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Inhibitory effect and mechanism of 'Taizi Yangrong Decoction'on oral mucositis after radiotherapy for nasopharyngeal carcinoma in vivo and in vitro

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Abstract: Objective: To explore the effect and mechanism of 'Taizi Yangrong Decoction'on the function of oxidative stress inflammatory response axis after radiotherapy for nasopharyngeal carcinoma. Methods: The rat model of radiation oral mucositis was established.The weight loss rate and behavioral score of rats in each group were observed in vitro;HE staining and immunohistochemistry were observed at the tissue level; At the cell level, the expression changes of oxidative stress and inflammatory factors were observed by ELISA. Results: Compared with the control group, the low-dose and high-dose groups of 'Taizi Yangrong Decoction' can improve the weight loss rate and behavior score of rats,and the HE staining and immunohistochemical score decreased significantly.Further in vivo experiments showed that after the intervention of 'Taizi Yangrong Decoction', the expression level of oxidative stress related indexes decreased significantly; Similarly,inflammatory factor related indexes the expression level also decreased significantly,and its expression level was closely related to the dosage. Conclusion'Taizi Yangrong Decoction' can significantly improve the local mucosal injury and systemic inflammatory response of rats after nasopharyngeal radiotherapy,and the effect of high dose group is the most significant.

Keywords: Nasopharyngeal carcinoma; Radiotherapy; Taizi Yangrong Decoction; Oxidative stress; Inflammatory reaction

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1.Introduction

Radioactive oral mucositis is the most common adverse reaction of radiotherapy for nasopharyngeal carcinoma, which is mainly manifested as oral mucositis, oral ulcer,local pain,local infection,eating difficulties and malnutrition,and is also the main limiting factor of radiotherapy for nasopharyngeal carcinoma [1]. At present, it is believed that the pathogenesis of radiation oral mucositis is based on the signal pathway of oxidative stress inflammatory factors,which is also different from other general inflammatory reactions [2]. Research shows that after radiation acts on oral mucosa tissue, it first triggers oxidative stress reaction[3],and then further activates inflammatory factors, finally leading to oral mucosa damage and inflammation [4].

According to the dialectical view of traditional Chinese medicine, nasopharyngeal carcinoma is a 'loss of honor', and radiation belongs to heat poison. Radiation is actually the evil of fire poison.The evil of fire poison is straight in the head, and the heat poison burns the tongue, meridians, qi, and blood, causing dysfunction. It hurts the qi component. Fire is a yang evil,and it strengthens the fire and eats qi. It is most likely to consume the qi and yin of the human body. The main causes and pathogenesis of this disease are the accumulation of heat toxin,yin deficiency,internal heat,and qi and yin deficiency [5~6]. Therefore, traditional Chinese medicine is used to prevent and treat the adverse reactions of radiotherapy, mainly in accordance with the principles of nourishing yin and yang, clearing heat and detoxifying, promoting blood circulation and cooling blood. In addition, the Yellow Emperor's Canon of Internal Medicine for the first time proposed the idea of 'treating diseases before they occur', that is, to give preventive treatment at the stage of disease occurrence and development, in order to reduce the risk of disease occurrence and the degree of harm at an early stage [7]. Through long-term front-line clinical work and summing up experience, our research group has made 'Taizi Yangrong Decoction'(which has been applied for national invention patent protection),playing the role of promoting fluid production, clearing heat and detoxification.

The purpose of this study was to explore the role and mechanism of the signal pathway based on oxidative stress inflammatory factors in radiotherapy related oral mucositis of nasopharyngeal carcinoma, and to observe the intervention effect of 'Taizi Yangrong Decoction',so as to provide basis for improving the tolerance and therapeutic effect of radiotherapy for nasopharyngeal carcinoma.

2. Materials

2.1 Animals

60 SPF female Kunming rats, 8 weeks old, with an

average weight of 21.3±2.1g, were provided by the Central Laboratory of the Affiliated Hospital of Qingdao University, and were raised in the SPF mouse feeding room of the laboratory. The certificate number is SDGP370000201902003609. Feeding conditions: room temperature 22±2℃, humidity 55±5%, 12h light/12h dark cycle environment, free diet. This experiment strictly follows the requirements of the Regulations on the Administration of Laboratory Animals (Ministry of Science and Technology of the People's Republic of China) and the Ethics Committee of the Affiliated Hospital of Qingdao University.

2.2 Drugs

The composition of 'Taizi Yangrong Decoction' is shown in Table 1.

Table 1. Composition of soup'Taizi Yangrong Decoction'

Component	Dose	Supplier
Radix Pseudostellariae	30g	The Affiliated Hospital of Qingdao University
Astragali	30g	The Affiliated Hospital of Qingdao University
Ophiopogon japonicus	12g	The Affiliated Hospital of Qingdao University
Polygonatum odoratum	12g	The Affiliated Hospital of Qingdao University
Ligusticum ligusticum	12g	The Affiliated Hospital of Qingdao University
Poria cocos peel	15g	The Affiliated Hospital of Qingdao University
Radix Angelicae	6g	The Affiliated Hospital of Qingdao University
Scutellaria baicalensis	12g	The Affiliated Hospital of Qingdao University
Licorice root	3g	The Affiliated Hospital of Qingdao University
Dexamethasone sodium phosphate injection	1ml:5mg	Zhengda Tianqing Pharmaceutical Group Co., Ltd.

