel and a key mediator of inflammation and bone responses (North, 2002). It can be activated by extracellular ATP, a danger signal from cells under mechanical stress (Milner et al., 1992; Bodin and Burnstock, 1998; Patel et al., 2005; Schneider et al., 2006). This causes the accumulation of intracellular calcium, and the release of chemical mediators, such as PGE2 and IL-1 α and β , that have utmost importance in inflammation and bone biology (Brough et al., 2003; Gudipaty et al., 2003; Li et al., 2005; Ferrari et al., 2006; Lister et al., 2007).

Proper function of P2X7R guarantees a proper acute phase response, helping to modulate the later chronic inflammatory reaction, the lack of which could lead to overwhelming macrophage infiltrate, even more apoptotic and necrotic cells, and generalized tissue damage (Lister et al., 2007).

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At the bone level, P2X7R activation has been shown to be an important mechanism in mechanotransduction. For instance, femurs of *P2X7R* KO mice had morphometric characteristics compatible with a lower formation and an increased resorption activity, leading to the conclusion that this KO mouse has a bone disease compatible phenotype (Ke et al., 2003). Recently, it has been shown that *P2X7R* KO mice tibia have reduced sensitivity to mechanical loading, especially in males (Li et al., 2005).

It has been suggested that IL-1 might have a role in tooth development and eruption, because IL-1 receptors are expressed by ameloblasts during the secretory phase of amelogenesis, and odontoblasts during dentinogenesis (Wise et al., 1995; Xu et al., 1998). These characteristics make *P2X7R* a particularly suited candidate gene to investigate a possible mechanical and genetic interaction in tooth and bone development. In addition, the importance of P2X7R has been extensively studied in fibroblasts and osteoblasts, which have fundamental roles in the initiation of tooth movement (Yee et al., 1976; Yee, 1979). Orthodontic tooth movement involves two fundamental processes possibly mediated by P2X7R: the metabolism of an associated necrotic-type tissue (hyalinized periodontal ligament tissue) and bone modeling/remodeling (Rygh, 1974, 1976; Roberts et al., 1981, 2004; Reitan, 1994; Roberts, 2005). External root resorption, an undesirable side effect of tooth movement, has been associated with the presence of macrophages, possibly linking it to a chronic inflammatory response (Rygh, 1974, 1976; Saito et al., 1991; Brudvik and Rygh, 1993a,b, 1994a,b). A strong association has been reported between IL-1 beta and root resorption (Al-Qawasmi et al., 2003, 2004).

Orthodontic responses are dependent on mechanical stimuli (stresses), which, in turn, largely depend on the morphology of the dentoalveolar structures and the applied load vectors (Tanne et al., 1991; Choy et al., 2000). Any alteration in dentoalveolar morphology, due to the lack of the P2X7R for example, would directly affect the PDL (periodontal ligament) stress patterns, and therefore all PDL stress-linked biological phenomena. This could complicate the comparison of bone modeling and root resorption in different mouse strains. When these responses are analyzed in a specific region, they could be caused solely by mechanical environment changes, not by purely inflammatory effects, thus not characterizing a true physiological difference at the reactive inflammatory level.

Appropriate structural models are essential for FEM (Finite Element Method) calculations of the stresses and strains within dentoalveolar structures. Thus, this preliminary study compares the dentoalveolar morphologies of *P2X7R* KO (in the C57B/6 strain) and C57B/6 WT mice using microCT-based and histological methods. Specifically, a protocol for the evaluation of their maxillary first molars, the subject of a subsequent experimental study, and their surrounding trabecular bone was developed. We hypothesized that there would be no gross differences in their dentoalveolar morphology. Failure to reject the hypothesis would justify a single structural model to represent both strains. On the other hand, two structural models, one specific to each strain, would be necessary if the hypothesis is rejected. Furthermore, the latter would require the development of a strategy to normalize biological responses according to the stresses in each strain of mouse.

