Bortezomib Facilitates Reparative Dentin Formation after Pulp Access Cavity Preparation in Mouse Molar

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Abstract

Introduction: The aim of this study was to evaluate *in vitro* and *ex vivo* roles of bortezomib, a proteasome inhibitor that binds to the active site of the 26S proteasome, in tertiary dentin formation. **Methods:** We established pulpal access cavity preparation that was treated with or without bortezomib before direct pulp capping with a calcium hydroxide–based material. We also analyzed bone morphogenetic protein (Bmp)- and Wnt-related signaling molecules using quantitative real-time polymerase chain reaction. **Results:** In the short-term observation period, the bortezomib-treated pulp specimens showed the period-altered immunolocalization patterns of nestin, CD31, and myeloperoxidase, whereas the control specimens did not. The bortezomib-treated group showed a complete dentin bridge with very few irregular tubules after 42 days. The micro–computed tomographic images showed more apparent dentin bridge structures in the treated specimens than were in the controls. Quantitative real-time polymerase chain reaction analysis showed up-regulated Bmp and Wnt. **Conclusions:** These findings revealed that treatment with 1 μmol/L bortezomib induced reparative dentin formation that facilitated the maintenance of the integrity of the remaining pulpal tissue via early vascularization and regulation of Bmp and Wnt signaling. *(J Endod 2017;■:1–7)*

Key Words

Cavity preparation, dentin-pulp complex, reparative dentin formation, vascularization, Wnt signaling

Significance

FDA-approved bortezomib facilitates reparative dentin formation through modulated vascularization and an inflammatory response via Bmp and Wnt signaling.

Dentin is produced by odontoblasts after their differentiation from dental papilla cells and occupies the entire inner surface of the pulp. Biochemically, dentin shares similarities with bone such as the composition of matrix proteins and the signaling pathway that regulates the differentiation of osteoblasts and odontoblasts (1). However, dentin differs morphologically from bone because of its calcification by the odontoblastic process and its inability to undergo remodeling. Dental pulp can differentiate into osteoblastic and odontoblastic cells (2) and is composed of a mixed population of fibroblasts, endothelial cells, immune cells, differentiated odontoblasts, stem cells, and progenitor cells (3).

After various injuries to the dentin and pulp, odontoblasts and pulp cells react to the damage by undergoing odontoblast differentiation from the stem and progenitor cells in the dental pulp, subsequently secreting the reparative dentin matrix osteodentin (4). Various signaling molecules are involved in the odontogenic process as well as the regeneration of dentin and pulp (5). The Wnt signaling pathway, one of the key signaling regulators for organogenesis, is reported to be particularly important for the morphogenesis, maintenance, and regeneration of dentin structure in the developmental and postnatal periods (6).

Bortezomib is the first therapeutic proteasome inhibitor and is approved in the United States for the treatment of multiple myeloma (7, 8). Proteasome modulates the degradation of protein ubiquitination to regulate protein expression and function. Myeloma cells express Dickkopf-1, which antagonizes the Wnt signaling pathway, resulting in the inhibition of osteoblastogenesis and bone formation (9). Bortezomib is known to inhibit nuclear factor kappa B activation and interleukin 6–mediated cell growth (10). In addition, treatment with bortezomib has been reported to increase bone mass by inhibiting osteoclast activity and the osteoblastic differentiation of mesenchymal stem cells through activation of the beta-catenin/T-cell transcription factor pathway (11). These studies suggest that bortezomib treatment is involved in a range of molecular cascades, including Wnt and beta-catenin signaling, during hard

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tissue formation and maintenance (11–18). They focused on bone formation because bone is the major target organ in multiple myeloma. However, the functional role of bortezomib in the development and regeneration of dentin is yet to be elucidated. In this study, we evaluated the effects of bortezomib on reparative dentin formation and maintenance of the integrity of the remaining pulpal tissue using *in vitro* and *ex vivo* experiments.

Materials and Methods

All experiments were performed according to the guidelines of the Intramural Animal Use and Care Committee, Kyungpook National University, Daegu, Korea.

Animals

Adult ICR mice were housed in a temperature-controlled room (22°C) under artificial illumination (lights on from 05:00–17:00) with 55% relative humidity and free access to food and water. For the hanging drop experiment, 20 embryos were obtained from time-mated pregnant mice on embryonic day 14 (E14). For the pulpal access cavity preparation, at least 15 adult 8-week-old male ICR mice in each group were euthanized 3, 5, and 42 days after pulpal exposure. Fifteen maxillae in each group were dissected and fixed for histomorphometry and immunohistochemistry while the remaining samples were prepared for micro–computed tomographic (micro-CT) evaluations.

