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Original Article

Evaluation of a new mouse model for studying dental pulpal responses to GaAlAs laser irradiation

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ABSTRACT

Objectives: The molecular mechanisms regulating pulpal responses to GaAlAs laser irradiation remain to be clarified. This study aimed to assess the feasibility of a mouse model for studying pulpal responses to GaAlAs laser irradiation.
Methods: Maxillary first molars of 5-week-old ICR mice were irradiated at an output power of 1.0 W for 180 s, and samples were collected at intervals of 0, 1, 3, 5, 7, 10, and 14 days. The demineralized paraffin sections were processed for hematoxylin and eosin staining, immunohistochemistry for nestin (a marker for odontoblast differentiation) and Ki67 (a marker for cell proliferation), and a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay.
Results: The intense nestin immunoreactivity in the odontoblast layer of the mesial pulp was weakened immediately after irradiation and was almost lost on Days 1–3, although the extent of pulpal damage was variable among individual animals. At around Day 1, numerous TUNEL-positive cells appeared in the degenerative zone and gradually decreased in number by Day 14. Active cell proliferation occurred in the mesial pulp during Days 5–10. Nestin-positive odontoblast-like cells appeared along the pulp-dentin border by Day 10, resulting in tertiary dentin formation on Day 14.
Conclusions: The current output energy induced apoptosis in the affected dental pulp, followed by active cell proliferation, resulting in tertiary dentin formation. This is the first report regarding laser irradiation of teeth in an *in vivo* mouse model. This model could enable further understanding of the function of certain proteins, including transcriptional factors.

1. Introduction

The lasers used for the treatment of dentin hypersensitivity are divided into two groups: low output power lasers [helium-neon (He-Ne) and gallium/aluminum/arsenide (GaAlAs) lasers], and middle output power lasers [neodymium:yttrium-aluminum-garnet (Nd:YAG) and CO_2 lasers] [1]. Both groups of lasers appear to be efficacious in reducing dentin hypersensitivity [2]. In addition to the treatment of dentin hypersensitivity [1,3], the GaAlAs laser is applied for dental caries and subgingival calculus detection, tooth whitening, periodontal pocket disinfection, and root canal disinfection [4]. It has been suggested that GaAlAs laser irradiation at 830 nm has a pain suppressive effect by blocking the depolarization of C-fiber (but not Aδ-fiber) afferents in rats [5], whereas GaAlAs laser emissions at 904 nm have an analgesic effect on cat tongue [6]. Although the GaAlAs laser does not alter the structure of the tooth surface, the biological action of this type of laser on the dentin-pulp complex, especially in relation to its output energy, is not fully understood.

We have previously demonstrated that GaAlAs laser irradiation induces the formation of tertiary dentin by influencing the secretory activity of odontoblasts or the differentiation of odontoblast-like cells in a rat model [7,8]. The laser irradiation to the dental pulp causes a photobiomodulating effect by increasing the cellular metabolic activity of the odontoblasts and obliterating the dentinal tubules with the intensification of tertiary dentin

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production [9]. Smads and bone morphogenetic protein play important roles in the increase of alkaline phosphatase activity and calcification that occur after laser irradiation in human dental pulp cells *in vitro* [10]. Higher radiation energies induce apoptosis in the affected dental pulp, including odontoblasts, followed by active cell proliferation of the areas of intense heat-shock protein 25 immunoreactivity surrounding the degenerative tissue. This proliferation results in abundant tertiary dentin formation *in vivo* [8], and higher radiation energies in the rat model also cause irreversible changes of the pulp, often leading to the formation of an intrapulpal bone-like tissue [7].

Since a variety of transgenic/knockout mice are available for use in studies to reveal basic knowledge of molecular biology, it is important to establish experimental mouse models for injuries to teeth. These models can be used to understand the function of certain proteins, including transcription factors [11]. Therefore, this study aimed to assess the feasibility of a mouse model for studying pulpal responses to GaAlAs laser irradiation by clarifying the responses of the mouse maxillary first molar pulp lased at a certain power setting. The assessment methods used were as follows: (1) immunohistochemistry for markers of differentiated odontoblasts (nestin) and proliferative cells (Ki67); and (2) an apoptosis assay using terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL).

2. Materials and methods

2.1. Laser irradiation

Under anesthesia from an intraperitoneal injection of chloral hydrate, the mesial coronal surface of the upper first molar of 5-week-old ICR mice (Charles River Laboratories Japan Inc., Yokohama, Japan) was irradiated for 180 s with a pulsed 810-nm GaAlAs semiconductor laser (Lightsurge 3000, Osada Electronic, Tokyo, Japan) at an output power of 1.0 W, without any treatment for the enamel (Supplementary Video). The non-treated upper first molar of the animal was used as the control (n=4).

