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Oral mucosal irritation study in hamster to evaluate a therapeutic apparatus using hydrogen peroxide photolysis for periodontitis treatment

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ABSTRACT

We conducted an oral mucosal irritation study in hamsters to evaluate a therapeutic apparatus using hydrogen peroxide (H_2O_2) photolysis for periodontitis treatment (ISO 10993-Part 10, Annex B.3). The cheek pouches in 15 male hamsters were allocated to one of six groups. Group 1 received pure water treatment (control group). Group 2 received laser irradiation at 80 mW. Group 3 received 3% H_2O_2. Groups 4–6 received laser irradiation of 3% H_2O_2 at 80, 40, and 20 mW, respectively. The total treatment time was set at 7 min and treatment was repeated three times at approximately 1-h intervals. Macroscopic and microscopic histologic observations of the treated sites were performed immediately after each treatment and/or 24 h after the last treatment. The mean scores in macroscopic and histologic examinations in all six groups were 0. Accordingly, irritation indices calculated by subtracting the mean score in each experimental group from that in the control group (Group 1) were 0. Our results suggest that treatment with the apparatus has no mucosal irritation potential in hamster cheek pouches under test conditions simulating clinical conditions.

1. Introduction

We have developed a novel therapeutic apparatus to treat periodontitis, an inflammatory disease caused by pathogenic microbes in dental plaque leading to periodontal pocket formation, attachment loss, and loss of alveolar bone around the tooth (Darveau, 2010; Kinane et al., 2015; Socransky et al., 1998). The apparatus utilizes artificially generated hydroxyl radicals by hydrogen peroxide (H_2O_2) photolysis to kill pathogenic microbes. A schematic illustration of the microbicidal action of H_2O_2 photolysis is shown in Fig. 1. Hydroxyl radicals are a powerful oxidizing agent, inducing lethal oxidative damage to such microbes. However, as radicals cannot be formulated as a ready-made disinfectant due to their very short half-life in liquid (approximately 10^{-9} s) (Pryor, 1986; Sies et al., 1992), H_2O_2 photolysis in which 3% H_2O_2 is irradiated with light at a wavelength of 405 nm is applied in the apparatus. We previously reported that hydroxyl radical yield from H_2O_2 photolysis is dependent upon the irradiation time and laser output power, and this technique can kill pathogenic bacteria effectively through radical generation (Ikai et al., 2010; Sato et al., 2016; Shirato et al., 2011). Additionally, this technique is unlikely to induce bacterial resistance, possibly because of non-specific oxidative damage of cell structures by hydroxyl radicals (Ikai et al., 2013).

Regarding the biological safety of H_2O_2 photolysis, the acute locally injurious property of our hydroxyl radical generation system was evaluated by examining the oral mucosa and healing of full thickness skin wounds in rats using a prototype apparatus (Yamada et al., 2012). We showed that no abnormal findings were observed in the buccal mucosal region after three treatments utilizing this disinfection technique. However, the output power of the laser in the prototype apparatus was limited to 7 mW. An apparatus with the ability to emit laser light with an output power of ≥40 mW was subsequently generated, and the acute locally injurious property of this latest model was also assessed in rats (Sato et al., 2016). This

Abbreviations: GLP, good laboratory practice; ISO, International Organization for Standardization.

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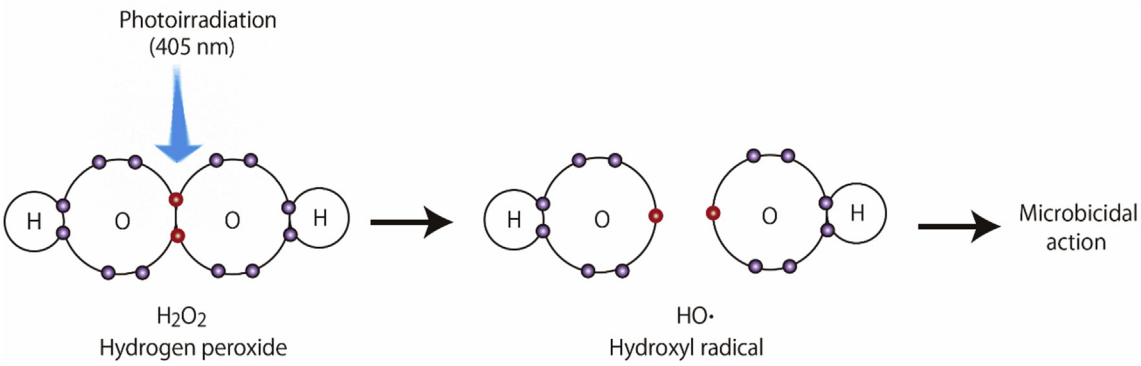

Fig. 1. Schematic illustration of the microbicidal action of H_2O_2 photolysis.

study also demonstrated that no abnormal findings were observed in the palatal mucosa, even when rats received three treatments of 3% H_2O_2 with laser irradiation at an output power of 40 mW.

