Effect of the antineoplastic agent busulfan on rat molar root development

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ABSTRACT
Objective: The antineoplastic bifunctional-alkylating agent busulfan (Bu) induces developmental anomalies. We examined histopathological changes in the molar roots of rats that received Bu at different stages of root formation.

Design: At different developmental stages, i.e., on postnatal days (P) 13, 15, and 19, rats were administered 7.5 mg/kg of Bu dissolved in dimethyl sulfoxide (DMSO) and then killed on P 30. After micro-computed tomography analysis, the maxillary first molars underwent immunohistochemical analysis for cytokeratin 14 (CK14), nestin, and dentin sialoprotein (Dsp). This was followed by histomorphometric analysis.

Results: The rats receiving Bu at an early stage (i.e., P 13 and P 15) showed osteodentin formation and complete destruction of the Hertwig’s epithelial root sheath (HERS). Cells around osteodentin showed nestin and Dsp immunoreactivity. The root lengths in rats treated with Bu at P 13 (1228.44 ± 62.17 µm) and P 15 (1536.08 ± 109.71 µm) were lower than that in the control rats (1674.10 ± 40 µm). A narrowed apical foramen and an increased amount of osteodentin were also present, depending on the rat’s age at the time of treatment (P < 0.05).

Conclusion: Busulfan treatment in juvenile rats resulted in abnormal root development, depending on the stage at which Bu was administered. This abnormal development may result from the destruction of the HERS. The administration of Bu caused a shortage of HERS cells, which are required for normal root development. This disturbs root formation, resulting in osteodentin formation and a narrowed apex foramen.

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1. Introduction

Advances in the treatment of childhood malignancies have dramatically improved survival.1 This progress has resulted in interest in the late sequelae of the disease and in the therapy administered for the disease.2 Children treated with chemotherapy at an early age show severe disturbances in odontogenesis such as tooth agenesis and microdontia, and disturbed development of the dental roots.3–9 Abnormal
events that occur during tooth development cause permanent sequelae; therefore, much clinical attention has been directed towards elucidating the effect of childhood chemotherapy on the development of the teeth.

Mammalian tooth root development is a long-term process. Certain periods during odontogenesis are sensitive to environmental insults. The sensitivity to the insults depends on the temporal and spatial emergence of developmental processes such as proliferation, migration, differentiation, and mineralization. The development of the tooth root and the periodontium involves a complicated sequence of events and requires the participation of various types of cells. The elongating apical portion of the developing root contains a bi-layered epithelial sheath, named the Hertwig’s epithelial root sheath (HERS), which subdivides the adjacent dental ectomesenchymal tissues into dental papilla and dental follicle. During root formation, this complex plays at least two important roles: mineralization (i.e., dentine formation and cementogenesis) and the induction of root organization.

Busulfan (Bu), a bifunctional alkylating agent, has been used to treat chronic myeloid leukaemia and used for the myeloablative-conditioning regimen before stem cell transplantation in children and in adults. In various foetal tissues or cultured cells, Bu has been shown to react with intracellular nucleophiles and proteins, which leads to DNA–DNA and DNA–protein crosslinks that cause DNA damage, and thereby inhibit cell proliferation and induce apoptosis.

Various methods have been used to examine the effects of antineoplastic agents on the developing tooth. Histomorphological investigations using cyclophosphamide have reported a correlation between the extent of injury in odontogenesis and the dose of the drug. Hølttä et al., in their epidemiological study, emphasize that a young age is a risk factor for late dental adverse effects in children who undergo high-dose chemotherapy. However, no study has reported the influence that the developmental stage at which antineoplastic agents are administered has on the HERS in the developing molar root. It is possible that a critical period exists during which antineoplastic agents have an effect on developing molar roots.

The aims of this study were to clarify the effects of administering Bu at different stages of rat molar root development and to examine the correlation between the severity of the damages and the developmental stages of root formation at which antineoplastic agents are administered.

2. Materials and methods

2.1. Experimental animals

All experiments were performed in accordance with the guidelines of the Niigata University Intramural Animal Use and Care Committee (approval no. 111). Pregnant rats were obtained from Charles River Japan, Inc. (Yokohama, Japan). They were provided a standard diet and water ad libitum and were maintained in a room with controlled temperature and humidity and a 12-h light–dark cycle.

