European Review for Medical and Pharmacological Sciences 2018; 22: 7020-7023

Oral ulcer healing and anti-Candida efficacy of an alcohol-free chitosan-curcumin mouthwash

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Abstract. – OBJECTIVE: The purpose of this study was to investigate oral ulcer healing and anti-Candida efficacy of an alcohol-free 0.1% chitosan-curcumin mouthwash.

MATERIALS AND METHODS: A buccal mucosal ulcer was induced in hamster by topical application of acetic acid. The test mouthwash was applied to the ulcer twice a day for 7 consecutive days beginning on the fourth day after the ulcer induction. The anti-Candida efficacy of the mouthwash was determined against both free floating and biofilm forms of *Candida albicans*.

RESULTS: The mouthwash significantly decreased the ulcer severity with a better ulcer healing efficacy than that of a standard benzydamine mouthwash. The mouthwash also exerted a comparable anti-Candida efficacy to a standard chlorhexidine mouthwash.

CONCLUSIONS: An alcohol-free 0.1% chitosan-curcumin mouthwash may serve as a safe and potential topical alternative agent in the management of oral inflammatory ulcer and of candidiasis.

Key Words:
Curcumin, Chitosan, Oral ulcer, Anti-Candida, Mouthwash.

Introduction

Oral ulcers are one of the common complaints of patients attending outpatient clinics. Their main causes include aphthae, chemotherapeutic agents, radiation and physical trauma. The onset of the ulcer is accompanied by a leukocyte infiltration in the epithelium focal vacuolation and an up-regulation in the production of inflammatory mediators and free radicals from infiltrating leukocytes[1]. *Candida albicans* has been found to be involved in the pathogenesis of secondary candidiasis of an oral ulcer, especially in immunocompromised patients[2]. Although chlorhexidine mouthwash is commonly used in dental clinical practice due to its anti-inflammatory and anti-Candida activities, it has not been recommended for use with cancer patients[3]. Anti-inflammatory: benzydamine mouthwash (Difflam®) is generally recommended for management of chemo/radiotherapy-induced oral ulcer in a clinical setting[4]. However, it may often require an oral antifungal agent to increase the therapeutic efficacy. The alcohol content in the mouthwash formulation may also often cause irritation to the inflamed mucosa. Curcumin has been claimed to be used as a safe antioxidant, anti-inflammatory and wound healing agent[5-7]. Additionally, from our preliminary antimicrobial study, curcumin powder (with no volatile oil content) in a concentration of 0.1% w/v, displayed potent antimicrobial effect against oropharyngeal organisms. Accordingly, an alcohol-free mouthwash of curcumin (0.1%) was formulated using a co-solvent system composed of chitosan (0.5%) and polyethylene glycol 400, and determined for its therapeutic potential in the management of oral inflammatory ulcers.

Materials and Methods

In Vivo Study on Hamster

24 male and female hamsters weighing 80-100 g were housed under normal laboratory condi-

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tions at 25 ± 1°C with a controlled 12-h light-dark cycle and maintained on standard rodent chow and tap water *ad libitum*. The experimental procedure was approved by the Committee on Animal Care and in accordance with the Guiding Principles for the Care and Use of Research Animal established by Prince of Songkla University (MOE 0521.11/283). The animals were divided into 4 groups of 6 each: Group 1, water control; Group 2, 0.15% benzydamine mouthwash (Difflam®) (positive control); Group 3, a formulation with chitosan only (vehicle control) and Group 4, 0.1% chitosan-curcumin mouthwash. Under anesthesia, a round filter paper of 5 mm in diameter was soaked in 15 µl of 99.7% acetic acid and then pressed onto the left site buccal mucosal of hamster for 60 s[8]. Each test compound was applied twice daily for 7 consecutive days beginning on the 4th day after the ulcer induction. Complete ulcer healing on the specimen obtained on day 12 was observed histologically using the histological scoring protocol[8] according to the following criteria: (1) presence of epithelial necrosis without signs of inflammation; (2) presence of inflammatory reaction but no appearance of angiogenesis; (3) presence of inflammatory reaction with new capillary proliferations at the ulcer base, but no epithelization at the ulcer surface; (4) decrease of inflammatory reaction, presence of new capillary proliferation and the beginning of epithelization at the ulcer surface; (5) presence of complete epithelization at the ulcer surface.