These results provide the basis to move forward to a clinical trial to assess the efficacy and safety of the apparatus in human patients with periodontitis. However, the biological safety of the apparatus must first be confirmed by a validation study with compliance of good laboratory practice (GLP) regulation. Therefore, the purpose of the present study was to assess the biological safety of the therapeutic apparatus using H_2O_2 photolysis for periodontitis treatment by a study validated under GLP regulation. Specifically, an oral mucosal irritation study in Syrian hamsters was conducted according to ISO 10993-Part 10.

2. Materials and methods

The protocol of the present study was designed according to "Tests for Irritation and Skin Sensitization, Annex B.3 Oral Mucosa Irritation Test" (ISO 10993–10, issued on August 1, 2010) and similar guidelines issued in Japan.

All experimental procedures were conducted in compliance with Japanese law and guidelines for animal protection. The experiment was also conducted in accordance with "Guidelines for Animal Experiments Adopted by General Incorporated Foundation, Food and Drug Safety Center (In-House Regulations) of October 1, 1990, followed by revision of April 1, 2013" (approval no. 1140051A).

2.1. Test apparatus

A test apparatus consisting of a control unit, handpiece, foot switch, and collateral components was made by AZ Co. Ltd. (Sendai, Japan). Functionally, it was equipped with a continuous-wave laser unit that emits light at a wavelength of 405 nm and had a water supply system to release 3% H_2O_2 or pure water. A hollow-type steel tip and a disposable plastic optical guide designed to be set inside the tip were fabricated for the device. A photograph and schematic illustration of the apparatus are shown in Fig. 2. The laser output power of the apparatus was validated by measuring it before the experiment. To simulate clinical conditions, the flow rate was set at 26.5 ml/min, which is within the proposed range for clinical use (10–30 ml/min).

2.2. Reference solution (control group) and 3% H_2O_2

As a reference solution, water for injection in the Japanese Pharmacopoeia (termed as pure water hereafter, Hikari Pharmaceutical Co., Ltd., Tokyo, Japan) was used. Three percent H_2O_2 without any stabilizer additives was prepared by Sanchemipha Co., Ltd. (Sendai, Japan). Stability of 3% H_2O_2 was assured according to the result of an acceleration test (40°C/75% relative humidity) for 6 months provided by Sanchemipha Co., Ltd. (data not shown). Since the accelerated storage conditions for six months recommended by an International Conference on Harmonization guideline (ICH, 2003) resulted in no degradation of H_2O_2, H_2O_2 concentration in the bottle container after each treatment was not measured.

2.3. Experimental animals

Seven-week-old male Syrian hamsters were purchased from Japan SLC Inc. (Hamamatsu, Japan), subjected to a 1-week quarantine period, and used after acclimatization for approximately 1 week. Animals individually kept in a plastic cage (235 mm width × 325 mm depth × 17 mm height) were housed at an approximate temperature and relative humidity of 21.5–25.0 °C and 40.0–75.0%, respectively, under 15 changes/day air ventilation and a 12-h light/12-h dark cycle. Animals were given access to food pellets (CE-2, CLEA Japan Inc., Tokyo, Japan) and tap water in a feed-water bottle *ad libitum*. The actual measured temperature and relative humidity were 23.5–24.0 °C and 51.0–64.0%, respectively.

Test animals were selected according to time course changes in body weight, general status, and gross appearance of cheek pouches. The body weight of selected animals on the treatment day was 109.3–136.5 g. When animals were selected and treated, they were subjected to mixed anesthesia consisting of medetomidine hydrochloride (Domitor®, Nippon Zenyaku Kogyo Co., Ltd., Koriyama, Japan), midazolam (Dormicum Injection 10 mg, Astellas Pharma Inc., Tokyo, Japan), and butorphanol tartrate (Vetorphale®, Meiji Seika Pharma Co., Ltd., Tokyo, Japan). At the observation and sacrifice time point (24 h after the last treatment), animals were anesthetized with sodium pentobarbital (Somnopentyl®, Kyoritsuseiyaku Corp., Tokyo, Japan). The cheek pouch mucosa was washed with physiological saline in the Japanese Pharmacopoeia (Otsuka Pharmaceutical Factory, Imizu, Japan), and extraneous substances attached to the mucosa such as feedstuffs were carefully removed by a cotton swab.

2.4. Experimental groups

Five experimental groups were set. Three experimental groups received laser light and 3% H_2O_2 concomitantly as in clinical application. That is, the maximum output power of the laser (80 mW) was set as the highest dose, and the other two doses were set at the common ratio 1/2 (40 and 20 mW). In addition, two experimental groups received laser treatment alone at the highest dose (80 mW) or 3% H_2O_2 treatment alone. Pure water treatment