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ORIGINAL ARTICLE

The effect of photobiomodulation on total amount of substance P in gingival crevicular fluid: placebo-controlled randomized clinical trial

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Abstract

To investigate the effect of photobiomodulation (PBM) and placebo on total amount of substance P in gingival crevicular fluid (GCF) pre- and postoperatively. Twenty-six patients having tooth with symptomatic apical periodontitis were enrolled in this study. GCF was collected preoperatively. The patients were assigned into two groups ($n = 13$), as follows: placebo and PBM. Sampling was repeated 7 days after root canal treatment. Two independent samples T test was used for analyzing of the differences between preoperative and postoperative substance P levels in GCF ($p = .05$). The Pearson correlation analysis was used for determination of correlation among substance P levels and other variables. For placebo group, there is no significant difference between preoperative and postoperative total amounts of substance P level ($p = 0.553$). For PBM group, postoperative total amount of substance P level was significantly higher than those of preoperative level ($p = 0.005$). Within the limitation of the present study, PBM has immunomodulation effect linked to the modulation of the total amount of substance P in the gingival crevicular fluid. Thai Clinical Trials Registry: TCTR20161228002

Keywords Substance P · Photobiomodulation · Postoperative pain · Gingival crevicular fluid

Introduction

Photobiomodulation (PBM) has become a successfully and widely used treatment option for almost every field in medicine and dentistry. The described main effects are antiinflammatory, immunostimulating, analgesic, and induction of cell proliferation [1]. PBM has been used for the prevention of swelling and trismus after the removal of impacted third molars, following periodontal surgery procedures, for reducing orthodontic post adjustment pain, as well as for the treatment of chronic facial pain, chronic sinusitis, gingivitis, herpes simplex, dentinal tooth hypersensitivity, and sensory aberrations in the inferior alveolar nerve [2–4]. PBM is also used for acceleration of healing process. Noda et al. [5] concluded that PBM enhanced soft- and hard-tissue healing of tooth extraction sockets. Bhardwaj et al. [6] used PBM for treatment of intra-osseous defect, and they reported that PBM is a useful method at accelerated healing.

Most of the effects of PBM can be explained by mitochondria, which are the initial site of light action in cell mitochondria and responsible for generation of cellular energy (ATP) from oxygen and pyruvate and cytochrome c oxidase (the terminal enzyme of the mitochondrial respiratory chain) [7]. In stressed tissues, mitochondria also generate nitric oxide, which competes with oxygen and binds to cytochrome c oxidase [8]. This causes decreased generation of ATP and increased oxidative stress, which leads inflammation [8]. After PBM application, cytochrome c oxidase absorbs light and nitric oxide released from the respiratory chain. Thus, generation of ATP is increased and oxidative stress is decreased [9, 10].

Substance P is a neuropeptide that is responsible for nociceptive transmission [11] signal [12–14]. Firstly, in 1931, Von Euler and Gaddum isolated the substance P from the brain and intestine of horses [15]. The importance of substance P as a

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mediator of pain and inflammation has been reported in some studies [16]. It is known that the concentration of the neuropeptide in the pulp of patients suffering from caries, pulpitis, and granulomas and during orthodontic treatment increases [16]. Substance P also simulates production of various cytokines involved in wound healing [17, 18]; it has an important role in angiogenesis, epidermal cell proliferation, and capillary and fibroblast proliferation [19, 20]. It is shown that its reduction directly caused to the abnormal healing response. These characteristics of substance P exhibit the role of substance P on healing process.

Substance P is present in the sulcular fluid; its concentration is assessable [21]. According to our literature research, there is no information about the effect of PBM on change of substance P level in gingival crevicular fluid (GCF) before and after root canal treatment. Thus, the aim of this study was to investigate the effect of PBM and placebo on total amount of substance P in GCF pre- and postoperatively. The null hypothesis is that there is no significant difference between pre- and postoperative substance P level in both groups.