Note:After decoction, filter the liquid medicine, dissolve it in purified water, and adjust the concentration to 2g/L.

2.3 Main reagents

Chloral hydrate (100ml, LZ-13621, Shanghai Lianzu Biotechnology Co., Ltd.), Ehong (100ml, KFS120, Beijing Biotech Co.,Ltd.), acetone (100g, 30169134, Tianjin Kemio Biotechnology Co., Ltd.), HE staining (500ml, G1005, servicebio), NF-κB p65 immunohistochemistry kit (100T,IH-13669R, Shanghai Yaji Biological Co., Ltd.), Trizol Agent (100ml, s00099, inhibitor), TGF- β ELISA kit (48T/96T, ZK-H042, Sigma), IL-6 ELISA kit (48T/96T, XG-P63231, Sigma), IL-10 ELISA kit (48T/96T, SJ191c2853634, Sigma), NrF-2 ELISA kit (50T/24S, AKPR014C, Beijing Boxing Biotechnology Co.,Ltd.), HO-1 ELISA kit (100T/48S, BA1068, Shanghai Shangbao Biological Co., Ltd.) NQO-1 ELISA kit (110T/100S, AKFA013M, Beijing Boxing Biotechnology Co., Ltd.).

2.4 Main instruments

SW-CJ-1F biological purification workbench (Suzhou Purification Equipment Factory, China), binocular optical microscope (OLYMPUS, Japan), TB-718 biological tissue embedding machine (Hubei Taiwei Technology Co., Ltd.,China), HM-325 paraffin microtome (Microm,Germany), GWGPSO remote radiotherapy machine (Nuclear Power Research and Design Institute, China).

3. Method

3.1 Preparation of rat model of radiation-induc

After adaptive feeding for 1week, 8-week old rats were randomly divided into control group, low-dose group and high-dose group, 20 rats in each group, and labeled. After the rats were anesthetized with 10%chloral hydrate, they were taken to the supine position, fixed

on the treatment bed, shielded the chest and abdomen, and irradiated after fully exposing the head. Exposure conditions: head, 3×1.5cm, 36MV photons, 3Gy/time, once a day, 20times in total. By observing the changes of oral mucositis, it was determined that the model was successful.

3.2 Grouping and administration

The control group (purified water), low-dose group (Taizi Yangrong Tang, 100mg/kg), and high-dose group (Taizi Yangrong Tang, 200mg/kg) all started to gavage corresponding drugs on the first day of irradiation until the end of irradiation.

3.3 General status

(1) Weight loss rate: from the first day of irradiation,10 rats in each group were randomly selected for weight measurement every week, and their average values were taken to observe the weight change and nutritional status until the end of irradiation.

(2) Behavioral score: From the first day of irradiation, 10rats in each group were randomly selected every week for behavioral score. The scoring criteria: 0 for asymptomatic, 1 for scratching, 2for local redness and swelling, 3 for dysphagia, 4 for malaise, and 5 for death. Finally, the average score was taken until the end of irradiation.

3.4 Sample

(1) Tissue specimen: Before and after irradiation, oral mucosa of rats in each group was taken, labeled and preserved for staining and immunohistochemistry.

(2) Blood sample: from the first day of irradiation, 5rats in each group were randomly selected every week, peripheral blood was drawn through the tail vein, and plasma was separated, labeled and stored for testing.

3.5 HE staining

Take the oral mucosa of rats for HE staining and observe under the light microscope (x100) and scoring. Scoring criteria: no inflammatory exudation 1 point, inflammatory cell infiltration area less than 20% of the full field 2 points, inflammatory cell infiltration area accounting for 20~50% of the full field 3 points, inflammatory cell infiltration area greater than 50% of the full field 4 points.

3.6 Immunohistochemical test

Take the oral mucosa tissue of rats after irradiation, and conduct NF through the test kit- κB p65 was detected by immunohistochemistry to observe its expression in tissues. The scoring method was the same as that of HE staining.

3.7 Detection of oxidative stress related indicators in oral mucosa by ELISA

Oral mucosa tissues of rats in each group were taken and homogenized with glass homogenizer.The content of glutathione peroxidase (GSH-PX) in the homogenate was detected by chemical colorimetry;The contents of superoxide dismutase (SOD) and malondialdehyde (MDA) in oral mucosa of rats in each group were detected by xanthine oxidase method and thiobarbituric acid colorimetry. The expression of NrF-2, HO-1, NQO-1, which constitute the NrF2-ARE signal pathway, in the oral mucosa of rats in each group was detected by ELISA to understand the changes of oxidative stress indicators before and after irradiation and the intervention effect of 'Taizi Yangrong Decoction'.

