CLINICAL RESEARCH

Effects of Kangfuxin Solution (康复新液) Combined with Low-level Laser on Cytokines and Immune Function in Patients with Radiation-induced Oral Mucositis

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ABSTRACT **Objective:** To investigate the effect of Kangfuxin Solution (康复新液) combined with low-level laser therapy (LLLT) on cytokines and immune function in patients with radiation-induced oral mucositis. **Methods:** There were 84 nasopharyngeal carcinoma patients with oral mucositis after intensity-modulated radiation therapy (IMRT) were randomly divided into the control group (CG) and the observation group (OG), with 42 cases in each group. The CG was given LLLT, and the OG was treated with Kangfuxin Solution in addition to LLLT for 10 consecutive days. The healing time of oral mucosa, mucositis grading, oral pain scores, cytokines (interleukin-6, interleukin-1β and tumor necrosis factor-α) and T lymphocyte subsets were compared between the 2 groups before and after treatment. **Results:** The healing time of oral mucosa in the OG was (6.8±1.4) d, which was significantly shorter than (8.6±1.9) d in the CG (t=4.943, P<0.01). After treatment, the grading of oral mucositis in the OG was better than that in the CG, with a statistically significant difference (Z=2.942, P<0.05). The oral pain scores of the OG was lower than that in the CG at different time points after treatment, and the difference was statistically significant (t=8.207, 11.017, P<0.01). After treatment, the levels of IL-6, IL-1β and TNF-α in peripheral blood of the OG were significantly lower than those in the CG (t=5.217, 2.775, 4.053, P<0.01). There were statistically significant differences in $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ between the OG and the CG after treatment (t=5.692, 6.093, 3.658, P<0.01). **Conclusion:** Kangfuxin Solution combined with LLLT can significantly shorten the healing time of oral mucosal, reduce the grading of oral mucositis, relieve oral pain, reduce inflammatory response and improve the immune function of patients.

KEYWORDS Radiation-induced oral mucositis; Nasopharyngeal carcinoma; Kangfuxin Solution; Low-level laser therapy; Cytokines; Immune function

In the era of intensity-modulated radiotherapy, the five-year local control rate of non-metastatic nasopharyngeal carcinoma was 86.0%-91.8%, and the 5-year overall survival rate was 77.1%-84.7%, and alleviating the sequelae of radiotherapy and improving the quality of life became the key to the management of nasopharyngeal carcinoma.[1] Radiation-induced oral mucositis (RIOM) is the main dose-limiting toxic reaction of intensity-modulated radiotherapy for nasopharyngeal carcinoma, which can cause interruption of radiotherapy, change of fractional dose, and reduce the efficacy of radiotherapy. The associated pain, dry mouth, loss of taste, and difficulty in eating seriously affect the quality of life of patients, prolong hospital stays, increase economic burden, and even lead to imbalance of water and electrolyte balance, threatening patients' lives.[2]

At present, there is still no specific drug for the prevention and treatment of RIOM, and clinical treatment with anti-infection, analgesia, nutritional support and other treatments aims to reduce symptoms and reduce the occurrence of complications.[3-4] Kangfuxin Solution (康复新液) is an ethanol extract of American cockroach, which has

the effects of eliminating edema, anti-infection, and repairing ulcers, and is often used in the prevention and treatment of radioactive oral mucositis in recent years.[5-7] Low-energy lasers are recommended by MASCC/ISOO for the treatment of radiation oral mucositis by regulating the production of reactive oxygen species and inflammatory factors.[8-10] At present, there is little literature on the treatment of radiation oral mucositis with rehabilitation liquid combined with low-energy laser. This study aimed to evaluate the efficacy of rehabilitation solution combined with low-energy laser on oral mucositis after intensity-modulated radiotherapy for nasopharyngeal carcinoma, and to explore the effects on inflammatory factors and T lymphocyte subsets.

DATA AND METHODS

RIOM Diagnosis and Grading Criteria

The diagnostic criteria refers to the "Diagnostic Criteria for Radiation Stomatitis" issued by the National Health Commission (GBZ162-2004).[11] Grading is based on the grading standards for acute radiation mucositis of the Radiotherapy Oncology Group (RTOG) in the United States. Grade 1: mucosal hyperemia, no pain or mild pain, no need for analgesics. Grade 2: flaky mucositis, mild to moderate pain, or secretion of serum/hemato inflammatory discharge, requiring analgesics. Grade 3: confluent fibromucositis, severe pain, requiring analgesics; Grade 4: mucosal ulceration, bleeding and even necrosis.