There are no morphometric data on BMD (bone mineral density), the structure of maxillary trabecular bone, or tooth morphology for the C57B/6 WT and P2X7R KO mice. Additionally, it has been demonstrated that the interaction of genetic and non-genetic factors influence bone quantity and architecture differentially in different bones, to the extent that, even within the same bone, information from a specific site cannot be extrapolated to another (Judex et al., 2004). Thus, the necessity for specific morphometric analysis of mineralized tissue at the site of interest for the purpose of estimating its material properties is further justification for this study.

Materials and Methods

This animal study was approved by the Indiana University School of Dentistry IACUC. A total of 31 adult male mice (19 WT and 12 KO), sacrificed at 17 weeks and 3 days (± 3 days) of age, were used in this study. The WT mice were purchased from Taconic Farms, (Germantown, NY). The KO mice were obtained from a colony maintained by the Orthopedic Biomechanics Laboratory at Indiana University School of Medicine (IUSM). After arrival, the animals were allowed at least 1 month of acclimation. They were euthanized by inhalation of carbon dioxide, and after decapitation, the heads were washed and put in neutral buffered formalin solution in the refrigerator for 1 day. Sections of the mice maxillas were extracted after dissection and fixed in the refrigerator for an additional day. Then, they were placed in 70% ethanol solution and kept at ambient temperature. The maxillary sections were coated with paraffin and scanned using a Skyscan 1072 microCT (Skyscan, Kontich, Belgium). Reconstruction of the teeth in 2D transverse slices was performed using NRecon (Skyscan, Kontich, Belgium) software. All scans and reconstructions were standardized with optimized settings as described previously (Viecilli et al., 2007).

Molar Morphology

The reconstructed data were imported into Mimics 11.1 (Materialise, Aarstelar, Belgium). This software was used to segment and perform comparative measurements of the teeth, segmented as shown in Fig. 1, and is defined as follows:

1. *Crown length*: distance between the midpoint of the mesial marginal ridge and distal marginal ridge.
2. *Crown width*: distance between the midpoints of the lines formed by union of the distal and mesial cusps, buccally and lingually.
3. *Crown height*: distance between the cemento-enamel junction and the occlusal plane of the maxilla.
4. *Root length [measured on each root, mesial (M), disto-buccal (DB) and disto-lingual (DL)]*: distance between the apex and the bone insertion line, measured along the root canal.
5. *Maximum root diameter (measured on each root)*: maximum root diameter below the furcation, measured perpendicular to the root canal.

6. *Minimum root diameter (measured on each root)*: minimum root diameter below the furcation, measured perpendicular to the root canal.
7. *Tooth volume*: total volume of the mineralized tissue of the tooth.

3D Bone Morphometry

CT Analyzer 4.1 (Skyscan, Kontich, Belgium) software was used to perform 3D bone morphometry. The bone used for analysis was sampled from a 0.5-mm diameter sphere (Fig. 2). The sphere was chosen so that it was equidistant to all roots and located at the midplane between the mesial root apex and the furcation, parallel to the occlusal plane. To segment the bone from the rest of the tissue inside this sphere, a global gray value threshold was standardized for all specimens. Bone mineral density and Hounsfield Unit calibrations were performed using scans of water and hydroxyapatite rods (CIRS, Norfolk-VA) of dimensions similar to the specimens and known mineral densities of 0.75 and 0.25 g/cm³.

Histology

Histology was conducted after the teeth were scanned to visually check for gross changes in tissue morphology such as disruptive developmental effects in cementum, dentin, enamel, and PDL. The spatial position of the section plane was standardized. The maxillas were embedded in methyl-methacrylate, and ground to 20- μ m sections using a series of papers up to 1,200 grit on an ExaKt system (ExaKt Technologies, Oklahoma City, OK). The sections were polished down to 8–10 μ m using papers of 2,500 and 4,000 grit. They were then deplasticized using acetone and xylene and stained using a modified Masson's trichrome stain protocol, which was optimized for methyl-methacrylate sections of teeth (Fig. 3).

