Bisphosphonates induce permanent abnormality in tooth root via activation of Notch and Hedgehog signaling pathway in PTHrP-expressing dental follicle cells

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Bisphosphonates are strong inhibitors of bone resorption and are used for bone loss diseases in pediatric patients, such as osteogenesis imperfecta or bone-destructing cancers. As adverse consequences, these patients often experience arrest in dental anomalies and tooth eruptions. However, how bisphosphonates exert negative effects on tooth root formation and disrupt tooth eruption remains 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 study aims to reveal how ZOL affects PTHrP+ DF cell fates in tooth root formation and eruption. 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. Both protocols induced delay in tooth eruption and truncation in tooth roots after 9 weeks following the completion of ZOL treatment at 3 months. Histologically, proliferation and differentiation of PTHrP^{+} DF cells into Periostin^{+} periodontal ligament cells and alveolar bone osteoblasts were significantly impaired in ZOL-treated molars. To reveal the difference in RNA expression between Zol-untreated molar and -treated molar, we performed Bulk RNA-seq of FACS-sorted DF cells. As a result, Hh and Notch signaling pathways in ZOL-treated mice were activated from gene set enrichment analysis. Therefore, we crossed Hh and Notch constitutional activation mice (*R26SmoM2* and *Rosa*$^{N1\text{-}IC}$) with *PTHrP-creER* mice and analyzed the tooth root morphology. The Hh- and Notch-activated mice recapitulated bumpy tooth-root surfaces of ZOL-treated mice, indicating that both signalings play important roles in the subsequent tooth-root surface abnormality in downstream of ZOL. In conclusion, bisphosphonates induce permanent abnormality in tooth roots by by activating Hh signaling and Notch signaling pathways.
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