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Dental alveolar bone defects related to Vitamin D and calcium status☆

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Abstract

Vitamin D is important for skeletal development, growth, and homeostasis but has been sparsely studied in the oro-facial bone. Dental alveolar bone anchors teeth to mandible and maxilla bones via a periodontal ligament. Its formation and maintenance are strictly dependent on the presence of tooth organs and it is characterized by a high turnover rate. In order to study the role of Vitamin D and the calcium status on dental alveolar bone formation, microradiographic and histologic comparison of wild-type, Vitamin D receptor null mutant (VDR (−/−) hypo- and normo-calcemic mice and tissues were performed at 2 months. In hypo-calcemic VDR (−/−) mice, alveolar bone was hypomineralized and demonstrated a cellular and matrix organization, similar to the immature woven bone. In normo-calcemic VDR (−/−) mice, mineralization of dental alveolar bone appeared normal, but bone was morphologically abnormal in some specific anatomical locations. These data show that Vitamin D and calcium status may control the formation of dental alveolar bone. The differences of phenotype between hypo- and normo-calcemic VDR null mutant mice suggested a specific Vitamin D control of alveolar bone formation by the Vitamin D nuclear receptor pathway.

Keywords: Vitamin D; VDR null mutant; Alveolar bone

1. Introduction

Dental alveolar bone corresponds to the bone surrounding dental roots that anchors teeth to mandible and maxilla bone. Dental root is composed of central dentin, pulp and peripheral acellular, and cellular cementum. Alveolar bone is connected to cementum via large collagen fibers of periodontal ligament. Tooth and its supporting tissues, including alveolar bone, form an unique developmental and physiological entity. Indeed, the development and the presence of tooth organs are necessary for the formation and maintenance of alveolar bone [1].

The role of Vitamin D during tooth development has been clearly evidenced by the phenotype of human Vitamin D-resistant rickets, as well as by the experimental data obtained in Vitamin D-deficient rodents (for review see [2]). During tooth formation, morphogenic abnormalities, matrix disorganization, and hypomineralization are the major features of the Vitamin D pathway disturbance. Dental tissue forming cells strongly express nuclear [3,4] and membranous [5] Vitamin D receptors, as well as Vitamin D-dependent dental matrix protein, such as amelogenins [6], and calbindins [3]. Vitamin D receptors [4,5] and Vitamin D-dependent bone proteins [7] are also strongly expressed in alveolar bone forming cells. However, the alveolar bone defects associated to Vitamin D pathway disturbance are less characterized.

An animal model of Vitamin D-resistant rickets, Vitamin D nuclear receptor (VDR) null mutant (VDR (−/−)) mice, has been described [8]. After weaning, VDR (−/−) mice display, a rickets phenotype with hypocalcemia, alopecia, long bone [8], and tooth defects [9] prematurely die at 5 months. Interestingly, bone phenotype of VDR mutant mice may be rescued by the use of a calcium rich diet [10]. In order to investigate the potential role of Vitamin D pathway in the specific dental alveolar bone, we have performed a comparative radiographic and histologic study in wild-type, VDR null mutant hypo- and normo-calcemic mice.

2. Material and methods

2.1. Hypo- and normo-calcemic VDR (−/−) transgenic mice

VDR/neoR transgenic mice have been produced by replacing a 1.1 kb fragment containing the second exon

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encoding DNA binding domain with the first zinc finger (necessary to VDR function) by a neoR sequence [8]. VDR null mutants ($n = 30$) were obtained by mating VDR heterozygous mice. To evaluate the impact of hypocalcemia in these mutants, half of VDR null mutants ($n = 15$) were fed immediately after weaning with a special diet, the composition of which is known to normalize calcium and phosphate levels (Diet 20 with 20% lactose, 4% calcium, and 1.26% phosphate from INRA, Jouy en Josas, France). Kindred wild-type mice were used as controls. Animals were sacrificed at 2 months by carbon dioxide asphyxiation.

2.2. Tissue processing, radiography, and histology

Two-month-old mice were sacrificed and mandibles were dissected out. Half-mandibles were then fixed overnight at 4 °C in a phosphate-buffered saline (PBS) buffer containing 4% paraformaldehyde (PFA) and microradiographs of half-mandibles were performed. Samples were then decalcified for 1 month at 4 °C in a PBS buffer containing 4.13% ethylene diamine tetraacetic acid (EDTA) and 0.2% PFA. Half-mandibles were then wax embedded and 8 μm sections were collected and stained according to a modified Van Gieson protocol [9].

3. Results

3.1. Microradiographic analysis of VDR null mutant mice

The comparison of VDR (−/−) hypo-calcemic (Fig. 1A) and wild-type (Fig. 1B) mice mandibles show that dental tissues and alveolar bone of VDR (−/−) hypo-calcemic mice were hypomineralized. Some minor differences were comparatively observed between mandibles of VDR (−/−) normo-calcemic (Fig. 1C) and wild-type mice (Fig. 1D).