In Vitro Anti-Candida Study

Anti-Candida Determination Against Free-Floating C. albicans

A stock solution of curcumin (5,000 µg/ml) was prepared in dimethyl sulfoxide (DMSO) and two-fold serial dilutions were done in 50°C of trypticase soy agar (TSA) to achieve a final concentration of 250-1 µg/ml. Chlorhexidine solution was used as a positive control. The suspension of *C. albicans* ATCC 10231 approximately 10^7 cfu/ml was inoculated on the agar surface of TSA with a loop calibrated to deliver 1 µl. The plate was incubated at 25°C for 48 h.

Anti-Candida Determination Against C. albicans In Biofilm

A modified methodology of a previously published bioprosthetic biofilm colonization model[9] was used. As a surface for biofilm adherence, the discs were soaked in artificial saliva without enzymes (Thailand Hospital Formulary), incubated overnight at 37°C and then placed into 50 ml tubes (6-7 pieces/tube) covered with the inoculum of 10^8 cfu/ml (10 ml). After incubation overnight at 37°C, the inoculated broth was removed and 10 ml of a 0.9% saline solution was added and incubated for another 30 min. The discs were then removed and placed into separate clean tubes containing 0.1% chitosan-curcumin mouthwash as well as 0.2% w/v chlorhexidine (positive control), blank formulation (negative control), formulation with chitosan only (vehicle control) and formulation with curcumin only. After soaking for 10 min in the shaker incubator at 37°C, the test solution was removed and the discs were transferred into the tubes, each containing 5 ml of 0.9% saline. 100 µl of the saline solution was pipetted from each tube, spread on sabouraud dextrose agar plates and incubated overnight at 37°C. Colonies in each plate were then counted.

Statistical Analysis

Comparisons between groups were made by one-way analysis of variance (ANOVA) followed by the Dunnett's test. Values of $p<0.05$ were considered to be statistically significant**.**

Results and Discussion

An acetic acid-induced buccal mucosal ulcer model is commonly used in animal studies of ulcers as its histopathological characteristics resemble clinical oral ulcer in term of both pathological features and healing process. It was found that repeated application of 0.1% chitosan-curcumin mouthwash significantly decreased ulcer severity and was more effective in accelerating the ulcer healing than either the formulation with chitosan only or benzydamine mouthwash (Table I). The histological evaluation found that the mouthwash decreased the infiltration of inflammatory leukocytes and enhanced the degree of fibrosis and regeneration of epithelial cell (Figure 1). The healing and prevented progression of the acetic acid-induced oral ulcer may be attributed to the ability of curcumin to modulate collagen and decrease the production of reactive oxygen species and pro-inflammatory mediators: tumor necrosis factor-α, nitric oxide and prostaglandins[7,10]. The addition of chitosan caused the curcumin to have comparable anti-Candida efficacy to a standard