3.8 Detection of inflammatory factor related indexes in plasma by ELISA

Detection of TGF-βin plasma of rats in each group by ELISA,the expression of IL-6 and IL-10 in order to understand the expression changes of inflammatory factor indexes before and after irradiation and the intervention effect of 'Taizi Yangrong Decoction'.

3.9 Statistical methods

Statistical analysis SPSS statistical software was used to analyze the data. The measurement data were expressed as mean±standard deviation, and one-way ANOVA was used. LSD-t test was used to compare the two. The difference was statistically significant with $P<0.05$.

4. Result

4.1 General state of rats

(1) Effect of 'Taizi Yangrong Decoction' on the rate of weight loss

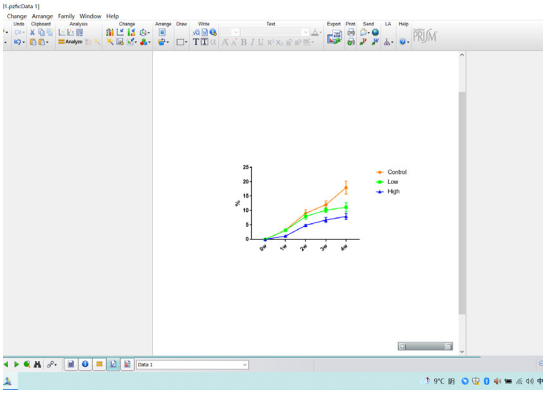

Figure 1. Change of weight loss rate (%) of rats in each group

As shown in Figure 1,with the irradiation time dose,the rate of weight loss of rats in each group gradually increased,and the two were positively

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correlated,with the highest in the control group and the lowest in the high-dose group.After irradiation, the difference between low-dose group and high-dose group was statistically significant compared with the control group (P<0.05, t was 8.03 and 12.99 respectively). The difference between the low-dose group and the high-dose group was still statistically significant (P<0.05, t=5.41).

(2) Effect of 'Taizi Yangrong Decoction' on behavioral score

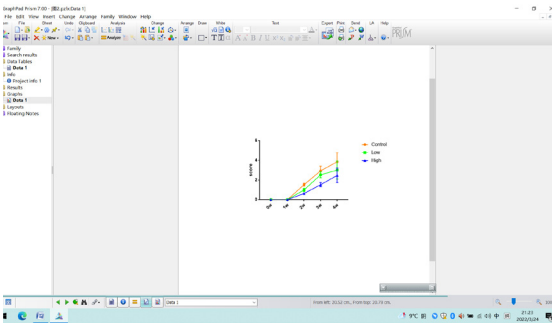

Figure 2. Behavior score changes of rats in each group

As shown in Figure 2,the behavioral score is positively correlated with the irradiation time dose,with the highest score in the control group and the lowest score in the high-dose group. After irradiation, there was no significant difference between the low-dose group and the control group (P>0.05, t=2.04); The difference between the high-dose group and the control group was statistically significant (P<0.05, t=3.70).

4.2 HE staining results and scores of oral mucosa

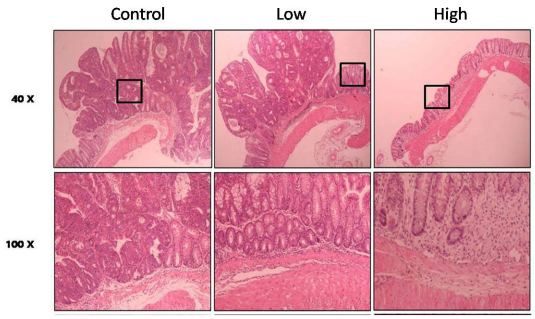

Figure 3. HE staining results of rats in each group after irradiation

HE staining is the hematoxylin eosin staining method. The hematoxylin dye is alkaline, which mainly makes the chromatin in the nucleus and the ribosome in the cytoplasm blue. The eosin dye solution is acidic, which mainly makes the components in the cytoplasm and extracellular matrix red. Inflammatory cells are characterized by large nucleus and large nucleocytoplasmic ratio. Therefore, blue inflammatory cells can be seen under microscope after HE staining, especially after 100x magnification.As shown in the HE staining results in Figure 3,a large number of inflammatory cells exudate in the control group, accounting for more than 50% of the field of vision. The exudate area of inflammatory cells in the low-dose group accounts for 20~50% of the field of vision, while the exudate area of inflammatory cells in the high-dose group accounts for less than 20% of the field of vision. According to the scoring standard, the scores from low to high are respectively 2 points for high dose group, 3 points for low dose group and 4 points for control group.