2.2. Experimental design

Wistar rats of both sexes were used. They had a known date of birth (the day of birth was designated as “Day 0”). Fig. 1 shows a schematic outline of the experimental protocol. We divided 72 littermates into 6 groups (n = 12), that received either Bu (i.e., the experimental groups) or dimethyl sulfoxide (DMSO) (i.e., the control groups) at the age of 13 days, 15 days, or 19 days. On postnatal days (P) 13, 15, and 19, the rats in the experimental groups received an intraperitoneal injection of 7.5 mg/kg body weight (b.w.) of Bu (Wako, Osaka, Japan) dissolved in DMSO (Sigma–Aldrich, St. Louis, MO). At each time point, the control rats for each group were injected with the same volume of DMSO. Rats were weighed at the age of 13 days and every 2 days thereafter. They were killed on postnatal day 30. The animals were anesthetized and transcardially perfused with physiological saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS). The maxillae were removed en bloc and immersed in the aforementioned fixative for an additional 12 h. For histological comparison with normal development, rats that had not undergone either treatment were killed at days 13, 15, 19, or 30.

2.3. Three-dimensional root structure analysis

To evaluate the three-dimensional sagittal views of the control molars and the experimental molars, multiple scans were obtained of the maxillae of some of the rats from each group by using micro-computed tomography (μ-CT) apparatus that was equipped with a microfocus X-ray tube with a focus size of 8 μm × 8 μm (MCT-100MF; Hitachi Medical Corporation, Tokyo, Japan). The tube voltage was 70 kV; tube current, 100 mA; magnification, 8× and voxel size, 17.0 μm × 17.0 μm × 17.0 μm. Three dimensional images of the upper first molars were reconstructed from 201 image slices by using imaging software (TRI/3D BON; Ratoc System Engineering Co. Ltd., Tokyo, Japan).

2.4. Histological analysis

After decalcification in 10% ethylenediamine tetraacetic acid disodium salt solution (pH 7.4) for 4 weeks at 4 °C, the tissue blocks were dehydrated in a graded ethanol series and then embedded in paraffin. The 5-μm sections were prepared and dewaxed with xylene. Some sections were stained with haematoxylin and eosin (H&E).

2.5. Immunohistochemistry

For the immunoperoxidase procedure, sections were processed for the avidin–biotin peroxidase complex (ABC) method by using either a mouse anti-cytokeratin14 monoclonal antibody (diluted 1:100) (Novoceastra, Newcastle, UK) or a mouse anti-nectin monoclonal antibody (diluted 1:2000) (Millipore, Billerica, MA). A solution of 0.01 M PBS (pH 7.4) was used to dilute the antibodies and to rinse the sections. Endogenous peroxidase was inactivated by treatment with 0.3% H2O2 in absolute methanol for 30 min. Any non-specific immunoreactivity was inhibited by pre-incubation in 2.5% normal goat serum (Vector labs, Burlingame, CA). After
incubation (4 °C, 24 h) with a primary antibody, the sections were consecutively reacted with biotinylated anti-mouse IgG for 90 min, and then reacted with the ABC complex (Vector labs) for 1 h at room temperature. Immunoreactivity was visualized by using an ENVISION kit (DAKO, Carpinteria, CA). The immunostained sections were counter-stained with 0.05% methyleneblue. Immunohistochemical controls were performed by replacing the primary antibodies with PBS. These immunostained sections contained no specific immunoreaction (Suppl. Fig. 1).

For double immunofluorescent staining for nestin and dentine sialoprotein (Dsp), the sections were treated by incubation with an anti-nestin monoclonal antibody (Millipore) and with a Cy3-conjugated anti-mouse IgG (diluted 1:250) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After washing with PBS, the sections were consecutively incubated with goat anti-Dsp polyclonal antibody (diluted 1:100) (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and Dylight 488-conjugated anti-goat IgG (diluted 1: 250) (Jackson ImmunoResearch Laboratories Inc.). The sections were examined with a fluorescent microscope (AXIO imager.M1; Carl Zeiss, Jena, Germany).