Finite Element Model

After the results of the morphological comparison were known, the reconstructed sections of a specimen representative of average dimensions were imported into Amira (Visage Imaging, Dusseldorf, Germany) software. Bone, PDL, and tooth were segmented into different bodies and smoothed to guarantee a mesh with high quality elements (free of ill-conditioned elements). The tissues were meshed with 4-noded tetrahedrons. The resulting model had strong anatomical fidelity, keeping the cortical bone and internal trabecular structure as distinct parts of the model (Fig. 4). Nodes of each material were shared at the interfaces. The model was imported into IDEAS 10, and saved in an IGES format to be imported into ANSYS 10, the finite element analysis software, where elements were converted into ~200K SOLID45 tetrahedrons. The material properties were established based on BMD of the representative specimen and previously determined rodent data (Kawarizadeh et al., 2003).

Statistics

Because of the reduced number of mice in the KO group, Mann-Whitney's nonparametric test was used to compare measurements between strains. The significance level was set at 5%.

Results

The results for molar morphology comparison are shown in Table 1. None of the measured dimensions revealed a statistical difference. The results for bone morphometry are depicted in Table 2. Although none of the measurements revealed statistical significance at the 5% level, there was a trend toward decreased trabecular number and increased trabecular separation in the KO group ($P = 0.18$ and 0.13 , respectively). The unexpected strongest trend was toward a higher bone mineral density (BMD) in the KO group, 0.91 g/cm^3 compared with 0.78 g/cm^3 in the WT ($P = 0.1$).

Evaluation of the histological sections, performed with assistance from an experienced pathologist, did not reveal any typical noticeable difference in structural integrity of the tissues. Two KO mice demonstrated some mild level of enamel malformation, evidenced by brownish colored enamel spots similar to amelogenesis imperfecta that was detectable under the dissection microscope. Despite these findings, closer evaluation revealed no consistent differences in the mineral content or structure of teeth in the microCT or histology.

The lack of significant results in the morphological comparison leads to modeling of both the WT and KO mice with the same mechanical characteristics for orthodontic purposes. A single finite element model, representative of the approximate average morphological characteristics, was constructed to evaluate the stresses and strains in the PDL, and establish a rationale for histological cuts and biomechanical analyses in future studies (Fig. 5).

Discussion

The results were not statistically significant, but demonstrated a trend toward increased structure model index (SMI), trabecular pattern factor (Tr.Pf), and trabecular separation (Tr.Sp) in KO mice, suggesting that KO mice have a tendency toward increased trabecular spacing, decreased connectivity and a weaker, rod-like trabecular structure. On the other hand, there was a trend toward increased bone mineral density (BMD) in the KO mice.

This study is in partial contrast to previous studies that showed significant differences in bone morphometric parameters between the *P2X7R* WT and KO mice in other bone sites. Studies performed in the femur and tibia revealed a tendency toward lower BMD, higher catabolic, and lower anabolic bone activity in the KO mouse under unloaded and artificially loaded conditions (Ke et al., 2003; Li et al., 2005).

In this study, adult mice (17 weeks and 3 days of age) were used, whereas in the original femur and tibia morphology comparison study by Ke et al., younger (2 months) and older adult mice (9 months) were used. The mechanosensitivity study by Li et al. also used older mice (16 weeks of age), to prevent interference of growth-associated bone modeling in the data. Although it is possible that differences in the developmental age might have caused the contrast between this study and the ones mentioned above, other biomechanical complexities are probably the best candidates to explain it, as described below.