4.3 Oral mucosa NF- κ B p65 immunohistochemical results

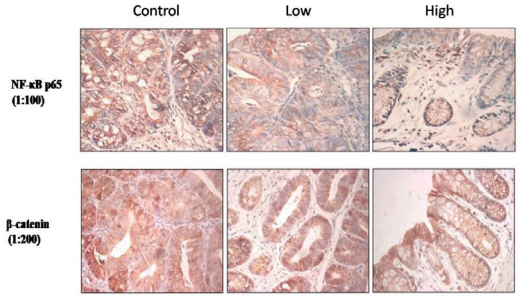

Figure 4. NF of rats in each group after irradiation- κ B p65 immunohistochemical results

The immunohistochemical results showed that the expression area of NF-κB p65 in the control group occupied 20~50% of the visual field, while the expression area of NF-κB p65 in the low-dose group and high-dose group was less than 20% of the visual field. According to the scoring standard, the scores from low to high are respectively 2 points for high dose group, 2 points for low dose group and 3 points for control group. Further comparison between low-dose group and high-dose group shows that NF-κB p65 in low-dose group was higher than that of high dose group.

4.4 Expression changes of oxidative stress related indicators

As shown in Table 2~4 and Figure 5, the expression levels of NrF-2, HO-1 and NQO-1 in plasma of rats in each group were positively correlated with the irradiation time,that is,they were the lowest at the beginning, showing a gradually increasing trend and reaching the highest value at the end of irradiation. Further comparison between two groups showed that there was no statistical difference between the groups at the beginning (P>0.05), but there was significant difference between the groups at the beginning of the third week of irradiation (P<0.05).

4.5 Expression changes of inflammatory factor related indicators

As shown in Table 5~7 and Figure 6, the expression levels of IL-4 and IL-10 in plasma of rats in each group

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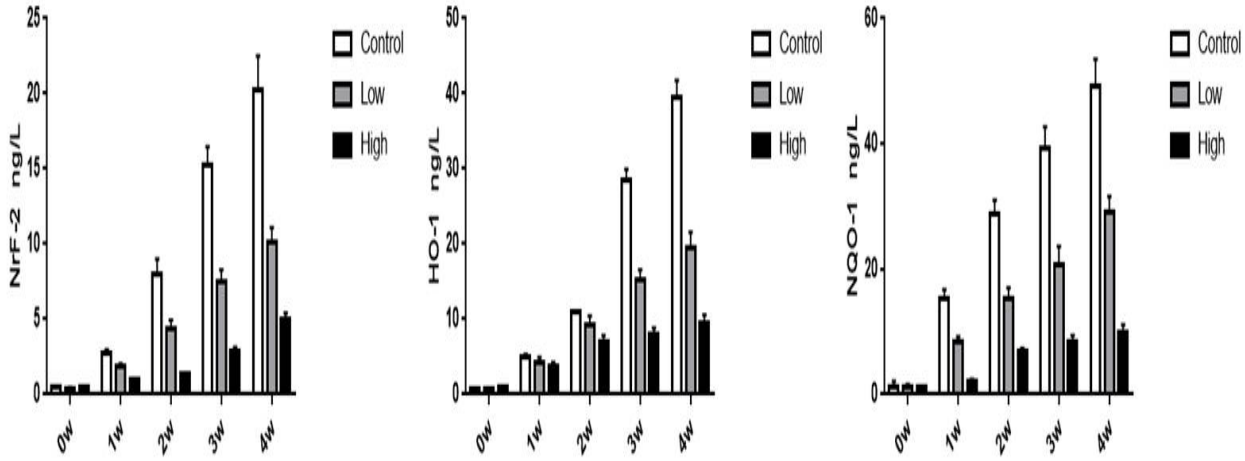

Figure 5. Expression changes of oxidative stress related indicators in plasma of rats in each group

Table 2. Detection results of NrF-2 in plasma of rats in each group

Group	Control	Low	High
0w	0.51±0.06(n=10)	0.43±0.03 (n=10)	0.46±0.04(n=10)
1w	2.71±0.26(n=10)	1.82±0.21 (n=10)	0.94±0.11(n=10)
2w	8.02±0.98(n=10)	4.43±0.52 (n=10)	1.33±0.12(n=10)
3w	15.23±1.23(n=10)	7.48±0.82 (n=10)	2.82±0.31(n=10)
4w	20.03±2.22(n=10)	10.08±1.04 (n=10)	4.94±0.49(n=10)

Note: Measurement data are expressed by mean ± standard deviation, and t-test is used to analyze the difference of expression among groups.

Table 3. Detection Results of HO-1 in Plasma of Rats in Each Group

Group	Control	Low	High
0w	0.79±0.08(n=10)	0.73±0.06 (n=10)	0.92±0.08 (n=10)
1w	4.88±0.42(n=10)	4.32±0.65 (n=10)	3.82±0.46 (n=10)
2w	10.91±1.08(n=10)	9.24±1.21 (n=10)	6.92±0.89 (n=10)
3w	28.41±1.56(n=10)	15.32±1.25 (n=10)	7.99±0.88 (n=10)
4w	39.52±2.26(n=10)	19.62±1.98 (n=10)	9.44±1.14 (n=10)

Note: Measurement data are expressed by mean ± standard deviation, and t-test is used to analyze the difference of expression among groups.