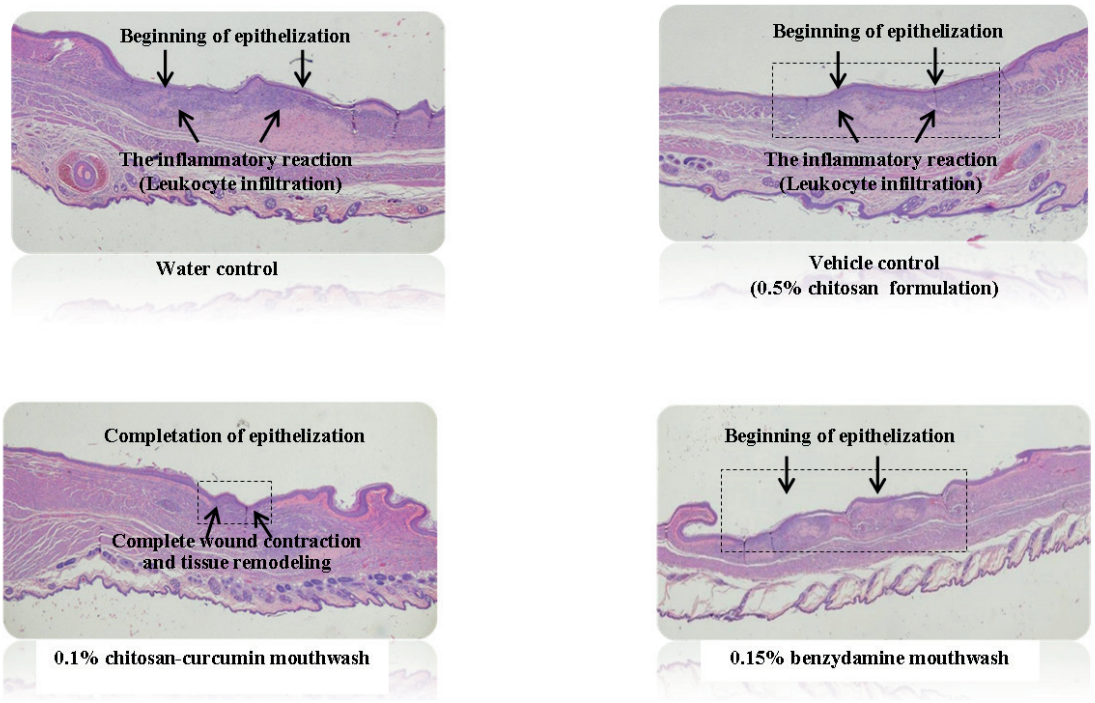

Figure 1. The samples of the histological presentation of the oral ulcer area in each treatment group on day 12 after the ulcer induction (the square show size of wound area).

Table I. Effect of 0.1% chitosan-curcumin mouthwash on buccal mucosal ulcer induced by topical application of acetic acid in hamster.

Samples	Ulcer area (mm^2) (mean±SEM)		Histological healing scores (mean±SEM)
	Day 4	Day 12	
Water control	51.43±0.64	4.61±0.58	3.75±0.25
Vehicle control (0.5% chitosan without curcumin)	54.67±4.92	2.71±0.25	3.83±0.17
0.1% chitosan-curcumin mouthwash	50.14±0.89	1.20±0.05	4.80±0.20*
0.15% benzydamine (Difflam®) mouthwash	54.85±1.36	4.52±0.54	3.67±0.33

Values represent the mean±SEM obtained with 6 animals in each group. *p<0.05 compared with those of the water control, vehicle control and Difflam® solution (Dunnett's test). Histological healing scores determined as described in the Materials and Methods section.

Table II. Average amount of *C. albicans* after mixing with 0.1% chitosan-curcumin mouthwash for 10 minutes.

Formulations	Average *C. albicans* number (cfu/ml)	
	Suspension	Biofilm
Mouthwash vehicle	7.0×10^5	7.0×10^3
0.1% w/v curcumin mouthwash	0	2.8×10^3
0.5% w/v chitosan mouthwash	1.1×10^5	0
0.1% w/v curcumin and 0.5% w/v chitosan mouthwash	0	0
0.2% w/v chlorhexidine mouthwash	0	0
0.9% normal saline solution	9.5×10^5	6.9×10^3

0.2% chlorhexidine mouthwash in complete eradication of both free-floating form and biofilms of *C. albicans* (Table II). The obtained results indicated the potential for further clinical investigation of this alcohol-free chitosan-curcumin mouthwash for oral care of cancer and immunocompromised patients.

Conclusions

We found that an alcohol-free 0.1% chitosan-curcumin mouthwash may serve as a safe and potential topical alternative agent in the management of oral inflammatory ulcer and candidiasis.

Acknowledgements

This work was supported by a grant from Prince of Songkla University. Special thanks to Mrs. Maria Mullet for assistance with the English.

Conflict of Interest

The authors declare that they have no conflict of interest.

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