General Information

With the approval of the ethics committee of Huangshi Central Hospital of EDong Healthcare, 84 patients with nasopharyngeal cancer who developed oral mucositis after the end of intensity-modulated radiotherapy in Huangshi Central Hospital of EDong Healthcare from January 2018 to January 2021 were selected as research subjects, and were divided into the control group and the observation group according to the random number table method, with 42 cases in each group. The age in the control group was 42-70 years old, with an average (49.26 ± 12.48) years old, 32 cases of males and 10 cases of females, and the clinical stage of the eighth edition of nasopharyngeal carcinoma:[12] 6 cases of stage II., 22 cases of stage III., 14 cases of stage IV.A, 18 cases of oral mucositis grade 2, 18 cases of grade 3, and 6 cases of grade 4. The age in the observation group was 38-69 years old, with an average (48.45 ± 10.65) years old, 30 males and 12 females. Clinical stage: 7 cases of stage II., 25 cases of stage III., 10 cases of stage IV.A, 16 cases of oral mucositis grade 2, 22 cases of grade 3, and 4 cases of grade 4. There was no statistical difference in the general data of the 2 groups (P>0.05), which was comparable. All participants volunteered to participate in the study and signed an informed consent form.

Inclusion Criteria: (1) Met the diagnostic criteria of RTOM and the grading criteria of grade 2-4 mucositis. (2) Nasopharyngeal tissue biopsy under nasal endoscopy was clearly diagnosed as nasopharyngeal carcinoma. (3) Exclude distant transfers. (4) Newly treated patients. (5) Receiving radical radiotherapy. (6) Voluntarily participate in this trial and sign the informed consent form.

Exclusion Criteria: (1) Patients with recurrent or residual nasopharyngeal carcinoma. (2) Pregnant and lactating women. (3) Oral mucosal lesions before radiotherapy. (4) Those who are allergic to the test drug. (5) Serious heart, liver, kidney, hematopoietic system and nervous system diseases. (6) Those who have participated in clinical trials of other drugs within 6 months. (7) There are other researchers who believe that they are not suitable to participate in this trial.

Interventions

All patients received routine care such as hygiene education, oral hygiene, toothbrushing with soft bristles and fluoride toothpaste, intravenous nutrition as appropriate, anti-infective therapy, and no use of glucocorticoids and analgesic drugs. The control group used HJZ-2C laser irradiator (Chengdu Guoxiong Optoelectronic Technology Co., Ltd.) for laser treatment, irradiated 1 time/d, continuous irradiation for 14 days, continuous wave, output power 300 mw, wavelength 810 nm, spot diameter 5 mm. The observation group was given rehabilitation solution more. After cleaning the mouth, take 10 mL of Kangfuxin Solution (Sichuan Good Doctor Panxi Pharmaceutical Co., Ltd., batch number: Z51021834), rinse for 5 min alternately with

Table 1. Comparison of the Grading of RTOM Before and After Treatment in the 2 Groups (*n*=42)

Group	Before treatment					After treatment					*Z* value	*P* value
	0	1	2	3	4	0	1	2	3	4		
Control group	0	0	18	18	6	16	12	8	6	0	5.989	0.000
Obserbation group	0	0	16	22	4	26	14	2	0	0	7.800	0.000
Z value			0.118					2.942				
P value			0.906					0.003				

cheek and sucking, then slowly swallow, 4 times/d (after three meals and before going to bed), fast for 30 min after swallowing, avoid gargling, and take medicine continuously for 14 days.

Observation Indicators

(1) Compare the healing time of oral mucosa in the two groups. (2) Compare the grades of oral mucositis in the two groups before and after treatment. (3) Visual analogue scoring (VAS) was used to evaluate oral pain before, during and after treatment. (4) Serum inflammatory factor detection: collect 5 mL of venous blood in the fasting state, centrifuge at low speed for 10 min, take the supernatant and store it in the -80 ℃ refrigerator for later use. The ELISA method was used to detect the levels of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in serum before and after treatment, and the ELISA kit was purchased from PeproTech in the United States. (5) T lymphocyte subset detection: the automatic flow cytometry analyzer (Navios, Coulter Company) was used to detect $CD4^+$ and $CD8^+$ before and after treatment, and calculate the $CD4^+/CD8^+$ value.

Statistical Analysis

SPSS 19.0 software was used for statistical analysis, the measurement data was expressed in $\bar{x} \pm s$, and the *t*-test was used for the comparison between groups. Count data are expressed as percentages (%), using χ^2 test or rank sum test. $P<0.05$ was statistically significant.

RESULTS

Healing Time of Oral Mucosa

The healing time of oral mucosa in the control group was (8.6±1.9) d, and the healing time of oral mucosa in the observation group was (6.8±1.4) d, and the difference between the two groups was statistically significant (t=4.943, $P<0.01$).