Table 4. Detection Results of NQO-1 in Plasma of Rats in Each Group

Group	Control	Low	High
0w	1.22±0.28(n=10)	1.33±0.31 (n=10)	1.19±0.14(n=10)
1w	15.32±1.47(n=10)	8.36±0.89 (n=10)	2.18±0.22(n=10)
2w	28.77±2.24(n=10)	15.39±1.62 (n=10)	6.85±0.54(n=10)
3w	39.34±3.34(n=10)	20.77±2.92 (n=10)	8.45±0.92(n=10)
4w	49.22±4.25(n=10)	28.97±2.64 (n=10)	9.97±1.14(n=10)

Note: Measurement data are expressed by mean ± standard deviation, and t-test is used to analyze the difference of expression among groups.

are positively correlated with the irradiation time, that is, they are lowest at the beginning, showing a gradually increasing trend and reaching the highest value at the end of irradiation; However, TGF-β The expression level increased significantly at the first week of irradiation, decreased first and then increased at the second to third weeks, and increased again at the fourth week.Further comparison between two groups showed that there was no significant difference in the expression of IL-6 and IL-10 between the groups at the initial stage (P>0.05), but there was significant difference in the expression of IL-6 and IL-10 between the groups at the beginning of the second week of irradiation (P<0.05); TGF-β There was a statistical difference between the control group

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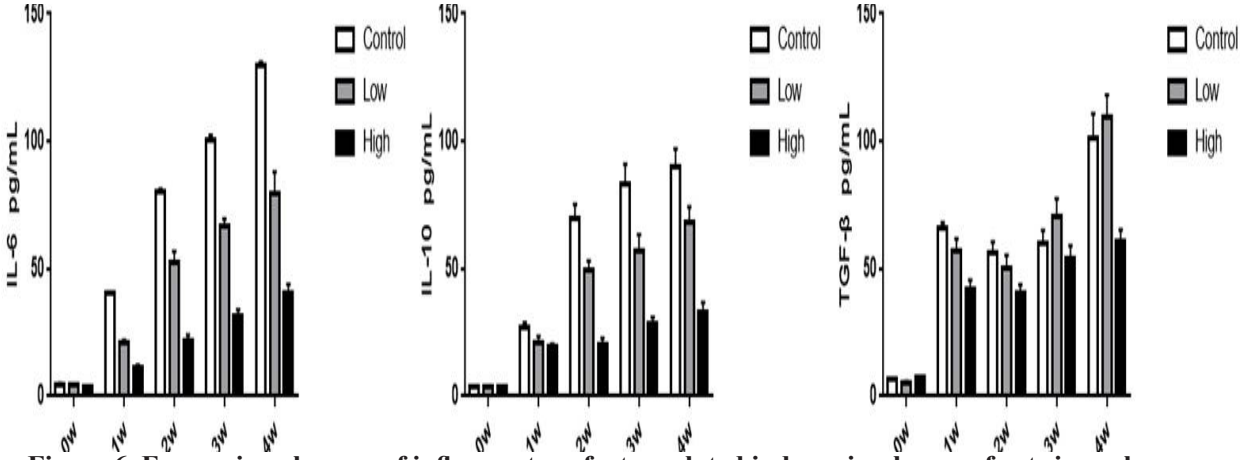

Figure 6. Expression changes of inflammatory factor related indexes in plasma of rats in each group

Table 5. Detection Results of IL-6 in Plasma of Rats in Each Group

Group	Control	Low	High
0w	4.71±0.31(n=10)	4.21±0.28 (n=10)	3.74±0.33(n=10)
1w	40.51±4.21(n=10)	20.87±3.02 (n=10)	10.91±1.09(n=10)
2w	80.41±7.92(n=10)	52.71±4.98 (n=10)	21.75±2.31(n=10)
3w	100.81±9.29 (n=10)	66.44±3.25 (n=10)	31.84±2.84(n=10)
4w	129.42±10.21 (n=10)	79.82±8.14 (n=10)	40.33±3.58(n=10)

Note: Measurement data are expressed by mean ± standard deviation, and t-test is used to analyze the difference of expression among groups.

Table 6. Detection Results of IL-10 in Plasma of Rats in Each Group

Group	Control	Low	High
0w	3.52±0.39(n=10)	3.66±0.27 (n=10)	3.63±0.23(n=10)
1w	26.74±2.21(n=10)	21.36±2.22 (n=10)	19.34±1.24(n=10)
2w	69.74±5.51(n=10)	49.84±3.37 (n=10)	20.57±2.22(n=10)
3w	83.54±7.52(n=10)	57.21±6.24 (n=10)	28.76±2.19(n=10)
4w	90.37±6.66(n=10)	68.39±5.88 (n=10)	32.95±3.82(n=10)

Note: Measurement data are expressed by mean ± standard deviation, and t-test is used to analyze the difference of expression among groups.

