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Evaluation of topical external medicine for 5-fluorouracil-induced oral mucositis in hamsters

Hiromi Mitsuhashi, Katsuya Suemaru *, Bingjin Li, Ranji Cui, Hiroaki Araki

Department of Clinical Pharmacology and Pharmacy, Neuroscience, Ehime University Graduate School of Medicine, Shitsukawa Toon, Ehime, 791-0295, Japan

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Abstract

Oral ulcerative mucositis is a common and painful toxicity associated with chemotherapy for cancer. Current treatment for chemotherapy-induced oral mucositis is largely palliative, and no adequate treatment with conclusive evidence exists. The purpose of this study was to evaluate the potential effectiveness of the topical external medicines used in clinical settings, and the authors investigated the effects of 1% azulene ointment, 0.12% dexamethasone ointment, and polaprezinc–sodium alginate suspension on an animal model for oral mucositis induced by chemotherapy. Oral mucositis was induced in hamsters through a combination treatment of 5-fluorouracil and mild abrasion of the cheek pouch. Each drug was administered topically to the oral mucosa of hamsters, and the process of healing of damaged oral mucositis was examined by measuring the size of the mucositis. Azulene ointment did not reduce the size of the mucositis compared with the vaseline-treated control group. Polaprezinc–sodium alginate suspension significantly improved the recovery from 5-fluorouracil-induced damage. In contrast, local treatment with dexamethasone exacerbated the mucositis markedly. These results suggested the healing effect of polaprezinc–sodium alginate suspension and the risk of steroids to severe oral mucositis induced by chemotherapy.

Keywords: Oral mucositis; Polaprezinc; Dexamethasone; Azulene; 5-fluorouracil

1. Introduction

Oral ulcerative mucositis is a frequent complication of chemotherapy or radiotherapy for cancer, and chemotherapy-induced mucositis has increasingly become a common dose-limiting toxicity for a number of chemotherapeutic regimens. In general, these ulcerative lesions are painful, limit oral intake, and act as portals of entry for indigenous oral microbial flora (Sonis and Clark, 1991).

Presently, there is some evidence for the prevention of oral mucositis in cancer patients. For example, oral cryotherapy using ice chips prevents mucositis induced by bolus doses of 5-fluorouracil (Mahood et al., 1991; Cascinu et al., 1994). Mouthwash with benzydamine, a non-steroidal drug with anti-inflammatory, anesthetic and antimicrobial properties, is more effective when used prophylactically to prevent mucositis rather than therapeutically once mucositis is present (Schubert and Newton, 1987). Chlorhexidine, an antiseptic, has been reported to prevent radiation-induced mucositis (Dodd et al., 1996). Recently, biologically active factors are now being considered for their potential efficacy in preventing and/or treating mucositis. Granulocyte-colony stimulating factor (Saarilahti et al., 2002), keratinocyte growth factor (Spielberger et al., 2004; Freytes et al., 2004), interleukin-11 (Sonis et al., 1997a) and transforming growth factor-beta 3 (McCormack et al., 1997; Sonis et al., 1997b) reportedly reduce the severity of mucositis when applied as pre-treatments to chemotherapy and/or in the repair phase. Unfortunately, however, there are no established treatments for oral mucositis. The major current treatments for oral mucositis in clinical settings are local anesthetics (Yamamura et al., 1998), agents that coat the oral mucosa with sodium alginate (Oshitani et al., 1990), anti-ulcer agents, such as polaprezinc (Matsukura and Tanaka, 2000; Fujiwara et al., 2002) or rebamipide (Matsuda et al., 2003), and anti-inflammatory agents, such as azulene or steroids (Rubenstein et al., 2004). However, the precise efficacy of these drugs remains unclear. Thus, in the present study, to evaluate the potential effectiveness of topical ointments used

* Corresponding author. Tel.: +81 89 960 5731; fax: +81 89 960 5745.
E-mail address: suemaru@m.ehime-u.ac.jp (K. Suemaru).

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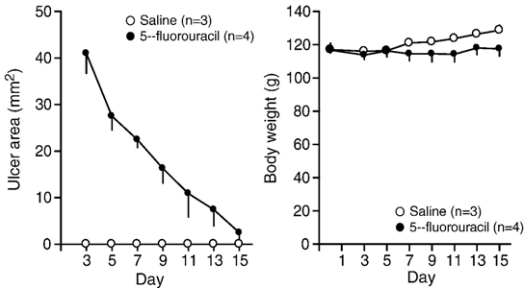

Fig. 1. Changes in ulcer area and body weight in the hamster. Hamsters received two intraperitoneal injections of 5-fluorouracil ($n=4$) or saline ($n=3$) on days 0 and 2. Each point represents the mean±S.E.M.