2.6. Histomorphometry

Microscopic images were projected onto a screen tablet by using a microscope with a CCD camera (Pixera 150CL-CU; Fremont, CA) integrated with image analysis software (System Supply Co. Ltd., Ina, Japan). Histomorphometric measurements of the dimensions of the developing medial root of the maxillary first molars were performed on the tablet. As illustrated in the schematic drawing in Fig. 2, the root length (RL), width of apical foramen (WAF), thickness of root dentine (TRD), area of cellular cementum (ACC), and area of osteodentin (AOD) were measured at magnification 40× (for RL) and magnification 100× (for WAF, TRD, ACC, and AOD). The mean values of each parameter were assessed by multiple measurements of the H&E-stained serial sections, which were spaced equidistantly (50 μm, 5×, n = 5). A single examiner conducted the measurements in a blinded fashion and without previous knowledge of the study groups.

2.7. Statistical analysis

Comparisons of the histomorphometric data of the developing root from the experimental and control groups were performed by using one-way analysis of variance (ANOVA). When ANOVA suggested a significant difference between the groups, a post hoc Tukey–Kramer multiple comparison test was performed. All statistical analyses were performed by the statistical software JMP, version 5.01 (SAS Institute Inc., Cary, NC). A P < 0.05 was considered statistically significant.

3. Results

3.1. Body weight

The rats treated with Bu at postnatal day 13 (P13) began to lose weight (from 53.33 ± 0.57 g) at 23 days of age and thereafter. They had lost approximately 6.42% of their weight by 30 days of age (45.00 ± 2.64 g). Other experimental groups gained
weight in the same manner as the control rats during the experimental period (Fig. 3).

3.2. Reconstructed three-dimensional root apex structure

Fig. 4 shows representative reconstructed three-dimensional (3D) images of the maxillary first molars from the study groups. The 3D reconstructed sliced views of the experimental molars and the control molars showed disturbed root elongation in the experimental rats (Fig. 4a, c and e). The DMSO control groups did not exhibit any marked changes (Fig. 4b, d and f). Compared to each of the control rat groups, the experimental rats exhibited a shortened root length and a narrowed apical foramen (Fig. 4a, c and e). No apparent changes in the thickness of dentine or in the degree of mineralization existed between the experimental rats and the control rats (Fig. 4a–f).

3.3. Morphological observations

During normal development, root dentine formation began at postnatal day 13 (Day 13) (Fig. 5a). At postnatal day 15 (Day 15),
the root dentine had reached approximately one-half of the complete root length (Fig. 5b). At postnatal 19 (Day 19), the root dentine had further elongated and increased in thickness (Fig. 5c). At postnatal day 30 (Day 30), the entire root length of the first molar was nearly complete and the apical end of the root dentine was still incomplete (Fig. 5d).

The experimental rats showed morphological anomalies in the apical region. The degree of destruction was more severe in the rats treated with Bu at young ages. The apical edges of the advancing dentine were completely destroyed and showed osteodentin-structure (Fig. 6a, c and e). At higher magnification, the osteodentin appeared more compact and contained fewer cells in the rats that had received Bu at more advanced ages (i.e., P 15 or P 19 days) than in rats that had received Bu injection at P 13. The differentiating odontoblast layer was completely damaged and the typical columnar odontoblasts were no longer in the apical region. The cellular cementum had formed in the experimental groups (Fig. 6b, d and f). Compared to those under normal development, all the experimental rats had an increase in their dentine thickness after the Bu administration (Figs. 5a–c and 6a, c, e).

There was no morphological difference between the control animals that received DMSO at P 13 and the normal rats at Day 30 (Figs. 5d and 6g). Animals from the other control groups showed similar results (Suppl. Fig. 2d and g).

3.4. Cytokeratin 14 analysis

To assess the morphological damage on the HERS and the epithelial rests of Malassez (ERM) structures after Bu administration, we detected epithelial cells by using cytokeratin 14 (CK14) immunohistochemistry. In the rats treated with Bu at younger ages (i.e., P 13 or P 15), the HERS structure was completely destroyed and no CK14 immuno-reactivity was present in the apical region. At the periphery of the root surface, the CK14-positive ERM cells had almost disappeared in the coronal aspect and the inter-radicular aspect of the root surface (Fig. 7a and c). However, a presumed CK14-immunoreactive remnant of the HERS cells was observed as a cluster in the apical periodontal ligament (PDL) region (Fig. 7b and d). In the rats that received Bu at P 19, numerous ERM cells were sparsely present in the periphery of the root surface (Fig. 7e). A large cluster of CK14-positive cell—presumably the deformed remnant of the HERS—was present at the tip of the osteodentin and some CK14 positive cells were within the cellular cementum (Fig. 7f).