Materials and methods

Study population

Patients between 18 and 65 years old referred for root canal treatment were selected for the study population. The selection criteria at baseline were (i) having mandibular molar tooth with symptomatic apical periodontitis without having swelling or sinus tract, severe periodontal disease or periodontal probing depth of more than 3 mm, or periapical radiolucency, (ii) no systemic problems or allergic reactions, (iii) not taking analgesics until at least 3 days, and (iv) not having root canal treatment performed previously. For determination of the patients' pain levels, 10 cm, visual analog scale (VAS) was used, and patients who have severe preoperative pain and preoperative percussion (VAS > 60) were included in the study. The protocol was approved by the Research Ethics Committee.

Randomization

For patients' randomly distribution into two groups, a web program (www.randomizer.org) was used. According to allocation and sequence, 13 patients for per group and totally 26 patients received treatment.

Clinical procedures

Firstly, each patient signed the informed consent forms. Before beginning of the root canal treatment, for GCF sampling, supragingival plaque was carefully removed from experimental tooth and contralateral tooth using a curette. The tooth was isolated using cotton wool rolls and a saliva ejector. Before introducing the Periopaper strip (Oraflow, Smithtown, NY, USA) in the gingival sulcus, the tooth was dried with warm air for 10 s. Two Periopaper strips were introduced in mesial and distal interproximal gingival crevice of the tooth for a time of 30 s. The absorbed GCF volume of each strip was determined by a volume quantifying device (Periotrons 8000; Oraflow) which had previously been calibrated as described by Chapple et al. [22], and the strips were stored at − 80 °C until the laboratory procedures. One investigator collected the GCF samples. The strip was put into Eppendorf tubes containing 500 μL of phosphate-buffered saline (PBS) and kept at − 80 °C until use.

After mandibular block anesthesia was performed and lip numbness was obtained, access cavity was prepared and the rest of the root canal treatment procedure was performed under rubber-dam isolation. The working length was verified with an electronic apex locator (Propex Pixi; Dentsply Maillefer, Ballaigues, Switzerland). Biomechanical preparation of the canals was performed with Reciproc instruments. Irrigation with 2 mL 1% sodium hypochlorite (NaOCl) was performed between pecking in-out motions. Size of preparation was determined according to manufacturer's instructions. After preparation, canals were irrigated with 5 mL of 1% NaOCl for 1 min and 5 mL of 5% EDTA for 1 min. Matched single cones and two seal sealers (2Seal; VDW, Munich, Germany) were used for filling of the root canals. Then the restoration of the tooth was completed with a flowable composite resin (3M-ESPE, St. Paul, MN, USA) and a nanohybrid composite resin (3M-ESPE). Then, the quality of the root filling was evaluated via radiographs and categorized as acceptable and unacceptable according to the criteria used by Khabbaz et al. [23] All of the teeth were followed for 1 month.

Group characterization

The patients were assigned into two groups ($n = 13$), as follows:

Photobiomodulation After root canal treatment and restoration of the tooth, a 970 ± 15-nm diode laser with a 10-W source power (SIROLaser Xtend; Sirona Dental Systems GmbH, Bensheim, Germany) was activated at 0.5 W and 10 Hz (power density ≈ 2.86 W/cm^2) at a distance of approximately 10 mm to the tissue around the apex of the root (Fig. 1). The laser was used in gated mode; circular movement was performed during application. Pulse duration was 0.5 s, and pulse pause was 50%. The spot size was approximately 1.1569 cm^2, major diameter was 1.252 cm, minor diameter was 1.241 cm, and circularity was 0.70 (calculated on real photo of laser). Total