3.2. Histologic analysis of VDR null mutant mice

In wild-type mice, bone trabeculae were formed with a dense lamellar bone matrix containing few osteocytes (Fig. 2A). Trabeculae of alveolar bone of VDR (−/−) hypo-calcemic mice appeared larger and were formed with a disorganized bone matrix containing numerous osteocytes (Fig. 2B). The global aspect of alveolar bone trabeculae in VDR (−/−) normo-calcemic and wild-type mice was similar. However, in some specific location, such as interradicular zone, a chondroid bone was observed with a mineralized matrix, containing cell columns (Fig. 2C). Interestingly, while the insertion of periodontal ligament fiber bundles in alveolar bone was similar in wild-type and VDR (−/−) hypo-calcemic mice, periodontal fibers were not inserted in this chondroid bone of VDR (−/−) normo-calcemic mice (Fig. 2A–C).

4. Discussion

This study shows that dental alveolar bone formation was impaired by the absence of a functional VDR. In VDR (−/−) hypo-calcemic mice, dental alveolar bone was affected very clearly when compared to the mandible. This responsiveness to Vitamin D disturbance could be due the high turnover of alveolar bone comparatively to mandible bone [11], or the presence of various site specific osteoblast subpopulations, as suggested by the expression pattern of an Msx1 gene [12].

Mineralized tissue defects observed in human rickets or experimental Vitamin D deficiency have been related to indirect effects via hypocalcemia. In both situation calcium

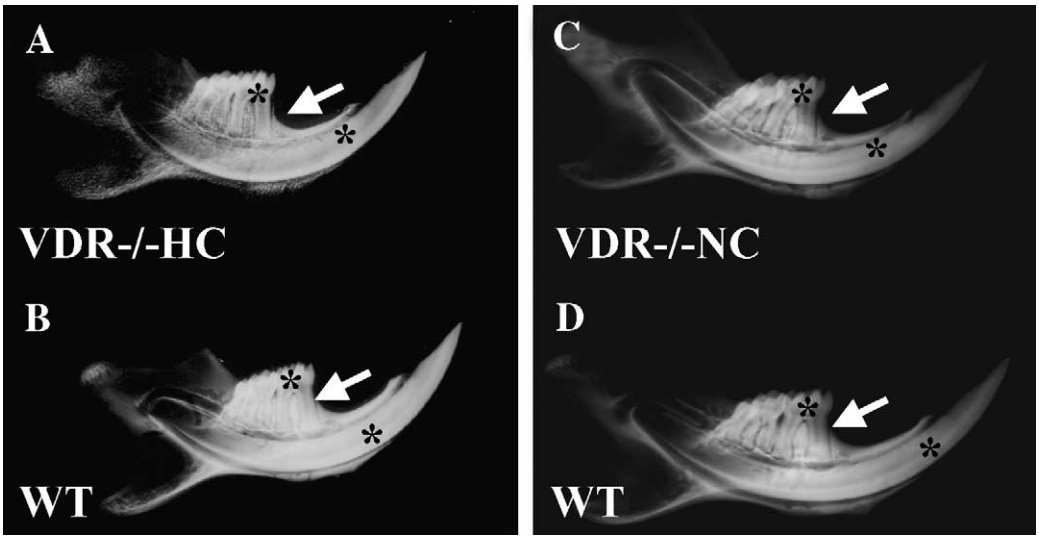

Fig. 1. Microradiographies of half-mandible from wild-type, VDR (−/−) hypo-calcemic and normo-calcemic 2-month-old mice. (A) Alveolar bone (arrow) and teeth (asterisk) of VDR (−/−) hypo-calcemic mice (VDR (−/−) HC) were hypomineralyzed comparatively to wild-type half-mandible (WT) (B). No mineralization differences were observed between VDR (−/−) normo-calcemic (VDR (−/−) NC) (C) and wild-type (WT) half-mandibles (D).