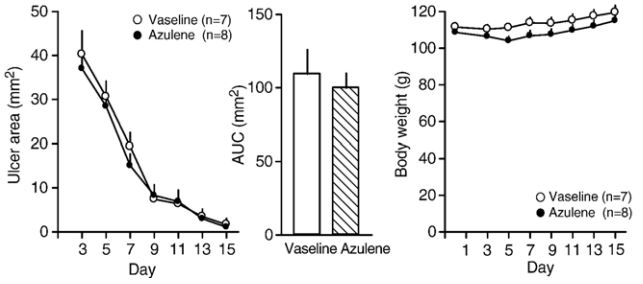

Fig. 2. Changes in ulcer area and body weight in hamsters injected with 5-fluorouracil and the effect of azulene. Vaseline ($n=7$) or 1% azulene ointment ($n=8$) was administered topically to the oral mucosa of hamsters. Each point represents the mean±S.E.M. The bar chart represents the AUC between day 3 and day 15 for each group.

clinically, we investigated the effects of topical treatments with azulene, dexamethasone and a polaprezinc–sodium alginate suspension in a hamster model for oral mucositis induced by 5-fluorouracil.

2. Materials and methods

2.1. Animals

Seven-week-old Golden Syrian hamsters (Japan SLC, Inc, Shizuoka, Japan) weighing 90–120 g were used in all experiments. All animals were housed in a room maintained at 22±2 °C under a 12/12 h light/dark cycle with lights on at 07:00 a.m. They were fed with a standard rodent diet and water *ad libitum*. The experimental protocol was conducted according to the Guidelines of the Ethics Review Committee for Animal Experimentation of Ehime University Medical School.

2.2. Drugs

The following drugs were used: 5-fluorouracil injection (Kyowa-hakko Co., Ltd. Tokyo, Japan), 0.12% dexamethasone ointment (Voalla ointment; Maruho Co., Ltd. Osaka, Japan), vaseline (White vaseline; Merck Hoei Ltd. Osaka, Japan), sodium alginate (Alloid G; Kaigen. Co., Ltd., Osaka, Japan), polaprezinc (15% Promac granules; Zeria Pharmaceutical Co., Ltd, Tokyo Japan), and sodium azulene sulfonate (Wako Pure Chemical Industries, Ltd., Tokyo, Japan).

Azulene ointment (1%) was prepared by mixing with vaseline. Polaprezinc–sodium alginate suspension was suspended in sodium alginate at a concentration of polaprezinc 1 g/Alloid G 3 ml. The control group was administered with saline. All of the drugs used were applied to the intraoral lesions at doses of 20 mg or 20 μl once daily.

2.3. Oral mucositis model

The hamster model for chemotherapy-induced oral mucositis was based on a modified method of Sonis et al. (1990). Hamsters received two intraperitoneal injections of 5-fluorouracil (60 mg/kg) on day 0 and day 2. To induce mucosal ulceration, hamsters were anesthetised with diethyl ether, and the left cheek pouches were everted and lightly scratched with a small wire brush on days 1 and 2. External medicines (20 mg or 20 μl) were applied to the left cheek pouch every day under anesthesia with diethyl ether. Ulcers were assessed every other day immediately prior to the application of drugs. Assessment of the hamster cheek pouch included measuring the length and width of the ulcer with calipers (mm).

2.4. Statistical analysis

All results are represented as group means and standard errors of the mean. Two-way analysis of variance (two-way ANOVA), with drug treatment as the between-subjects factor and time as the within-subject factor, was used. Whenever the drug treatment factor or the drug treatment factor×time interaction was significant, *post hoc* comparison was carried out. The *post hoc* individual comparisons were performed with Student's *t*-test. The significance level was set at $P<0.05$.

3. Results

Fig. 1 shows the development of oral mucositis induced by 5-fluorouracil in hamsters. The combination of intraperitoneal injections of 5-fluorouracil and mild abrasion of the cheek pouch caused oral mucositis on day 3. Thereafter, the observation of mucositis showed that the lesions decreased in size with time. However, a combination treatment of saline and

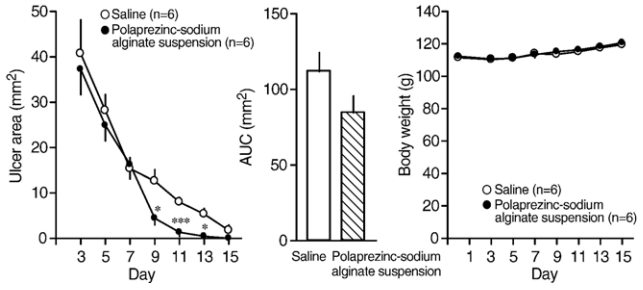

Fig. 3. Changes in ulcer area and body weight in hamsters injected with 5-fluorouracil and the effect of polaprezinc–sodium alginate suspension. Saline ($n=6$) or polaprezinc–sodium alginate suspension ($n=6$) was administered topically to the oral mucosa of hamsters. Each point represents the mean±S.E.M. The bar chart represents the AUC between day 3 and day 15 for each group. *$P<0.05$, ***$P<0.001$ (two-way ANOVA followed by Student's *t*-test).