Classification of Oral Mucositis

Before treatment, there was no significant difference in the grade of oral mucositis between the two groups (Z=0.118, $P>0.05$), and after treatment, the grade of oral mucositis in both groups was reduced compared with that before treatment (Z=5.989, 7.800, $P<0.05$), but the grade of mucositis in the observation group was better than that in the control group, and the difference between the two groups was statistically significant (Z=2.942, $P<0.05$, Table 1).

Oral Pain Score

Before treatment, there was no significant difference in oral pain score between the two groups (t=1.088, $P>0.05$), and the oral pain score in the two groups during and after treatment was lower than that before treatment, and the oral pain score in the observation group was lower than that in the control group at the same time point, and the difference between the two groups was statistically significant (t=8.207, 11.017, $P<0.01$, Table 2).

Table 2. Comparison of VAS Scores Before and After Treatment in the 2 Groups (*n*=42, $\bar{x} \pm s$)

Group	Before treatment	Being treatment	After treatment
Control group	6.24±1.20	4.44±0.72	2.10±0.48
Observation group	5.98±0.98	3.22±0.64	1.08±0.36
t value	1.088	8.207	11.017
P value	0.280	0.000	0.000

Inflammatory Factor Levels

Before treatment, there were no significant differences in peripheral blood IL-6, IL-1β and TNF-α between the two groups (t=0.989, 0.734, 0.514, $P>0.05$). After treatment, the peripheral blood levels of IL-6, IL-1β and TNF-α in both groups were lower than those before treatment (the observation group: t=10.410, 8.069, 17.221, $P<0.01$. The control group: t=7.675, 4.570, 13.739, $P<0.01$), and the peripheral blood levels of IL-6, IL-1β and TNF-α

in the observation group after treatment were significantly lower than those in the control group (t=5.217, 2.775, 4.053, P<0.01, Table 4).

Table 4. Comparison of Serum Level of Cytokines Before and After Treatment in the 2 Groups (n=42, $\bar{x} \pm s$, mg/mL)

Group	Treatment time	IL-6	IL-1 β	TNF-α
Control group	Before treatment	28.43±4.21	46.64±9.78	52.84±8.12
	After treatment	22.92±1.98	37.52±8.46	32.49±5.12
Observation group	Before treatment	27.56±3.84	45.25±7.39	51.94±7.94
	After treatment	20.84±1.66	32.93±6.58	28.48±3.86

Note: P<0.01, compared with the same group before treatment, and P<0.01, compared with the control group after treatment.

Comparison of the Proportion of T lymphocyte Subsets

The test results of T lymphocyte subsets ($CD4^+$, $CD8^+$ and $CD4^+/CD8^+$) before and after treatment showed statistically significant differences between the 2 groups (the observation group: t=13.565, 4.911, 11.114, P<0.01. The control group: t=9.125, 10.769, 8.719, P<0.01), and the difference of $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ after treatment between the two groups was statistically significant (t=5.692, 6.093, 3.658, P<0.01, Table 5).

Table 5. Comparison of T Lymphocyte Subsets Before and After Treatment in the 2 Groups (n=42, $\bar{x} \pm s$)

Group	Treatment time	$CD4^+$/%	$CD8^+$/%	$CD4^+/CD8^+$
Control group	Before treatment	25.98±4.42	40.15±5.68	0.78±0.20
	After treatment	35.44±5.06	28.49±4.12	1.18±0.22
Observation group	Before treatment	26.75±4.52	39.94±5.32	0.79±0.18
	After treatment	42.24±5.86	34.48±4.86	1.39±0.30

Note: P<0.01, compared with the same group before treatment, and P<0.01, compared with the control group after treatment.

DISCUSSION

Nasopharyngeal carcinoma is a common malignant tumor in southern China, with the highest incidence in Guangdong Province, also known as "Guangdong cancer". The unique anatomy of the nasopharynx and the high sensitivity of nasopharyngeal carcinoma to radiation determine that radiotherapy is the main radical treatment for nasopharyngeal carcinoma. Intensity-modulated radiotherapy has the characteristics of good target conformance and protection of normal tissues, and has become the mainstream radiotherapy technique for nasopharyngeal carcinoma. In the era of intensity-modulated radiotherapy, the incidence of radiation oral mucositis is still as high as 80%, and about 56% of patients can develop grade 3-4 mucositis, which affects the implementation of radiotherapy plan and restricts the improvement of radiotherapy for nasopharyngeal carcinoma.[13-14] Although granulocyte colony-stimulating factor, keratinocytes growth factor, aluminum hydroxide gel, and cryotherapy are often used in the prevention and treatment of radioactive oral mucositis, there is still no uniform standard.[10] Therefore, the search for effective treatment methods is an urgent problem to be solved in clinical practice.

