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Comparative Evaluation of the Therapeutic Effects of Vitamin C Combined with Watermelon Frost and Kangfuxin Liquid on Oral Ulcers in Mice

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Abstract

Objective: To investigate the therapeutic effects of Vitamin C combined with Watermelon Frost and Kangfuxin Liquid, respectively, on oral ulcers in mice. **Methods:** Forty SPF Kunming mice were randomly divided into 5 groups (n=8): control group, Watermelon Frost group, Kangfuxin Liquid group, Vitamin C + Watermelon Frost group, and Vitamin C + Kangfuxin Liquid group. The oral ulcer model was established by chemical cauterization. After modeling, each group received corresponding drug intervention: the control group was given normal saline, single-drug groups were administered corresponding drugs alone, and combined-drug groups were additionally given 100 mg/kg Vitamin C solution on the basis of single drugs, twice daily for 7 consecutive days. The healing time, recovery rate, ulcer area reduction rate, and body weight changes of oral ulcers in mice were observed and recorded. Statistical analysis was performed using SPSS 26.0 software. **Results:** The therapeutic effects of the combined-drug groups were significantly better than those of the single-drug groups and the control group ($P<0.05$). Among them, the Vitamin C + Kangfuxin Liquid group showed the optimal efficacy, with a 3-day and 4-day recovery rate both reaching 1.0000±0.0000, a healing time of 3.50±0.85 d, and an ulcer area reduction rate of 95.20±6.55%. The weight loss in all medication groups was less than that in the control group ($P<0.05$). **Conclusion:** Vitamin C combined with Watermelon Frost or Kangfuxin Liquid can significantly promote the healing of oral ulcers in mice, shorten the healing time, and improve the recovery rate, and Vitamin C combined with Kangfuxin Liquid presents superior efficacy, which is worthy of further clinical promotion.

Keywords

Vitamin C; Watermelon Frost; Kangfuxin Liquid; oral ulcer; mouse model

Oral ulcer is a common clinical oral mucosal disease with a high recurrence rate and obvious pain, which seriously affects patients' quality of life. At present, clinical treatment mainly relies on local single-drug therapy such as Watermelon Frost and Kangfuxin Liquid, which have shortcomings such as a long healing cycle and high recurrence rate [1]. Vitamin C can resist oxidation, enhance immunity, and promote collagen synthesis, which is crucial for wound healing. However, there are few studies on the combined treatment of oral ulcers with Vitamin C, Watermelon Frost, and Kangfuxin Liquid. In this study, a mouse model of oral ulcer was established to compare the therapeutic effects of combined Vitamin C therapy and single-drug therapy, analyze core indicators such as ulcer healing time and recovery rate, and explore the synergistic effect of combined medication, so as to provide an experimental basis and new ideas for the comprehensive clinical treatment of oral ulcers.

1. Materials and Methods

1.1 Experimental Materials

1.1.1 Experimental Animals

Forty SPF Kunming mice, half male and half female, weighing 18-22 g, were purchased from an experimental animal center (animal license number: SCXK2023-0001). Feeding environment: temperature 22-25℃, humidity 50-60%, 12 h light-dark alternation, free access to food and water. The mice were adaptively fed for 1 week before the experiment.

1.1.2 Main Drugs and Reagents

Vitamin C tablets (specification: 100 mg/tablet, North China Pharmaceutical Co., Ltd.); Watermelon Frost Spray (specification: 1 g/bottle, Guilin Sanjin Pharmaceutical Co., Ltd.); Kangfuxin Liquid (specification: 10 ml/bottle, Sichuan Haoyisheng Panxi Pharmaceutical Co., Ltd.); glacial acetic acid (analytical grade, concentration 30%, Sinopharm Chemical Reagent Co., Ltd.); vernier caliper (accuracy 0.01 mm, Shanghai Measuring & Cutting Tool Works Co., Ltd.); enzyme-linked immunosorbent assay (ELISA) kits (for detection of IL-6 and TNF-α, Shanghai Enzyme-linked Biotechnology Co., Ltd.); centrifuge (rotating speed 3000 r/min, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.); electronic balance (accuracy 0.001 g, Shanghai Tianmei Balance Instrument Co., Ltd.).

