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The traditional Japanese medicine hangeshashinto alleviates oral ulcer-induced pain in a rat model

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ABSTRACT

Objective: Recent studies have demonstrated that mouthwash made with the traditional Japanese medicine hangeshashinto exhibits anti-inflammatory action and alleviates oral mucositis scores, including pain complaints, in patients undergoing chemoradiotherapy. However, no study has demonstrated the mechanism underlying how hangeshashinto provides pain relief in oral ulcers.
Design: The analgesic effects on pain-related behaviors following the topical application of hangeshashinto were evaluated in an oral ulcer rat model treated with acetic acid using recently developed methods. Indomethacin, the representative anti-inflammatory agent, was intraperitoneally administered. The tissue permeability of the oral mucosa was histologically evaluated after applying the fluorescent substance FluoroGold.
Results: The topical application of hangeshashinto in ulcerative oral mucosa suppressed mechanical pain hypersensitivity over 60 min, without any effects on healthy mucosa. The same drug application also inhibited oral ulcer-induced spontaneous pain. Indomethacin administration failed to block the mechanical pain hypersensitivity, though it did largely block spontaneous pain. Topical anesthesia with lidocaine showed hyposensitivity to mechanical stimulation in healthy mucosa. In the ulcer regions in which the oral epithelial barrier was destroyed, deep parenchyma was stained with FluoroGold, in contrast to healthy oral mucosa, in which staining was limiting to the superficial site.
Conclusions: Hangeshashinto leads to long-lasting analgesic effects, specifically in the ulcer region by destroying the epithelial barrier. Hangeshashinto alleviates oral ulcer-induced pain in inflammation-dependent and/or independent manner.

1. Introduction

In dental practice, oral ulcers are the most common painful mucosal lesions. They are iatrogenically caused by unsuitable denture bases and orthodontic appliances (Baricevic et al., 2011; Geckili, Bektas-Kayhan, Eren, Bilgin, & Unur, 2012; Munoz-Corcuera, Esparza-Gomez, Gonzalez-Moles, & Bascones-Martinez, 2009). In head and neck cancer patients undergoing chemo-radiotherapy, oral ulcerative mucositis is one of the most serious side effects, and mucositis-induced pain affects nutrition, talking and swallowing, ultimately resulting in poor patient quality of life. Sometimes, severe pain forces the delay and/or interruption of therapy (Donnelly, Blijlevens, & Verhagen, 2003; Sonis, 2004; Trotti et al., 2003). Currently, topical anesthesia with lidocaine to the oral cavity has been used to treat oral ulcer in patients (Descroix et al., 2011; Khanal, Baliga, & Uppal, 2010; Saunders et al., 2013). However, lidocaine application induces significant side effects on oral functions such as the loss of touch and taste sensations during meals (Khanal et al., 2010).

Hangeshashinto, a traditional Japanese (kampo) medicine, is composed of 7 herbal extracts (Coptis Rhizome, Ginseng, Glycyrrhiza, Jujube, Pinellia Tuber, Processed Ginger, and Scutellaria Root), and it has been approved by the Ministry of Health, Labor and Welfare of Japan and widely used to treat acute and chronic gastrointestinal catarrh, fermentative diarrhea and acute gastro-enteritis and oral mucositis as a pharmaceutical-grade drug in Japan (Kase, Saitoh, Ishige, & Komatsu, 1998; Yamashita et al., 2014). To date, hangeshashinto has been reported to exhibit anti-

Abbreviations: OPAD, orofacial pain assessment device; TRPV1, transient receptor potential vanilloid 1; TRPA1, transient receptor potential ankyrin 1.

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inflammatory, anti-oxidant and anti-bacterial effects (Fukamachi et al., 2015; Kase et al., 1998; Kono et al., 2014; Matsumoto et al., 2015; Nakazono, Ara, Fujinami, Hattori, & Wang, 2010). Recently, some clinical studies have reported that using mouthwash made from hangeshashinto for more than one week considerably reduces oral mucositis scores in cancer patients and improves chemoradiation completion rates (Hatakeyama et al., 2015; Kono et al., 2010; Yamashita et al., 2014). Furthermore, in a double-blind, placebo-controlled, randomized comparative trial of patients with colorectal cancer treated chemotherapy, hangeshashinto significantly reduced the median duration of grade ≧2 mucositis (WHO oral mucositis scale)(Matsuda et al., 2015). The Common Terminology Criteria for Adverse Events (CTCAE) ver.4.0, which is frequently used as the WHO oral mucositis scale, involves pain complaints as an inclusion criterion for higher grade scores (Kono et al., 2010; Yamashita et al., 2014). To date, however, it is not clarified whether hangeshashinto has analgesic effects on pain models including oral mucositis-induced pain.

Berberine and ferulic acid, which are ingredients of Coptis Rhizome (Chen et al., 2012; Kim, 2015), reportedly alleviate mechanical pain hypersensitivity in neuropathic pain models after chronic construction injury and spinal nerve ligation (Zhang et al., 2010). Together with less sensitivity to neuropathic pain in the anti-inflammatory agent models (Kitagawa et al., 2013; Sung et al., 2007), direct application of hangeshashinto to oral ulcers may induce an analgesic effect on mechanical pain hypersensitivity in an inflammation-independent manner and this effect may be one reason why it reduces the oral mucositis scores.