In the control rats that received DMSO at P 13, numerous CK14-positive cells were present in the periphery of the root surface (Fig. 7g) and a CK14-positive bi-layered structure was present (Fig. 7h). Animals from the other control groups showed similar results (Suppl. Fig. 2e and b).

3.5. Nestin analysis

To assess the influence of Bu on odontoblasts, we employed nestin immunohistochemistry. The experimental animals displayed a similar distribution pattern of nestin-positive cells in the osteodentin areas (Fig. 8a, c and e). Intense immunoreactivity for nestin was present in cuboidal cells that were adhered to the osteodentin and in cells embedded in the

Fig. 5 – Midsagittal views of developing maxillary first molar medial root at (a) Day 13, (b) Day 15, (c) Day 19, and (d) Day 30. (a) The root dentine formation has begun. (b) The root dentine reaches approximately one-half of the complete root length. (c) The root dentin has elongated further and increased in thickness. (d) The entire root length of the first molar is nearly complete and the apical end of the root dentine is still incomplete. Bars 200 µm.
Fig. 6 – A comparison of the morphological differences of the apical regions in rats administered Bu at different ages and in a control rat at Day 30. (a, c, e) Apical edges are destroyed in all experimental rat groups. (b, d, f, h) Enlarged views of the boxed areas in figures a, c, e, and g. (b, d, f) The differentiating odontoblast layer is completely damaged and osteodentin (•) is present. (f) Osteodentin is more compact and contains fewer cells. (g) A control animal that received DMSO at P 13. (b, d, f, h) Cellular cementum is visible on the root surface. AB, alveolar bone; D, dentine; DP, dental pulp; OB, odontoblasts; •, osteodentin. Bars 200 μm (a, c, e, g), 50 μm (b, d, f, h).
Fig. 7 – CK14 immunoreactivity in rats administered Bu and in the control rats. (b, d, f, h) The enlarged views of the boxed areas in figures a, c, e and g. (a, c) No CK14 immuno-reactivity is present in the apical region. The CK14-positive cells have nearly disappeared from the periphery of the root surface. (b, d) Clusters of CK14-immuno-reactive cells are visible in the PDL (arrowheads). (e) In the periphery of the root surface, fewer ERM cells are present in the experimental rats than in the control rats. (f) A large cluster of CK14-positive cells is present at the tip of the osteodentin (arrowhead). Numerous CK14-positive cells are embedded in the cellular cementum (arrows). (g) The CK14-positive cells are sparsely spread adjacent to the root surface. (h) A CK14-positive bi-layered structure is discernible (arrowhead) and numerous CK14-positive cells are embedded in the cellular cementum (arrows). AB, alveolar bone; D, dentine; DP, dental pulp; OB, odontoblasts; *, osteodentin. Bars 200 μm (a, c, e, g), 50 μm (b, d, f, h).
Fig. 8 – Nestin immunoreactivity in rats administered Bu and in the control. (b, d, f, h) The enlarged views of the boxed areas in figures a, c, e and g. (a, c, e) Columnar odontoblasts adjacent to the root dentine show nestin immunoreactivity. (b, d, f) Intense immunoreactivity for nestin is present in the cuboidal cells adhering to the osteodentin and in the cells in the osteodentin (arrows). Cells embedded in the cellular cementum display no nestin immunoreactivity (arrowheads). (g) Columnar odontoblasts adjacent to the dentine matrix show nestin immunoreactivity. (h) Dental pulp cells adjacent to the odontoblasts are also positive for nestin (arrows). AB, alveolar bone; D, dentine; DP, dental pulp; OB, odontoblasts. Bars 200 μm (a, c, e, g), 50 μm (b, d, f, h).
osteodentin; however, they did not appear to be morphologically typical odontoblast-like cells (Fig. 8b, d and f). Cells adjacent to and embedded in the cellular cementum displayed no nestin immunoreactivity (Fig. 8b, d and f).

In the control rats that received DMSO at P 13, columnar odontoblasts and differentiating odontoblasts adjacent to the dentine showed nestin-immunoreactivity (Fig. 8g and h). Animals from the other control groups showed similar results (Suppl. Fig. 2f and i).