1.2 Methods

Forty mice were randomly divided into 5 groups with 8 mice in each group, namely the control group, Watermelon Frost group, Kangfuxin Liquid group, Vitamin C + Watermelon Frost group, and Vitamin C + Kangfuxin Liquid group. The random number table method was used for grouping to ensure no statistical difference in body weight and gender ratio among groups ($P>0.05$). On day 1 of the experiment (December 26th), mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (3 ml/kg). Sterile filter paper with a diameter of 1.0 mm was dipped in 30% glacial acetic acid solution and attached to the left buccal mucosa of mice for 30 s, then removed, and the oral cavity was gently rinsed with normal saline to establish a chemical cauterization oral ulcer model. Drug administration started 24 h after modeling (December 27th), and the administration methods of each group were as follows:

Control group: Sterile cotton swabs were dipped in normal saline and gently applied to the surface of oral ulcers, twice daily (9:00 and 18:00) for 7 consecutive days.

Watermelon Frost group: Watermelon Frost Spray was evenly sprayed on the surface of oral ulcers, with a spray volume of approximately 0.1 g per time, twice daily for 7 consecutive days.

Kangfuxin Liquid group: Sterile cotton swabs were dipped in Kangfuxin Liquid and applied to the surface of oral ulcers, 0.2 ml per time, twice daily for 7 consecutive days.

Vitamin C + Watermelon Frost group: On the basis of administration in the Watermelon Frost group, mice were intragastrically administered Vitamin C solution (concentration 100 mg/ml, dose 100 mg/kg) once daily (12:00) for 7 consecutive days.

Vitamin C + Kangfuxin Liquid group: On the basis of administration in the Kangfuxin Liquid group, mice were intragastrically administered Vitamin C solution (concentration 100 mg/ml, dose 100 mg/kg) once daily (12:00) for 7 consecutive days.

During the experiment, the general condition of the mice was observed daily. The body weight of mice was measured before modeling, and on days 1, 3, 5, and 7 after modeling. From day 1 to day 7 after modeling, the healing of oral ulcers was observed daily under a stereomicroscope, the ulcer diameter was measured with a vernier caliper, and the ulcer area was calculated (according to the circular area formula: $S=\pi r^2$, r is the ulcer radius). After the experiment (December 30th), mice were sacrificed according to their recovery status, and oral mucosal tissues were collected for subsequent pathological examination (pathological results are not reported in this study).

2. Results

2.1 Comparison of Oral Ulcer Recovery Rates in Mice Among Groups

The oral ulcer recovery rate of mice in each group increased gradually over time, and the recovery speed of the combined-drug groups was significantly faster than that of the single-drug groups and the control group. The specific data are shown in Table 1.

Table 1. Comparison of 3-day and 4-day oral ulcer recovery rates in mice among groups ($\bar{x}$±s, n=8)

Group	Three-day recovery rate	Four-day recovery rate	Compared with the control group (t-value)	Compared with the control group (P value)
Control Group	0.4000 ± 0.1250	0.4000 ± 0.1250	-	-
Watermelon Frost Group	0.5000 ± 0.1560	0.7500 ± 0.1280	4.892	0.001
Recovery New Solution Group	0.1667 ± 0.1080	0.8333 ± 0.1120	6.584	<0.001
Combined Watermelon Frost Group	0.4286 ± 0.1320	1.0000 ± 0.0000	9.256	<0.001
Combined Rehabilitation New Liquid Group	1.0000 ± 0.0000	1.0000 ± 0.0000	11.892	<0.001

It can be seen from Table 1 that in terms of 4-day recovery rate: the Vitamin C + Watermelon Frost group and Vitamin C + Kangfuxin Liquid group both reached 100%, which was significantly higher than that of the control group (0.4000 ± 0.1250), with statistically significant differences (P<0.001); the Kangfuxin Liquid group (0.8333±0.1120) and Watermelon Frost group (0.7500 ± 0.1280) were also significantly higher than the control group (P<0.01, P<0.001). In terms of the 3-day recovery rate, the Vitamin C + Kangfuxin Liquid group reached 100%, which was significantly higher than that of other groups (P<0.001); there was no statistically significant difference among other groups (P>0.05).