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.job.2016.10.002.

2.2. Tissue preparation

Materials were collected from groups of 3–6 maxillae at Days 0 (n=3), 1 (n=5), 3 (n=3), 5 (n=6), 7 (n=3), 10 (n=4), and 14 (n=6) after laser irradiation (total number of samples=30). The animals were perfused with physiological saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The maxillae, including both the lased and control teeth, were removed *en bloc* and immersed in the same fixative for additional 12 h. Following decalcification in a 10% ethylenediaminetetraacetic acid disodium salt solution, the samples were embedded in paraffin and cut sagittally at a thickness of 4 μm. Sections were processed for hematoxylin and eosin (H&E) staining and immunohistochemistry.

2.3. Immunohistochemistry and TUNEL assay

Immunohistochemistry procedures were conducted essentially according to our previous report [12], using a mouse anti-nestin monoclonal antibody diluted to 1:500 (EMD Millipore, Billerica, MA, USA) and a rat anti-Ki67 monoclonal antibody diluted to 1:100 for the cell proliferation assay (DAKO Japan, Tokyo, Japan). Apoptosis was quantified by TUNEL assay using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (EMD Millipore).

2.4. Statistical analysis

The percentage of nestin-positive perimeters in the mesial coronal perimeter of the pulp-dentin border was calculated in the control and lased teeth on Days 0–14 using ImageJ software (ImageJ 1.45 s; National Institutes of Health, Bethesda, MD, USA). The numbers of Ki67- and TUNEL-positive cells in the pulp chambers of each specimen (within a $175 \times 123\ \mu m^2$ grid) were calculated separately. Data were obtained from the samples of 34 teeth [control (n=3), at Days 3 (n=5), 5 (n=3), 10 (n=3), and 14 (n=4) after the procedures for the cell proliferation assay using Ki67 immunoreactivity; and control (n=3), at Days 3 (n=3), 5 (n=3), 10 (n=3), and 14 (n=4) after the procedures for the TUNEL assay]. Specimens that were not undergoing the healing process were excluded on the basis of the immunohistochemistry results for nestin. All data are presented as the means and standard deviations of each group. Furthermore, the number of cells in the pulp chamber at different periods after the procedures (0–14 days) and the control were compared by one-way analysis of variance, with multiple comparisons adjusted by the Scheffé *post hoc* test using statistical software (SPSS 16.0J for Windows; SPSS Japan, Tokyo, Japan).

3. Results

3.1. Histological and nestin immunohistochemical changes in the dental pulp following laser irradiation

Coronal odontoblasts showed pseudostratified features, and blood capillaries were located in the odontoblast layer (Fig. 1a, b). Intense immunoreactivity for nestin occurred in the odontoblasts within their cell bodies and processes (Fig. 1c, d). Immediately after irradiation, the odontoblast layer was disturbed, and pulpal hemorrhage was observed, in addition to aspiration of odontoblasts into the dentinal tubules in the mesial pulp (Fig. 1e, f). Nestin immunoreactivity was weakened in the mesial coronal pulp. Some odontoblasts retained their positive reactions for nestin in their cell bodies and processes (Fig. 1g, h). The nestin-positive reaction in the odontoblast layer disappeared in the mesial coronal pulp during Days 1–3, when inflammatory cell infiltration (including neutrophils), odontoblast degeneration, and pulpal hemorrhage were evident (Fig. 1i–p). The extent of pulpal damage was variable among individual animals.

At Days 5–7, the inflammatory reaction ceased and nestin-positive newly differentiated odontoblast-like cells started to appear along the mesial pulp-dentin border, in addition to an increase in intensity of the nestin-positive reaction in the mesial dental pulp (Fig. 2a–d). Tertiary dentin deposition was evident, and nestin-positive odontoblast-like cells were located beneath the deposited dentin matrix. Occasionally, a pulp stone (denticle) surrounded by nestin-positive odontoblast-like cells was observed in the mesial pulp chamber (Fig. 2e–j). The tertiary dentin formation progressed until Day 14 in most cases (5/6 samples) (Fig. 2k–n), whereas ectopic bone matrix deposition surrounded by nestin-negative cells also occurred in addition to dentin formation in the mesial dental pulp (1/6 samples) (Fig. 2o, p). The tooth with bone formation in the pulp chamber showed considerable root resorption (Fig. 2o). The changes in the percentage of nestin-positive perimeters in the mesial coronal perimeter of the pulp-dentin border in the control and lased teeth on Days 0–14 are shown in Fig. 3i.

3.2. Cell proliferation in the dental pulp following laser irradiation as determined by Ki67 immunohistochemistry

Ki67-positive cells were rarely detected in the dental pulp of