Radiation oral mucositis belongs to the categories of TCM aphthous, laryngeal paralysis, the basic pathogenesis is heat and yin, clinical yin deficiency and deficiency type, and also see heat evidence, qi stasis and other symptoms, treatment is mainly to support the right and dispel evil, invigorating qi and nourishing yin and moisturizing as the basic principle, supplemented by clearing heat, cooling blood detoxification and removing stasis.[15] Kangfuxin Solution is an ethanol extract of American cockroach, which is a TCM mucosal protectant, whose medicinal history can be traced back to the *Shennong Materia Medica* <<神农本草经>>, which has the effects of detoxification, nourishing yin and nourishing muscles, and is used to treat radiation oral mucositis in line with the pathogenesis of "heat and heat yin". Kangfuxin solution contains a variety of amino acids, polyols, epidermal growth factor, peptide active substances, mucionine, mucoprotein and other growth promoting factors, including desaprophytic muscle, promoting granulation tissue and angiogenesis, and repairing damaged mucosal tissue. Promote the deposition of collagen in the wound, increase the wound tension, promote tissue reconstruction, and promote wound healing. Oral mucosal diseases are treated by improving immunomodulatory function, anti-inflammatory, swollen, antioxidant and other

mechanisms.[16-18] Low-energy lasers, as an important local treatment, can eliminate edema, improve circulation, accelerate epithelial cell proliferation, and granulation tissue production of wounds, thereby reducing oral mucosal reactions and promoting wound healing.[8,9] Energetic lasers exert analgesic effects by inhibiting endogenous opioid release, reducing PGE2, COX-2 expression, and levels of plasminogen activators.[19] In this study, the grade of oral mucositis in both groups was lower than that before treatment (all $P<0.05$), and the grade of oral mucositis in the observation group after treatment was better than that in the control group ($P<0.05$). The oral pain score in the two groups was lower than that before treatment (both $P<0.05$), and the post-treatment in the observation group had a lower oral pain score than in the control group (both $P<0.05$). The healing time of oral mucosa in the observation group was shorter than that in the control group ($P<0.05$). It can be seen that the combination of rehabilitation solution on the basis of low-energy laser treatment can further reduce the oral mucosal reaction, reduce the oral pain score, and shorten the healing time of the oral mucosa.

The pathological process of radiation oral mucositis is extremely complex, Sonis.[20,21] divided it into five stages: initiation, injury response, signal amplification, ulceration and healing, and the inflammatory response runs throughout, and IL-6, IL-1β and TNF-α are considered to be the key factors in mediating radiation oral mucositis. When the oral mucosa is irradiated, the inflammatory signaling pathway dominated by nuclear transcription factor-κB is activated, and immune cells such as lymphocytes and macrophages are stimulated to release a large number of pro-inflammatory factors, such as IL-6, IL-1β and TNF-α, which accelerate the damage of the oral mucosa through cascade amplification.[22] Studies have shown that the levels of inflammatory factors such as IL-6, IL-1β, and TNF-α in peripheral blood correlate with the degree of damage to the oral mucosa, and inhibiting the release of these inflammatory factors can reduce mucosal reactions.[23] Patients with radiation oral mucositis often have a certain degree of immune dysfunction, manifested by a decrease in the number of $CD4^+$ T lymphocytes and an imbalance in the $CD4^+/CD8^+$ ratio, which affects the clinical outcome of patients.[24,25]

In this study, the plasma levels of IL-6, IL-1β and TNF-α in the 2 groups after treatment were lower than those in the 2 groups before treatment, and the levels of IL-6, IL-1β and TNF-α in the observation group were significantly lower than those in the control group (all $P<0.05$). After treatment, the number of $CD4^+$ T lymphocytes in the two groups was higher than that before treatment, the number of $CD8^+$ T lymphocytes decreased compared with before treatment, the proportion of $CD4^+/CD8^+$ increased compared with that before treatment, and the number of $CD4^+$ T lymphocytes and $CD4^+/CD8^+$ in the observation group after treatment were significantly higher than those in the control group, and the number of $CD8^+$ T lymphocytes was lower than that in the control group (all $P<0.05$). It can be seen that the combination of rehabilitation solution combined with low-energy laser treatment can reduce the inflammatory response of oral mucosa and improve the immune function.

In summary, on the basis of low-energy laser therapy, combined with rehabilitation new liquid for the treatment of radiation oral mucositis, it can shorten the healing time of oral mucosa, reduce the grade of oral mucositis, relieve oral pain, reduce inflammatory response, and improve the immune function of patients. The clinical efficacy needs to be expanded in prospective studies of the sample, and the specific mechanism needs to be studied in more depth.

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