Table 7. TGF in plasma of rats in each group- β detection result

Group	Control	Low	High
0w	6.71±0.33(n=10)	5.33±0.49 (n=10)	7.21±0.45(n=10)
1w	65.82±2.27(n=10)	57.23±4.55 (n=10)	42.36±3.37(n=10)
2w	56.12±4.46(n=10)	50.24±5.21 (n=10)	40.28±3.36(n=10)
3w	59.71±5.36(n=10)	70.84±6.64 (n=10)	53.67±5.36(n=10)
4w	100.95±9.97 (n=10)	109.37±8.87 (n=10)	60.88±4.49(n=10)

Note: Measurement data are expressed by mean ± standard deviation, and t-test is used to analyze the difference of expression among groups.

and the low-dose group (P>0.05). There was a statistical difference between the high-dose group and the low-dose group, and between the high-dose group and the control group (P<0.05).

5. Discussion

Both domestic and international guidelines unanimously recommend radical radiotherapy as the first treatment method for nasopharyngeal carcinoma, which can effectively improve the cure rate and reduce the distant metastasis rate.According to statistics,80~90% of the cure rate of nasopharyngeal carcinoma depends on radiotherapy [8]. Modern radiotherapy is based on the X-ray generated by the medical electron linear accelerator. After entering the human body,it directly acts on DNA molecules to break them,on the other hand,it first acts on the water molecules in cells to ionize them into H+ and OH -, and then acts on DNA molecules to break,eventually stopping the growth of tumor cells until necrosis [9]. But at the same time,it has brought radiotherapy related adverse reactions,which affect the quality of life and tolerance of patients,and even forced to discontinue treatment in severe cases. Radioactive oral mucositis is the most common adverse reaction of radiotherapy for nasopharyngeal carcinoma. At present,there is no effective intervention means,and usually only symptomatic treatment is given priority to.Its traditional Chinese and western medicine treatment has encountered a bottleneck [10].

Radiation is the evil of fire poison,which is easy to damage the human body's qi and yin.The main causes and pathogenesis are the accumulation of fire poison,yin deficiency and internal heat,and qi and yin deficiency[11]. According to the syndrome differentiation of traditional Chinese medicine, according to the etiology and pathogenesis of the disease,we should treat it by nourishing yin and yang,clearing heat and detoxifying,promoting blood circulation and cooling blood[12]. The theory of 'preventing disease' in Huangdi Neijing proposes that active intervention at the early stage of disease can effectively prevent the occurrence of disease and reduce the degree of harm [13].Review,analyze and sort out relevant studies: Zhu Siyu and others reported that Pseudostellaria can effectively prevent the occurrence and development of cardiovascular diseases by nourishing yin and promoting fluid production [14];Qu Jie and others reported that Astragalus membranaceus has become the first choice of traditional Chinese medicine for diabetes by supplementing qi and nourishing yin to treat diseases of deficiency of both qi and yin[15]; Han Xiao et al.confirmed by in vitro and in vivo tests that ophiopogon japonicus and polygonatum rhizome could reduce FFA,IL-6 and TNF-α in serum to reduce the inhibition of FINS secretion, thereby improving the islets β Cell function,thereby reducing blood sugar[16].The research group has been engaged in the first-line work of radiotherapy for nasopharyngeal carcinoma and intervention therapy of traditional Chinese medicine for a long time,and has accumulated rich clinical experience.Combined with the thought of TCM syndrome differentiation and the basis of previous research, the research group made 'Taizi Yangrong Decoction'(which has applied for a national patent),and achieved good intervention effect.This prescription is composed of Radix Pseudostellariae,Radix Astragali,Ophiopogon japonicus,Polygonatum odoratum,Rhizoma Ligustici,Poria cocos peel,Radix Angelicae, Radix Scutellariae, and Liquorice.In the prescription, Radix Pseudostellariae and Radix Astragali have the effect of nourishing yin and replenishing qi.They are both sovereign medicines for treating the loss of yin fluid and the loss of positive qi caused by radiotherapy;Ophiopogon japonicus and Polygonatum odoratum can strengthen the power of nourishing yin and promoting fluid production of Pseudostellaria heterophyllum,which can be used as the official medicine;Ligusticum sinense is used to clear the nostrils and remove dirt,dissipate cold and remove dampness,and Poria cocos peel is used to promote water and reduce swelling.Radix Angelicae and Radix Scutellariae are used to clear heat,detoxify,cool blood and stop bleeding.They are also used as adjuvants to treat nasal obstruction,pus bleeding,edema and inflammation caused by radiotherapy;Licorice can strengthen the power of Astragalus to replenish qi,and regulate the medicinal properties with stomach.It is the drug of this prescription.All shakes are used together to play the role of promoting fluid production,clearing heat and detoxification.During the treatment,they are treated according to symptoms and signs.

