Zoledronic acid administration disrupts tooth root formation and tooth eruption through inhibition of PTHrP expression in dental follicle

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Objectives: Pediatric patients with bone fragility caused by osteogenesis imperfecta or bone-destructing cancers are frequently treated with a bisphosphonate, zoledronic acid (ZOL). As an adverse consequence, these patients often experience arrest in tooth eruption. In preclinical studies, ZOL administration to young pre-weaning rodents results in delay in tooth eruption and tooth root abnormalities. However, how bisphosphonates exert negative effects on tooth root formation and disrupt tooth eruption remain incompletely understood. Cells in the dental follicle (DF), a sac-like membranous surrounding developing teeth, express parathyroid hormone-related protein (PTHrP, thereafter PTHrP$^+$ DF cells) and regulate tooth root formation and tooth eruption through autocrine PTHrP-PTH1R signaling. The objective of the study is to reveal how ZOL affects PTHrP expression and PTHrP$^+$ DF cell fates in tooth root formation and tooth eruption.

Experimental Methods: We treated *PTHrP-creER*; *R26R*-tdTomato mice (lineage-marked at postnatal day (P) 3) with ZOL during the pre-eruptive stage (between P5 and P23) under two different protocols: high-dose (3µg/g b.w. once a week) and low-dose (0.05µg/g b.w. every other day) regimens.

Results: Both protocols induced delay in tooth eruption and truncation in tooth roots after 9 weeks following the completion of ZOL treatment at 3 months, indicating long-lasting disturbance in tooth eruption associated with irreversible structural defects of tooth roots. Histologically, proliferation and differentiation of PTHrP$^+$ DF cells into Periostin$^+$ periodontal ligament cells and alveolar bone osteoblasts were significantly impaired in ZOL-treated molars. Interestingly, TRAP staining revealed that the number of osteoclasts appears to be unchanged in ZOL-treated mandibles. Flow cytometry analysis of *PTHrP*$^{mCherry/+}$ molars at P10 revealed that PTHrP-mCherry expression levels were significantly reduced in ZOL-treated molars, demonstrating that ZOL inhibits PTHrP expression in DF cells.

Conclusion: Bisphosphonates induce permanent defects in tooth root formation and long-term disruption in tooth eruption possibly by inhibiting PTHrP-expressing dental follicle in growing molars.

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