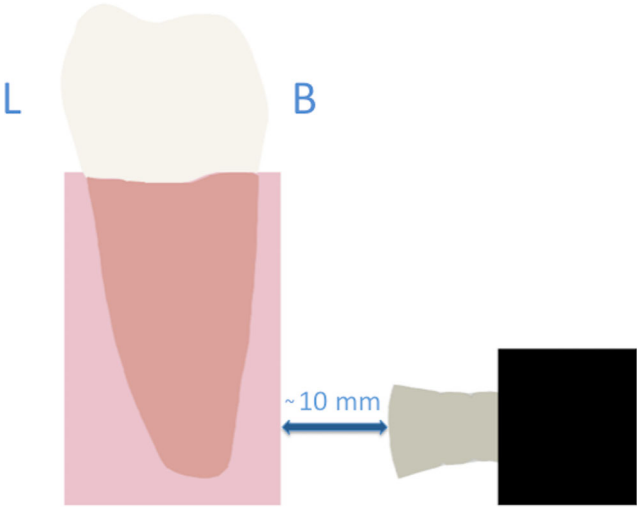

Fig. 1 Laser application (PBM)

application time was 60 s for each tooth. For this application, a 200-μm optical tip was used.

Placebo Diode laser was placed similarly PBM group; however, it was not activated.

Pain level at postoperative days 1, 3, 5, and 7 was signed on VAS by the patients. During 1 week, the need of analgesic was noted. Patients were recalled for postoperative GCF sampling after 1 week. At this visit, palpation sensitivity, percussion pain level, presence or absence of swelling, and sinus tract were noted.

Substance P assay

Substance P levels in the gingival crevicular fluid samples were measured by enzyme linked immune assay (ELISA) kit (Cayman, Ann Arbor, MI). ELISA kit was chosen according to its sensitivity. Reagents provided by the ELISA kit and all sample were waited until they reach room temperature. The assay is based on the competition between substance P and a substance P acetylcholinesterase conjugates for a limited number of binding sites coated in the wells. ELISA buffer, substance P ELISA standards, samples, and substance P tracer and antiserum were added to the appropriate wells of microtiter plate and incubated for 18 h at + 4 °C. After washing the plates five times with wash buffer, 200 μL of Ellman's reagent was added to the each well and 5 μL of tracer reagent was added to the total activity wells. Then, plates were incubated for 2 h in the dark in microplate shaker (Thermo Fisher Scientific, Waltham, MA, USA). After incubation, the absorbance of each well was read by spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Fisher, Waltham, MA, USA) at 410 nm. Concentration of the samples was calculated with the software provided by the reader's manufacturer, using a four-parameter logistic fit.

Statistical analysis

Two independent samples *T* test was used for analyzing of the differences between preoperative and postoperative substance P levels in GCF for each group ($p = .05$). The Pearson correlation analysis was used for determination of correlation among substance P levels and other variables (age, sex, preoperative pain, preoperative percussion, preoperative palpation, vitality) ($p = .05$).

Results

Twenty-six patients enrolled in this study (Fig. 2). There were 13 women and 13 men (for placebo group 7 women and 6 men, for PBM group 6 women and 7 men), with a mean age 27.54 ± 9.15 (for placebo group 27.77 ± 9.25, for PBM group 27.31 ± 9.41). There were no significant differences between the groups in terms of age ($p = 0.901$) and sex ($p = 0.695$).

Substance P levels pre- and postoperatively are shown in Table 1. There was no sample that had undetectable levels in both groups. For placebo group, preoperative total amount of substance P level was 3.971 ± 2.301 pg/30 s, and postoperative level was 3.437 ± 2.230 pg/30 s. There is no significant difference between preoperative and postoperative total amount of substance P level for placebo group ($p = 0.553$). For PBM group, preoperative total amount of substance P level was 4.798 ± 1.971 pg/30 s, and postoperative level was 7.378 ± 2.285 pg/30 s. Postoperative total amount of substance P level was significantly higher than preoperative level for PBM group ($p = 0.005$).