To clarify the analgesic effect of hangeshashinto, we investigated pain-related behavior following the topical application of hangeshashinto on ulcer regions in a rat model treated with acetic acid on oral mucosa. Oral ulcer-induced pain was assessed from pain-related behaviors using our recently developed methods (Hitomi et al., 2015) and another operant pain assay system (Anderson, Jenkins, Caudle, & Neubert, 2014; Anderson et al., 2013; Neubert et al., 2005; Rossi, Vierck, Caudle, & Neubert, 2006). To examine its inflammatory effects on oral ulcer-induced pain, the representative anti-inflammatory agent indomethacin was administered prior to ulcer development.

2. Materials and methods

2.1. Animals

Male Wistar rats (150–350 g, Kyudo, Saga, Japan) were used for all experiments, except for an experiment using the operant orofacial pain assessment device (OPAD) system (Stoelting Co., Wood Dale, IL, USA), which used male Sprague-Dawley rats (220–260 g, SLC Co., Ltd., Shizuoka, Japan). The rats, which were randomly used for each experiment in the present study, were maintained on a light–dark cycle (L:D, 12:12-h) in a temperature- and humidity-controlled room (21–23 °C and 40–60%, respectively) with food pellets and water provided ad libitum. All experiments were conducted in accordance with the EU Directive 2010/63/EU and the guidelines of the International Association for the Study of Pain (Zimmermann, 1983), and they were approved by the Animal Experiment Committee of Kyushu Dental University and the Laboratory Animal Committee of Tsumura & Co.

2.2. Oral ulcer model

According to our previous study (Hitomi et al., 2015), under pentobarbital anesthesia (50 mg/kg, intraperitoneally; Kyoritsu Seiyaku, Tokyo, Japan), eight-week-old rats were treated with 50% acetic acid soaked in a filter paper (3 mm × 3 mm, Whatman, Maidstone, UK) in the labial fornix region of the inferior incisors for 30 s. The acetic acid treatment induced obvious ulceration in the treated oral mucosal region on days 2–3. The oral ulcer was visually evaluated. As a sham treatment, some rats received only pentobarbital anesthesia, without acetic acid treatment.

2.3. Evaluation of mechanical pain hypersensitivity

To measure the withdrawal mechanical threshold in the oral mucosa in conscious rats, the stable intraoral opening method was performed in a handmade black box (6 × 6 × 13 cm) constructed from plastic rectangular bottle using a set of von Frey filaments (0.02–0.6 g, North Coast Medical, Morgan Hill, CA, USA) and 0.2 and 0.3 g handmade filaments, according to our recent study (Hitomi et al., 2015). To expose the oral mucosa, the mental skin of five-week-old rats under pentobarbital anesthesia (50 mg/kg, intraperitoneally) was pierced with a magnetized ring (22-gauge-like size, Daiso Sangyo, Hiroshima, Japan). The rats were then trained to stably expose the labial fornix region while conscious by attaching a small neodymium magnet with a 4 g weight to the pierced ring for 2–3 weeks prior to the measurements. Mechanical threshold was defined as the minimum pressure required to evoke an escape attempt in at least 3 of the 5 tests. In the experiment, the test was conducted not only on day 2 but also on day 3 to reduce the number of rats tested. Although mechanical pain hypersensitivity in many animals in the oral ulcer model tended to recover on day 3, from day 2 (Hitomi et al., 2015), 4 of the models that showed the same threshold or lower on day 3, compared with day 2 were tested with a different drug from that used on day 2.

The rats in the experimental group were stable in the restrainer; therefore, while they were conscious, a topical drug was applied for 5 min by placing a 20 μL-soaked cotton swab on the labial fornix region. Hangeshashinto (Lot no. 2100014010, containing seven crude drugs (Pinellia Tuber [tuber of Pinellia ternate Breitenbach, Araceae], Scutellaria Root [root of Scutellaria baicalensis Georgi, Labiatae], Glycyrrhiza [root or stolon of Glycyrrhiza uralensis Fisher, Leguminosae], Jujube [fruit of Zizyphus jujuba Miller var. inermis Rehder, Rhamnaceae], Ginseng [root of Panax ginseng C.A. Meyer, Araliaceae], Coptis Rhizome [rhizome of Coptis japonica Makino, Ranunculaceae] and Processed Ginger [steamed rhizome of Zingiber officinale Roscoe, Zingiberaceae]), was obtained from Tsumura & Co. (Tokyo, Japan) was diluted to 100 mg/mL in distilled water in the present study. As a control solution, distilled water was similarly applied. Furthermore, 1% lidocaine, a general local anesthetic drug (1% Xylocaine, AstraZeneca K.K, Osaka, Japan), was topically applied on healthy oral mucosa. The concentration of lidocaine is clinically used for oral ulcer patients (Descroix et al., 2011). To examine an anti-inflammatory effect on oral ulcer-induced pain, indomethacin (Wako, Osaka, Japan) at 5 mg/kg diluted in 0.1 M Tris buffer was intraperitoneally administered once per day for three days prior to the measurements on day 2 following the acetic acid treatment. For the vehicle injection, 0.1 M Tris buffer was administered at the same volume. The administration route of the drug has been reported to suppress inflammatory pain sufficiently in many studies (Calil et al., 2014; Harano et al., 2010).

2.4. Evaluations of spontaneous and evoked pain

Mouth rubbing behavior with both forelimbs was measured in a clear plastic cage (30 × 30 × 30 cm) on day 2 after the acetic acid or sham treatment. All rats were acclimated to the clear plastic cage at least three times prior to the behavioral measurements.

Sixty minutes after the topical drug application on the oral mucosa for 5 min (i.e., with a cotton swab held under 2% isoflurane anesthesia), spontaneous pain was evaluated for 10 min based on the mouth-rubbing behavior. In some oral ulcer model animals,