3.6. Dentine sialoprotein analysis

To evaluate the co-localization of nestin and dentine sialoprotein (Dsp) in the osteodentin area, we employed nestin/Dsp double-immunohistochemistry. The cuboidal cells on the osteodentin and cells embedded in the osteodentin extended nestin-positive cellular processes into the osteodentin. These cells showed cytoplasmic immuno-reactivity for Dsp. The osteodentin matrix also showed an intense immuno-positive reaction for Dsp (Fig. 9a–c).

3.7. Histomorphometric analysis

The length of the medullary root was significantly shorter in rats treated with Bu at P 13 (1228.44 ± 62.17 μm) or at P 15 (1536.08 ± 109.71 μm) than in the control rats (1674.10 ± 40.94 μm) (Fig. 10a). There was no significant root shortening in rats treated at P 19. The difference in the width of the apical foramen in the experimental rats and in the control rats was statistically significant (Fig. 10b). However, there were no significant differences in the root dentine thickness (Fig. 10c) or in the area of the cellular cementum (data not shown). Osteodentin formation was virtually absent in the control group; however, a significant difference existed between the rats that received Bu at a young age (i.e., P 13 and P 15) and the rats that received Bu at P 19 (Fig. 10d).

4. Discussion

The present study clearly demonstrated that administering Bu at different stages of root development results in severe destruction of the apical root structure, depending on the age at which the animals received the drug. These results show good agreement with the findings of clinical reports describing reduced root length and early closure of the apical foramen associated with childhood chemotherapy treatment.4-29 The reduction in body weight in the group treated with Bu at 13 days of age probably resulted from prominent damage in the hematopoietic system. However, the reduced root length and diameter of the apical foramen in the Bu-treated groups were not believed to have resulted from a systemic disorder caused by Bu administration. This reduction may have instead resulted from Bu-induced damage on the developing root since the root dentine thickness and cellular cementum formation were not affected in the group treated with Bu at P 13. To our knowledge, this is the first report that systematically shows a correlation between the age of infant rat teeth and Bu-induced pathological changes that are similar to changes seen in clinical cases.

Busulfan produces crosslinks in DNA through creating a four-carbon bridge by displacing two methylsulfonate molecules. The cells are most sensitive to the effect of Bu in the late G1-phase. The cells pass normally through the S-phase, but are unable to undergo normal mitosis.30 The progression through the cell cycle is blocked in the G2-phase, which is similar to the effect of other alkylating agents.31,32 Furukawa et al.34 have shown that Bu induces apoptosis and inhibits mitosis in the neuroepithelium, the retina, and the lens, which have high cell proliferative activity, and leads to microencephaly, microphthalmia and cataracts in the rat foetus. The apical end of the developing root and the surrounding periodontium proliferate throughout the development of the root.35 With regard to the HERS, to provide a sufficient number of cells to organize an extending epithelial cell structure along

Fig. 9 – Fluorescent micrographs indicating the co-localization of nestin and Dsp. Cells on and in the osteodentin are immunofluorescent for nestin (shown in red) and Dsp (in green) in rats administered Bu at (a) P 13, (b) P 15, and (c) P 19 (arrows). The osteodentin (*) also shows Dsp immunoreactivity (arrowheads). *, osteodentin. Bars 20 μm (a, b, c).
the advancing dentin edge, continuous proliferation activity has been demonstrated in the apical end in rats. Recently, the existence of stem cells in the developing apical complex has been suggested. Moreover, the cultured HERS cells have been reported to express stem cell markers such as Bmi-1, Nanog, SSEA-4, and Oct3/4. The exclusive destruction of the HERS in Bu-treated rats indicates that, unlike odontoblasts and cementoblasts, which are probably supplied with plenty of progenitor cells from putative dental pulp and follicle stem cells after Bu administration, the number of putative stem cells in the HERS is limited. The existence of epithelial stem cells in the developing HERS and ERM has not been confirmed in vivo; however, it is reasonable to speculate that Bu-related damage to the proliferating HERS stem cells caused a shortage of the cells and made them incapable of maintaining the organized HERS structure. This then resulted in the destruction or the disappearance of the HERS and the ERM. However, our preliminary observation failed to detect an increased number of TUNEL-positive or pyknotic cell nuclei in Bu-treated rats (data not shown). This may result from the elimination of the apoptotic cells during the observation period. In a chronological experiment that used a cyclophosphamide-treated rat model, Näsman and Hammarström reported that cell-free areas emerged in the proliferating zone of odontoblasts in the root apex immediately after (within 2 days) the drug’s administration and that these areas turned into mineralized osteodentin. We confirmed a similar osteodentin formation in our experimental rats; however, we could not evaluate the immediate effects of the Bu administration. Further detailed investigations of Bu-induced pathological changes in the developing root by using shorter observation periods would reveal the dynamics of the apoptotic cells.