Four patients and five patients had preoperative palpation sensitivity for placebo and PBM group, respectively. Palpation sensitivities for groups were statistically similar ($p = 0.680$). Five teeth and two teeth were nonvital for placebo and PBM group, respectively. There were no significant differences between groups in terms of vitality ($p = 0.185$). For placebo group, preoperative pain level was 88.54 ± 11.62 and preoperative percussion level was 77.54 ± 10.79. For PBM group, preoperative pain level was 91.38 ± 7.18 and preoperative percussion level was 82.92 ± 9.69. There were no significant differences between two groups in terms of preoperative pain ($p = 0.460$) and preoperative percussion levels ($p = 0.193$). Pain and percussion levels are shown in Table 2. For placebo group, postoperative pain levels at days 1, 3, 5, and 7 were respectively 33.62 ± 31.62, 17 ± 23.12, 8.08 ± 10.3, and 2.54 ± 4.35. For PBM group, postoperative pain levels at days 1, 3, 5, and 7 were respectively 6.38 ± 6.52, 0.85 ± 1.34, 0.15 ± 0.55, and 0. Postoperative pain at days 1, 3, 5, and 7 were significantly lower in PBM group than placebo ($p < 0.05$). Postoperative percussion levels at day 7 were 7.92 ± 11.22 and 0.15 ± 0.55 for placebo and PBM