This study observed the changes of general state of rats in each group before and after irradiation and under the intervention of different doses of 'Taizi Yangrong Decoction'.The results showed that the weight of rats in each group decreased gradually before and after irradiation (Fig.1),and the behavioral score increased gradually (Fig.2),indicating that irradiation had brought negative changes to rats in each group;When 'Taizi Yangrong Decoction' was given in the course of irradiation,the rate of weight loss and behavioral score were alleviated,and the degree of remission was positively correlated with the dosage.The above results were confirmed by HE staining and immunohistochemistry (Fig.3-4).In order to further clarify the pathogenesis of radioactive oral mucositis and the protective mechanism of 'Taizi Yangrong Decoction',in vitro and in vivo experiments

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were conducted.At present,it is generally believed that the important pathogenesis of radiation damage is the oxidative stress reaction in the body,that is,the radiation acts as a harmful stimulus on the human body to produce living oxygen,which leads to cascade amplification reaction,and finally leads to local radiation damage[17].Many studies have reported that NrF-2/HO-1/NQO-1 signal pathway participates in the process of oxidative stress injury of lung tissue during radiotherapy of chest tumor,is one of the most important endogenous protection systems,and plays an important role in the protection of oxidative stress injury[18~20]. As the downstream target protein of Nrf-2,HO-1 has multiple functions, and overexpression of HO-1 can significantly reduce tissue damage [21];NQO-1 is a kind of flavoproteinase, which can release the toxicity of quinones to cells by catalyzing the intracellular double electron reduction reaction,thus playing the role of cell and organ protection [22].As shown in Table 2~4 and Figure 5 of the research results,the expression levels of Nrf-2,HO-1 and NQO-1 in plasma of rats in each group after irradiation were significantly higher than those before irradiation ($P<0.05$); After the intervention of 'Taizi Yangrong Decoction',the expression levels of Nrf-2,HO-1 and NQO-1 decreased significantly ($P<0.05$), and their expression levels were closely related to the dosage.The results show that 'Taizi Yangrong Decoction' may protect the body by inducing the expression of antioxidant protein through NrF-2/HO-1/NQO-1 signal pathway,thereby improving the local and systemic oxidative stress response of rats after irradiation.Inflammatory reaction is also an important pathogenesis of radiation injury.Oxidative stress will promote IL-6,IL-10 and TGF-β Isoproinflammatory factor synthesis,activation of NF-κB signal transduction pathway promotes the expression of proinflammatory factors, induces 'inflammatory waterfall' effect and forms a vicious circle,leading to the microenvironment of radioactive oral mucositis[23~24]. As shown in Table 5~7 and Figure 6 of the research results, the expression of IL-6 and IL-10 in plasma of rats in each group increased significantly after irradiation, and the expression level decreased significantly after the intervention of 'Taizi Yangrong Decoction' ($P<0.05$), and its expression level was closely related to the dosage;TGF-β shows a 'M' type dynamic change of initial stage rise,middle stage decline and later stage rise, which may be related to TGF-β Is related to the double effect of the.Research report,TGF-β/Smad signal pathway suppresses inflammatory response in the early stage and promotes inflammatory response in the late stage.This housekeeping effect makes the inflammatory response process appear double peaks[25]. When combined with 'Taizi Yangrong Decoction' during irradiation,its expression level can be reduced to varying degrees,further reducing the stimulation of inflammatory response signal transduction pathway and the release of downstream inflammatory factors.

In general,'Taizi Yangrong Decoction' may reduce the oxidative stress response to radiation and down regulate TGF through NrF-2/HO-1/NQO-1 signal pathway inhibits the degree of inflammatory reaction in vivo,and finally inhibits the occurrence and development of radioactive oral mucositis.The intervention strategy of 'Taizi Yangrong Decoction' and the signal mechanism of oxidative stress inflammatory reaction are expected to provide laboratory basis for the treatment of radioactive oral mucositis and escort the radical radiotherapy of nasopharyngeal carcinoma.

Reference

[1]Tseng M,Ho F,Leong YH,Wong LC,Tham IW,Cheo T,Lee AW. Emerging radiotherapy technologies and trends in nasopharyngeal cancer[J].Cancer Commun (Lond),2020,40(9):395-405.

[2]Gugnacki P, Sierko E.Is There an Interplay between Oral Microbiome, Head and Neck Carcinoma and Radiation-Induced Oral Mucositis?[J] Cancers (Basel), 2021,13(23):5902.

[3]Soutome S, Yanamoto S, Nishii M, Kojima Y, Hasegawa T, Funahara M, Akashi M, Saito T, Umeda M.Risk factors for severe radiation-induced oral mucositis in patients with oral cancer[J]. J Dent Sci,2021,16(4):1241-1246.

[4]Yonglong Jin, Huiyun Wang, Yingqian Zhu, Hui Feng, Guanqun Wang, Shasha Wang. miR-199a-5p is involved in doxorubicin resistance of non-small cell lung cancer (NSCLC) cells[J]. European Journal of Pharmacology,2020,878:173105.