Histology and Immunohistochemistry

Specimens were fixed in 4% paraformaldehyde in phosphate-buffered saline overnight at 4°C, embedded in paraffin wax, and then cut into 7-μm-thick sections. Thereafter, 10 randomly selected slides from 5 specimens were stained for each experiment. Masson trichrome (MTC) staining was performed following routine processes (19), and immunohistochemistry was performed as previously described (20). The primary antibodies used were CD31 (Abcam, Cambridge, UK), myeloperoxidase (MPO; Bioss Antibodies Inc, Woburn, MA), and nestin (Bioss Antibodies Inc). The secondary antibodies used in this study were biotinylated antirabbit or antimouse immunoglobulin G. Immunocomplexes were visualized using a diaminobenzidine tetrahydrochloride reagent kit (Zymed Lab, San Francisco, CA). All sections were analyzed using a stereomicroscope (DM2500; Leica, Wetzlar, Germany) by 2 trained investigators blinded to the section's group. Any differences were resolved by forced consensus. All morphologic data with a focus on the pulpal area adjacent to the access opening were semiquantitatively graded in MTC and nestin staining using the following score: −, none; +, weak; ++, moderate; and +++, strong staining. The number of positive cells per unit area was counted for CD31 and MPO immunostaining after a defined frame was drawn in identical topographic regions across each section.

In Vitro Mesenchymal Tissue Culture and Quantitative Real-time Polymerase Chain Reaction

Procedures for *in vitro* culture were performed on stage E14 for 1 day with modification to the procedure described in a previous study (21). Microdissected tooth germs were treated with Dispase-II (Roche Applied Science, Mannheim, Germany) for 20 minutes, and then the mesenchymal molar-forming tissues were collected. The harvested mesenchymal tissues were cultivated on the lid of a 35-mm Petri dish with 300 μL of 1 μmol/L bortezomib or 0.01% dimethyl sulfoxide (DMSO) in Dulbecco modified Eagle medium with 1% penicillin

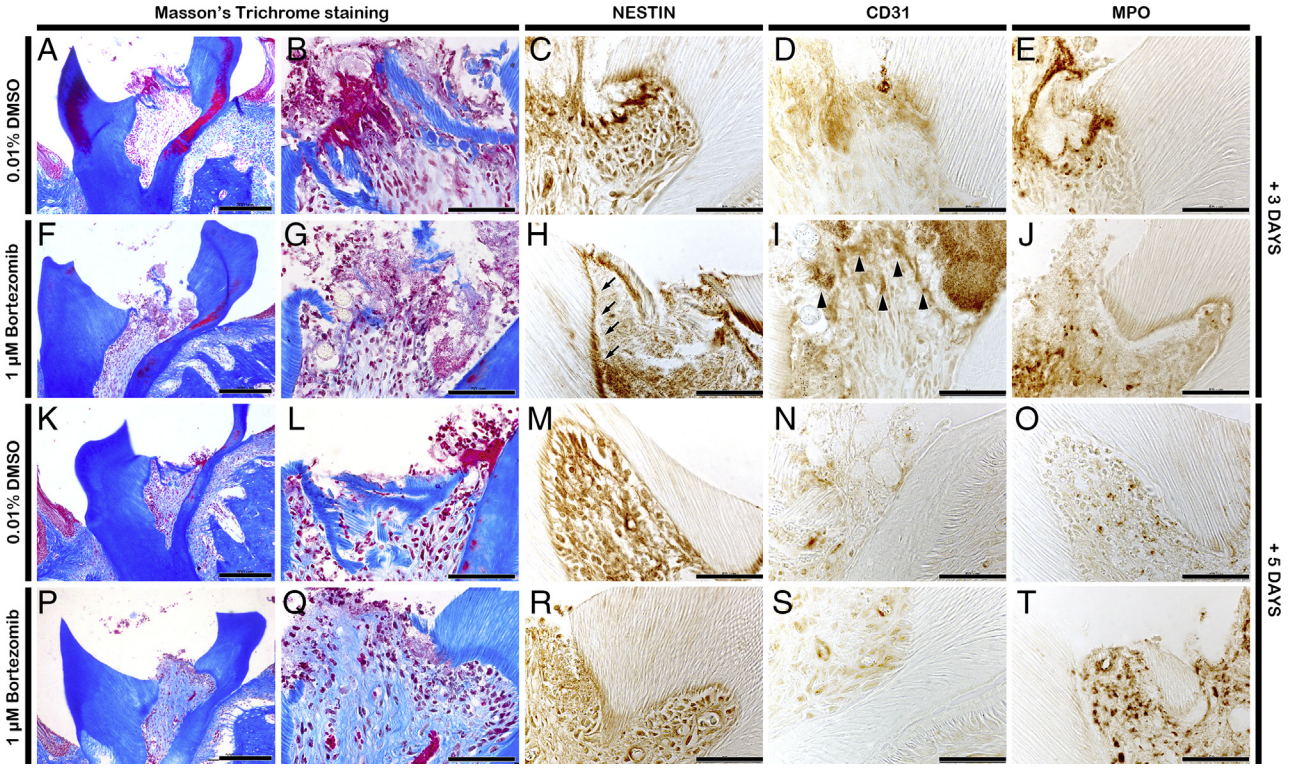

Figure 1. Histologic examination and immunostainings of nestin, CD31, and MPO after pulpal access cavity preparation. (*A*, *B*, *F*, *G*, *K*, *L*, *P*, and *Q*) MTC staining. Immunolocalization patterns of (*C*, *H*, *M*, and *R*) nestin, (*D*, *I*, *N*, and *S*) CD31, and (*E*, *J*, *O*, and *T*) MPO. Scale bars: *A*, *F*, *K*, and *P* = 200 μm and *B–E*, *G–J*, *L–O*, and *Q–T* = 50 μm.