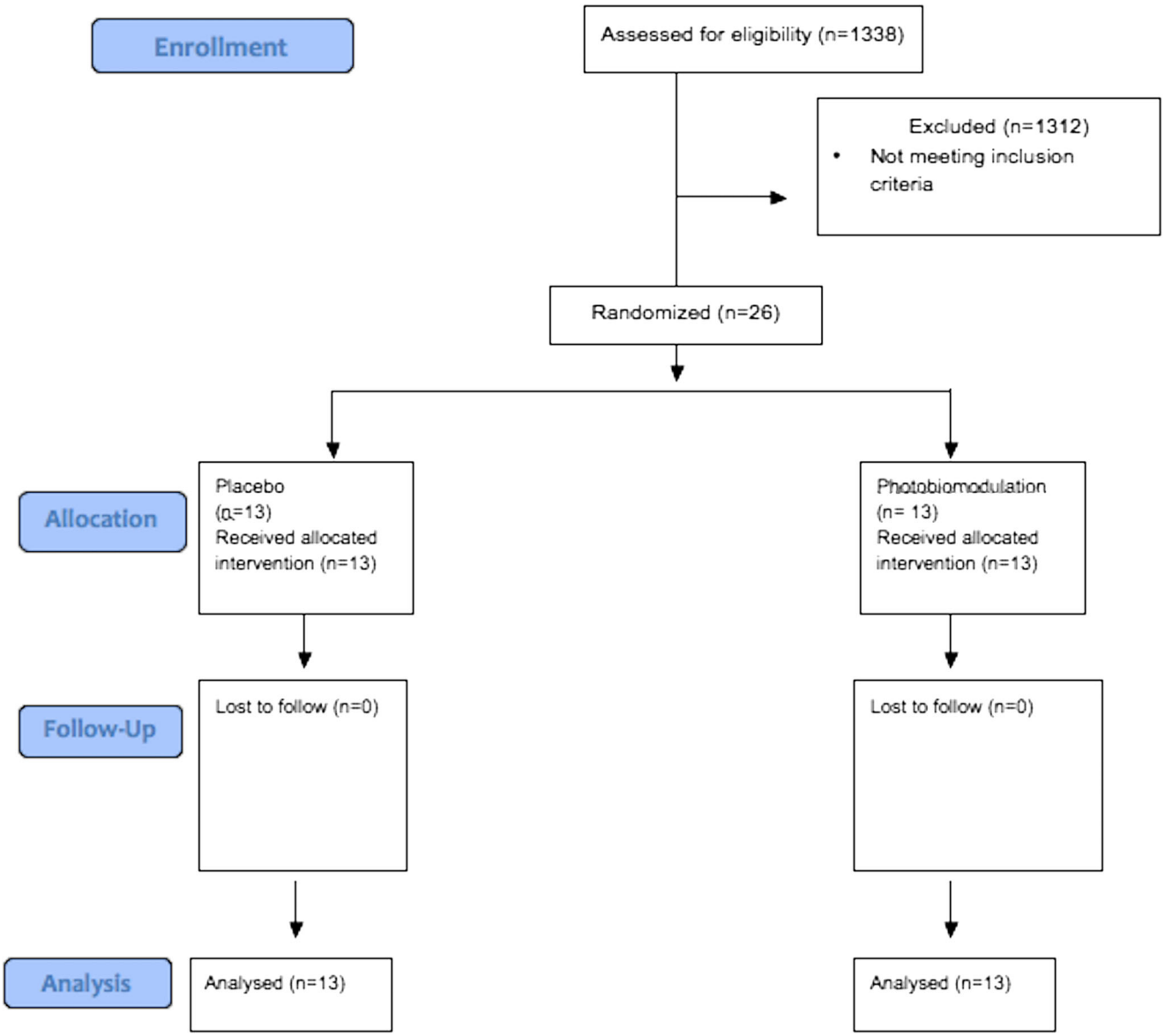

Fig. 2 CONSORT flow diagram

groups, respectively. Postoperative percussion level in PBM group was significantly lower than placebo group ($p = 0.20$). At day 7, no patient has palpation sensitivity, swelling, or sinus tract. No patient needed unscheduled appointment. Three patients in placebo group and 1 patient in PBM group needed analgesic use. No significant difference was found between groups in terms of need of analgesic ($p = 0.277$).

None of the root canal filling quality was categorized as unacceptable. At follow-up sessions, all of patients were asymptomatic.

According to the Pearson correlation, there was a weak relationship between preoperative total amount of substance P level and preoperative palpation ($p = 0.019$, $r = 0.458$).

Discussion

Yoo et al. [24] investigated the effect of Nd:YAG laser on pain and neuropeptide reduction in root canal exudate pre- and postendodontic treatment, and they concluded that Nd:YAG laser has favorable effects on pain and inflammation modulation. However, there is no data about substance P level in GCF after PBM for root canal treatment. Thus, the aim of this study was to investigate the effect of PBM and placebo on total amount of substance P in GCF pre- and postoperatively.

The results of the present study showed that although there is no significant difference between preoperative and postoperative total amount of substance P level for placebo group, postoperative total amount of substance P level was

Table 1 Preoperative and postoperative total amount of substance P levels in gingival crevicular fluid

	Preoperative (pg/30 s)	Postoperative (pg/30 s)	*p* value
Placebo	3.971 ± 2.301	3.437 ± 2.230	0.553
PBM	4.798 ± 1.971	7.378 ± 2.285	0.005

significantly higher than preoperative level for PBM group. Thus, the null hypothesis was partially rejected.

There is little information about substance P level associated with endodontics. Shin et al. [25] evaluated substance P levels in GCF during root canal treatment of nonvital teeth. The authors concluded that periradicular inflammation of endodontic origin can elevate substance P level in GCF. In another study, Awawdeh et al. [26] collected GCF samples before the treatment and then repeated 1 week after pulp extirpation. Awawdeh et al. [26] reported decrease in substance P level after pulp removal. These results are not harmonious with our results. In our study, there is no significant difference between preoperative and postoperative total amount of substance P level for placebo group. The difference between the results can be explained by the periapical injury in the second visit, different file systems, motion types, and irrigation solutions.