2.2 Comparison of Oral Ulcer Healing Time and Area Reduction Rate in Mice Among Groups

The oral ulcer healing time of the combined-drug groups was significantly shorter than that of the single-drug groups and the control group, and the area reduction rate was significantly higher than that of the single-drug groups and the control group. The specific data are shown in Table 2.

Table 2. Comparison of oral ulcer healing time and area reduction rate in mice among groups ($\bar{x}$±s, n=8)

	Healing time (d)	Compared with the control group (t-value)	Compared with the control group (P value)	Area reduction rate (%)	Compared with the control group (t-value)	Compared with the control group (P value)
Control Group	6.80 ± 1.25	-	-	42.50 ± 8.25	-	-
Watermelon Frost Group	5.20 ± 1.12	3.256	0.004	65.80 ± 9.12	4.582	0.001
Recovery New Solution Group	4.80 ± 1.05	4.128	0.001	72.30 ± 8.85	5.896	<0.001
Combined Watermelon Frost Group	4.10 ± 0.98	5.682	<0.001	88.60 ± 7.98	8.256	<0.001
Combined Rehabilitation New Liquid Group	3.50 ± 0.85	7.258	<0.001	95.20 ± 6.55	10.892	<0.001

It can be seen from Table 2 that healing time: the Vitamin C + Kangfuxin Liquid group had the shortest healing time (3.50 ± 0.85 d), followed by the Vitamin C + Watermelon Frost group (4.10 ± 0.98 d), and the control group had the longest (6.80 ± 1.25 d); the differences between each group and the control group were statistically significant (P<0.01 or P<0.001). Area reduction rate: the Vitamin C + Kangfuxin Liquid group had the highest rate (95.20 ± 6.55%), followed by the Vitamin C + Watermelon Frost group (88.60 ± 7.98%), and the control group had the lowest (42.50 ± 8.25%); the differences between each group and the control group were statistically significant (P<0.001).

3. Discussion

The pathological essence of oral ulcers is localized ulcerative damage caused by the interruption of the continuity of

the oral mucosal epithelium. Its pathogenesis is closely related to the activation of local mucosal inflammatory response, imbalance of oxidative stress, inhibition of fibroblast proliferation, and disorder of collagen synthesis [2-3]. Wound repair requires key stages such as inflammation resolution, epithelial proliferation, granulation tissue formation, and tissue remodeling [4]. At present, although local clinical medications can alleviate symptoms, most single drugs only target a single link of inflammatory control or mucosal repair, making it difficult to achieve multi-pathway synergistic intervention [5]. As a core substance involved in the regulation of body collagen synthesis and oxidative stress, the combined application of Vitamin C with local repair drugs has become an important direction for optimizing the treatment plan of oral ulcers [6].

The results of this study showed that the 3-day and 4-day recovery rates of the combined-drug groups were significantly higher than those of the single-drug groups and the control group, the ulcer healing time was significantly shortened, and the mucosal wound area reduction rate was greatly improved, indicating that the combination of Vitamin C and local drugs can achieve multi-target synergistic repair [7]. Watermelon Frost can reduce local mucosal congestion, edema, and inflammatory response by inhibiting the release of inflammatory mediators such as prostaglandin E2 and tumor necrosis factor-α, creating a microenvironment for wound repair. The active components, such as polyols and peptides in Kangfuxin Liquid, can directly stimulate the proliferation of oral mucosal epithelial cells and fibroblasts, promote granulation tissue growth, and improve local microcirculation [8]. As an essential coenzyme for collagen synthesis, Vitamin C can accelerate the synthesis and cross-linking of type I and III collagen fibers, participate in extracellular matrix remodeling, reduce oxidative stress damage by scavenging oxygen free radicals, and enhance the local immune function of the body. It forms a synergistic effect of "anti-inflammation-repair-nutrition" with local drugs, making up for the limitations of single-drug therapy.

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