[5] Zhou Xiaojun, Lan Qian Qi characteristics of nasopharyngeal carcinoma [J] Chinese Journal of Traditional Chinese Medicine, 2021,36 (08): 4587-4590

[6] Huang Yuanyuan, Quan Qi, Chen Ping, Lei Mengjie, Zhang Bei A report on the safety of 91 cases of nasopharyngeal carcinoma treated with traditional Chinese medicine after radiotherapy and chemotherapy [J]. Chinese Journal of Traditional Chinese Medicine, 2020,35 (08): 3902-3905

[7] Lv Peiwan, Zhao Guangsen, Zhou Hongfei, Hou Jianghong. Research on the method of "preventing disease" based on the theory of Huangdi Neijing [J]. Chinese Journal of Traditional Chinese Medicine, 2021,36 (12): 7383-7385 [8]Jing Zhou, Xiaojing Guo, [8]Weifen Chen, Liming Wang,Yonglong Jin*.Targeting survivin sensitizes cervical cancer cells to radiation treatment[J]. Bioengineered,

2020,11(1):130-140.
[9]Ganz JC.Radiobiology[J].Prog Brain Res,2022,268(1):23-48.
[10]Kenneth C W Wong,Edwin P Hui,Kwok-Wai Lo ,Wai Kei Jacky Lam ,David Johnson,Lili Li,Qian Tao,Kwan Chee Allen Chan , Ka-Fai To ,Ann D King,Brigette B Y Ma ,Anthony T C Chan. Nasopharyngeal carcinoma: an evolving paradigm[J].Nat Rev Clin Oncol,2021,18(11):679-695.
[11] Liang Xiaoya, Wang Lei, Wang Jian, Li Chaoqun, Chen Junjun, Yuan Feiyan, Zeng Kunjun, Qin Gan. Research overview of astragalus membranaceus in protection against ionizing radiation injury [J]. Journal of Clinical Chinese Medicine, 2021,33 (03): 593-596
[12] Liang Lu, Xie Yanda, Li Ziquan, Wang Tianbao. Research progress in the treatment of radiation oral mucositis with traditional Chinese medicine [J]. Modern Clinical Medicine, 2021,47 (03): 229-240
[13] Luo Bangshui, Yang Yitao, Zhu Yaqi, Chen Changyong, Cai Huazhu, Liu Zhizhen. Overview of the research on the theory of treating yin and yin diseases with yang in the Yellow Emperor's Internal Canon [J]. Modern Distance Education of Chinese Medicine, 2022,20 (01): 63-65
[14] Zhu Siyu, Yu Chunli, in the same month. Clinical application and dosage of Pseudostellaria heterophylla [J]. Jilin Journal of Traditional Chinese Medicine, 2021,41 (9): 1230-1233
[15] Qu Jie, Wen Na, Yang Jingfeng, Lei Liyan, Li Xiaohui, Yang Jun. Treatment of diabetes with the method of replenishing qi and nourishing yin [J]. Journal of Traditional Chinese Medicine, 2021,7 (7): 1378-1381
[16] Han Xiao, Kuang Yu, Zhang Shunjie, Zhao Yongyan, Dang Kairu, Peng Teng. Study on hypoglycemic pharmacodynamics of the compatibility of polygonatum, mulberry leaves and polygonatum odoratum [J]. Sichuan Journal of Traditional Chinese Medicine, 2018, 36 (5): 62-65
[17]Shimura T.The role of mitochondrial oxidative str ess and the tumor micro environment in radiation-related cancer[J].J Radiat Res,2021,62(1):i36-i43.
[18]Chao Yu,JianHui Xiao. The Keap1-Nrf2 System: A Mediator between Oxidative Stress and Aging[J]. Oxid Med Cell Longev,2021, 2021:6635460.
[19]Ohl K,Tenbrock K.Oxidative Stress in SLE T Cells, Is NRF2 Really the Target to Treat?[J].Front Immunol,2021,12:633845.
[20]Kaur G,Sharma A,Bhatnagar A.Role of oxidative stress in pathophysiology of rheumatoid arthritis: insights into NRF2-KEAP1 signalling[J]. Autoimmunity,2021,54(7): 385-397.
[21]An R,Li D,Dong Y,She Q,Zhou T,Nie X,Pan R,Deng Y.Methylcobalamin Protects Melanocytes from H(2)O(2)-Induced Oxidative Stress by Activating the Nrf2/ HO-1 Pathway[J].Drug Des Devel Ther,2021,15:4837-4848.
[22]Vendidandala NR,Yin TP,Nelli G,Pasupuleti VR,Nyamathulla S,Mokhtar SI.Gallocatechin-silver nanoparticle impregnated cotton gauze patches enhance wound healing in diabetic rats by suppressi ng oxidative stress and inflammation via modulating the Nrf2/HO-1 and TLR4/NF-kappaB pathways[J]. Life Sci,2021,286:120019.
[23]Zhang C,Liang Z,Ma S,Liu X.Radiotherapy and Cytokine Storm: Risk and Mechanism[J].Front Oncol,2021,11:670464.
[24]Chen Z,Wu Z,Muluh TA,Fu S,Wu J.Effect of low-dose total-body radiotherapy on immune microenvironment[J].Transl Oncol,2021,14(8):101118.
[25] Jin Yonglong, Liu Xiguang, Xiao Wenjing, Song Hao, Cui Yinshi, Pi Chunji. Inhibitory effect and mechanism of common wheat on OVA induced allergic reaction in mice [J]. Chinese Journal of Microbiology and Immunology, 2018, 38 (08): 572-581.

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