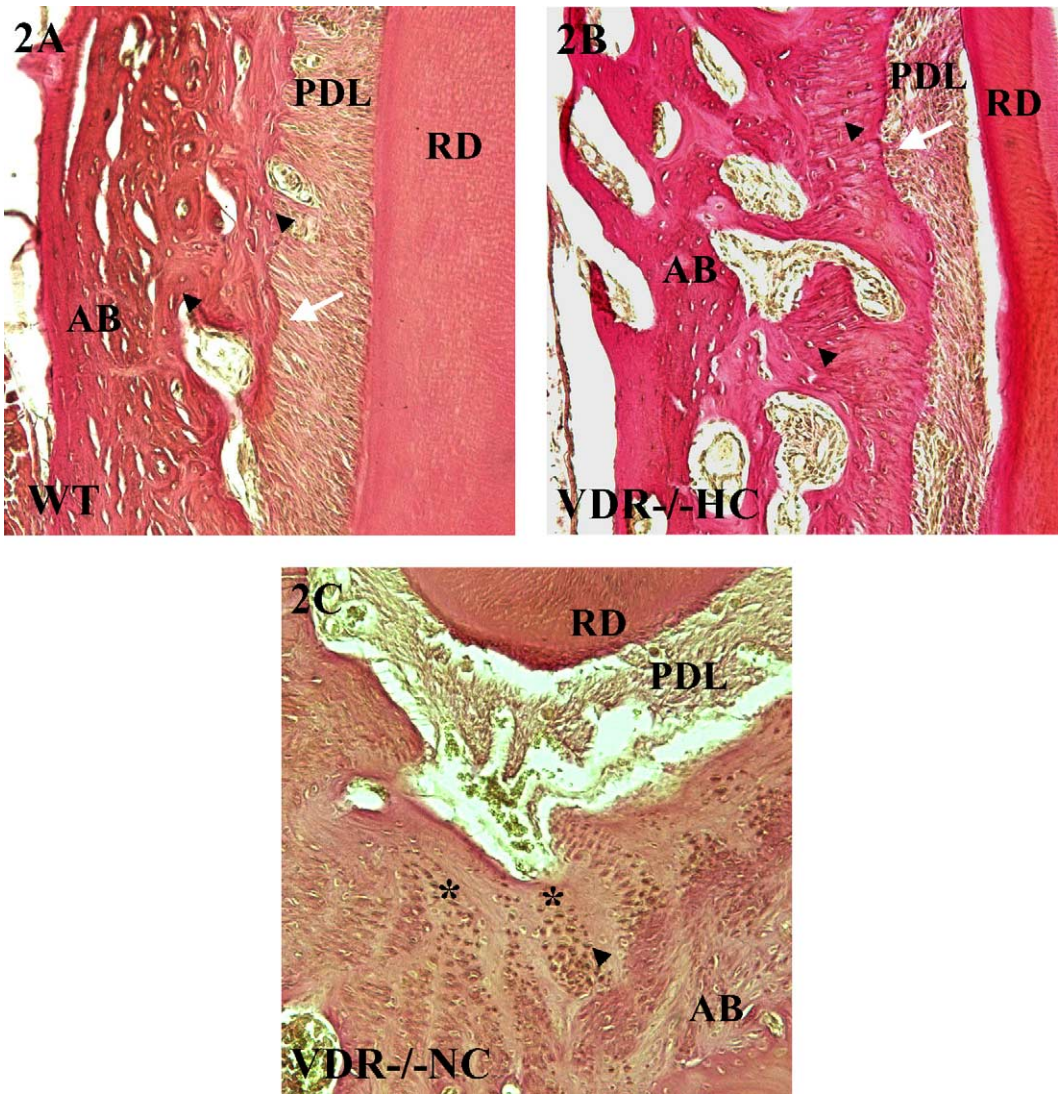

Fig. 2. Histology of molar alveolar bone of wild-type, VDR (−/−) hypo- and normo-calcemic 2-month-old mice. (A) View of the lingual aspect of the first molar alveolar bone of wild-type mice (WT). Bone trabeculae were formed with a dense lamellar bone matrix containing few elongated osteocytes (arrowheads). Periodontal ligament fiber bundles were inserted at the surface of the alveolar bone matrix (white arrow). (B) View of the lingual aspect of the first molar alveolar bone of VDR (−/−) hypo-calcemic mice (VDR (−/−) HC). Bone trabeculae were large and were formed with a disorganized matrix containing numerous rounding osteocytes (arrowheads). Inserted periodontal fiber bundles were observed in the depth of alveolar bone (white arrow). (C) View of the interradicular zone of the first molar of VDR (−/−) normo-calcemic mice (VDR (−/−) NC). Osteocytes were large and rounded (arrowheads). They were arranged as parallel columns (asterisk). Periodontal ligament fibers appeared disorganized and were not inserted in this chondroid bone. AB: alveolar bone, PDL: periodontal ligament, RD: root dentin; and magnification 50×.

supplementation heals rickets and restores a normal bone phenotype [8,10,13]. Our data suggest that some bone defects persisted in rescued VDR (−/−) normo-calcemic mice. Indeed, while appendicular skeleton appeared normal (data not shown) in VDR (−/−) normo-calcemic mice, dental alveolar bone displayed a chondroid aspect in some specific sites. Chondroid bone was associated to the rapid healing of bone fracture, as in case of mandibular distraction [14]. The presence of such a chondroid bone in VDR (−/−) rescued mice may suggest that osteoblast differentiation program could be modified in vivo by the disturbance of the Vitamin D signalization pathway, as shown by in vitro studies [15].

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