Exogenous stimuli (e.g., heat, topical lesions, irritating agents, allergens, ultraviolet light, and microbial agents) or endogenous stimuli (e.g., pH changes, cytokines, kinins, histamine, proteases, neurotransmitters, hormones, and stress) cause neurogenic inflammation [27, 28]. As a part of this process, neuropeptides, such as substance P, neurokinin A, calcitonin gene-related peptide, and vasoactive intestinal peptide, are released from the nerve endings of A-δ and C fibers [28–30]. In present study, although there is no significant difference between preoperative and postoperative total amount of substance P level for placebo group, postoperative total amount of substance P level was significantly higher than preoperative level for PBM group. However, postoperative pain was lower in PBM group than placebo group. Because PBM results in increased substance P level in GCF, someone can claim that PBM can provide wound healing through the substance P pathway.

Table 2 Preoperative and postoperative pain percussion levels

	Placebo	PBM	*p* value
Preoperative pain	88.54 ± 11.62	91.38 ± 7.18	0.460
Preoperative percussion	77.54 ± 10.79	82.92 ± 9.69	0.193
Postoperative pain at day 1	33.62 ± 31.62	6.38 ± 6.52	0.006
Postoperative pain at day 3	17 ± 23.12	0.85 ± 1.34	0.019
Postoperative pain at day 5	8.08 ± 10.3	0.15 ± 0.55	0.011
Postoperative pain at day 7	2.54 ± 4.35	0.00 ± 0.00	0046
Postoperative percussion	7.92 ± 11.22	0.15 ± 0.55	0.020

The role of substance P in wound healing is complex. Substance P causes physiological processes that include vasodilation, pruritus, plasma extravasation, activation of the immune system, and wound healing [27, 28, 31]. Another pathway of the substance P in wound healing was explained by the ability of substance P in mobilizing bone marrow mesenchymal stem cells from the bone marrow to wound sites [32]. In brief, the increase level of substance P in PBM group can contribute to wound healing. Future studies should be conducted to understand substance P on wound healing in endodontics.

PBM application can modulate neuropeptides and growth factors in GCF. Pamuk et al. [33] reported that PBM application provides reduction of transforming growth factor beta-1 and some enzymes in GCF. Kumaresan et al. [34] reported that PBM application was found to have additional benefits over root surface debridement with respect to clinical periodontal parameters and GCF periostin levels. Hochman et al. [35] investigated the effect of PBM and LED with different parameters on the secretion of substance P in rat skin and concluded that PBM which has 808-nm wavelength enhances substance P secretion in rat skin compared to 660-nm laser, 660-nm LED, and 470-nm LED. And they claimed that infrared rays penetrate deeper in skin than other wavelengths, thus achieving a larger amount of secretory nerve endings or secretory fibers more specific to substance P. In our study, we applied PBM that has a 970 ± 15-nm wavelength at a distance of approximately 10 mm to the tissue around the apex of the root. It may be claimed that this wavelength may provide reaching the periapical region and stimulate releasing substance P in GCF.

Samples for neuropeptide level evaluation can be collected from root canal [36], GCF [26], and periodontal ligament [37] after extraction of tooth. Because in our study, second samples were collected at postoperative day 7 and extraction was not performed; periodontal ligament cannot be used [37]. For collecting samples from root canal, it is needed not to complete the root canal treatment. In our study, not to increase of endodontic treatment visit, treatment was completed in one session. There are direct communication channels between the pulp cavity and the periodontal ligament (apical foramina, accessory lateral and furcation canals, and dentinal tubules) [38]. Substance P can diffuse into the periodontal ligament and, then, the gingival crevice. This long diffusion pathway between gingival crevice and apex may cause decrease in the amount of substance P [39]. Since in the present study, root canal treatment was completed in one session, GCF was appropriate for collecting samples.

Conclusion

Within the limitation of the present study, although there is no significant difference between preoperative and postoperative total amount of substance P level for placebo group, postoperative total amount of substance P level was significantly higher than preoperative level for PBM group. However, postoperative pain was lower in PBM group than placebo group. Because PBM has immunomodulation effect linked to the modulation of the total amount of substance P in the GCF without increased postoperative pain, this makes us think that PBM can provide wound healing through the substance P pathway.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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