From a biomechanical/environmental standpoint, the differences between maxilla versus femur/tibia results could also be attributable to complexities associated with the mechanism

of bone mechanotransduction. It has been shown that long bones are affected by the frequency and magnitude of strain, and the behavior of the surrounding tissues (Burr et al., 2002; Robling and Turner, 2002; Robling et al., 2006). The outcome of the loading patterns, bone formation or resorption, is dependent on the resulting expression of the biochemical regulators of bone remodeling. Absence of the P2X7R resulted in decreased secretion of PGE2 and bone formation in long bone under specific loading conditions. The alveolar bone is typically subjected to masticatory loads, which most likely have a different frequency and magnitude if compared with long bones. Because the loading frequency and magnitudes in the maxillary bones are typically smaller, the resulting small difference in PGE2 production would most likely not produce a different phenotype. It is also possible that mechanical loading of the PDL, as a result of occlusal loads, leads to a different gene expression and activation profile than the one that results from the loading of long bones. Because of different gene arrays controlling bone formation at different sites, the strain thresholds for an osteogenic response vary with location (Hsieh et al., 2001).

Our results confirmed previous findings that bone characteristics are very site specific (Judex et al., 2004). Biomechanical models of mineralized tissue should be constructed based on local analysis rather than on extrapolation of site-specific data to the entire skeleton.

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Fig. 1.
(a) Segmentation detail showing the three mouse roots and the different structures before and (b) after 3D reconstruction from microCT data (Viecilli et al., 2007). Yellow = tooth, blue = bone, and red = PDL.

Fig. 2.
3D Trabecular structure of the alveolar bone as shown by the microCT reconstruction. The circle represents the region used to evaluate bone morphology (Viecilli et al., 2007).

Fig. 3. Typical histological section, focusing on the root, and PDL. Bone and tooth root are stained blue, and PDL is stained pink. There were no noticeable differences between WT and KO sections.

Fig. 4.
Surface finite element model of a representative specimen showing highly organic internal structure, even after smoothing of tissues. Yellow = tooth, blue = bone, and red = PDL.

Fig. 5.
Resulting solid 3D finite element model obtained in ANSYS.

TABLE 1

Comparison of maxillary molar measurements in WT and KO mice

Measurement	WT			KO		
	Median	Range		Median	Range	<i>p</i> value
Tooth Volume (mm ³)	1.07	0.11		1.06	0.16	0.11
Crown Length (mm)	1.16	0.14		1.16	0.08	0.82
Crown Width (mm)	0.90	0.12		0.90	0.11	0.31
Crown Height (mm)	0.76	0.18		0.74	0.18	0.18
M Root Length (mm)	1.13	0.14		1.12	0.11	0.12
M Root mn Diam (mm)	0.37	0.19		0.38	0.08	0.13
M Root mx Diam (mm)	0.70	0.14		0.70	0.09	0.8
DB Root Length (mm)	0.82	0.13		0.83	0.16	0.97
DB Root mn Diam (mm)	0.23	0.13		0.23	0.08	0.75
DB Root mx Diam (mm)	0.67	0.28		0.67	0.17	0.67
DL Root Length (mm)	0.81	0.18		0.80	0.12	0.21
DL Root mn Diam (mm)	0.32	0.15		0.32	0.11	0.87
DL Root mx Diam (mm)	0.47	0.13		0.49	0.16	0.39

TABLE 2

Comparison of bone 3D morphometric parameters

Measurement	WT		KO		P value
	Median	Range	Median	Range	
BMD (g/cm ³)	0.78	0.15	0.91	0.20	0.10
BV/TV (%)	45	10	45	7	0.65
BS/BV (/mm)	50	15	49	16	0.99
Tr.Th (mm)	0.08	0.02	0.09	0.03	0.83
Tr.Sp (mm)	0.13	0.02	0.15	0.05	0.13
Tr.N (/mm)	5.6	1.1	5.1	1.4	0.18
Tr.Pf (/mm)	-3	10	-1	14	0.10
SMI	1.0	0.8	1.8	0.5	0.23
DA	2.2	1.3	2.1	1.1	0.29
FD	2.24	0.07	2.12	0.10	0.28

BMD, bone mineral density; BV/TV, bone volume fraction; BS/BV, bone surface-volume ratio; Tr.Th, trabecular thickness; Tr.Sp, trabecular separation; Tr.N, trabecular number; Tr.Pf, trabecular pattern factor; SMI, structure model index; DA, degree of anisotropy; FD, fractal dimension.