In tooth development and dental pathological conditions, dentine-producing odontoblasts express nestin. Quispe-Salcedo et al. recently demonstrated that nestin and Dsp are synchronically expressed in the odontoblast-lineage cells according to the progression of odontoblast differentiation and suggested that nestin can be a differentiation marker for odontoblasts and Dsp can be a functional marker for their secretory activity. The co-localization of nestin and Dsp in the cells on and within the osteodentin in the experimental rats indicates that they are derived from odontoblast progenitor cells and have participated in producing osteodentin, although these cells are morphologically different from mature odontoblasts.

It is well established that the HERS regulates the differentiation of the dental papilla cells into odontoblasts to form organized root dentin through epithelial–mesenchymal interactions. On the completion of root formation in normal root development, the HERS cells eventually disappear from the region and no organized root dentin is further elaborated. Xu et al. demonstrated in an ex vivo experiment that the developing apical complex produced osteodentin-like tissue without contamination of HERS cells. The osteodentin formation on the dentin edge in the rats showing HERS destruction conceivably imply that the absence of and/or the destruction of the HERS would attenuate the ability of developing apical odontoblast progenitor cells to form organized advancing dentin edges and would cause them to form osteodentin instead. We cannot conclude whether the nestin/
Dsp double-positive cells in the osteodentin area still possess normal odontoblast property; however, these cells apparently may have been odontoblast progenitor cells at the time of Bu administration, and have differentiated without undergoing interaction with the HERS. We speculate that the cessation of the downgrowth of the HERS reduced the root length and that the disappearance or destruction of the HERS triggered osteodentin formation from odontogenic progenitor cells on the edge of the advancing dentin. This then narrows the apex foramen. The mechanisms behind the late differentiation of odontoblast progenitor cells after the disappearance of the HERS remain unknown.

The possible role of the HERS in cementoblast differentiation and initiation of cementogenesis is a subject of controversy. Interactions between the dental follicle and the HERS, which would eventually lead to cementoblast differentiation, have not been elucidated, although these interactions have been repeatedly suggested. On the other hand, Heritier hypothesized that the disappearance of HERS epithelial cells is an essential requirement for the onset of cementogenesis. In the experimental rats, the early disappearance of or the severe or total destruction of the HERS did not appear to have adverse effects on cementum formation. The lack of a significant difference in the amount of cellular cementum formation suggests that HERS is unlikely to play a direct inductive role in the development of cellular cementum.

The primary effects of Bu on the developing root in rats were destruction of the HERS and the production of osteodentin. The drug also caused changes in the number of ERM cells. The decreased number of ERM cells implies that it has proliferative activity during root development as well as in physiological and pathological conditions, as reported previously. The ERM is preserved in the PDL throughout life and may have a number of functions such as preventing root resorption, inducing cementum formation, and maintaining homeostasis of the PDL. Because we sacrificed the rats at Day 30, at which point the PDL is not mature, we could not assess the tissue changes resulting from the absence of the ERM in comparison to the physiological condition of the mature periodontium. The treatment does not influence inert cells in the cell cycle at the time of Bu treatment, and the treatment enables long-term observation of mature rats with few or almost no ERM cells in the PDL. Therefore, Bu-treated rats may serve as a model for studying the role of the ERM in the homeostasis of the PDL. The influence of the disappearance of the ERM on PDL homeostasis at later stages needs further clarification.

In conclusion, this study morphologically demonstrated that the Bu-induced damages on the root formation are age dependent, i.e., the severity of the damage has correlation with the ages at which Bu was administered. The findings also clarified that the cytotoxic effects of Bu eventually led to early destruction and/or elimination of HERS and formation of osteodentin in teeth. Observing the time-course changes of osteodentin and cellular cementum formation with pathologically collapsing HERS could provide insights into the mechanisms of the development of the root apex.

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Competing interests

There are no conflicts of interest to declare.

Ethical approval

All experiments were performed in accordance with the guidelines of the Niigata University Intramanual Animal Use and Care Committee (approval no. 111).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.archoralbio.2013.09.009.

References


