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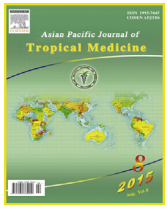

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Effect of Yupingfeng granules on HA and Foxp3$^+$ Treg expression in patients with nasopharyngeal carcinoma

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ABSTRACT

Objective: To investigate the effect of Yupingfeng on hyaluronic acid (HA) and Foxp3$^+$ Treg in patients with nasopharyngeal carcinoma.

Methods: A total of 58 cases of nasopharyngeal carcinoma were divided into two groups, 30 cases in the treatment group, 28 cases in the control group. Patients in two groups were treated with synchronous radiotherapy and chemotherapy treatment, the treatment group was treated with the Yupingfeng granules through oral administration, 10 g/time, tid for 2 courses. The serum Foxp3$^+$ Treg markers of each group were detected by flow cytometry assay before treatment and after treatment, and the level of HA in serum was detected by radio immunoassay.

Results: After radiotherapy and chemotherapy, the contents of Foxp3$^+$ Treg and HA were significantly decreased in two groups ($P < 0.05$), and the decrease of treatment group was more significantly ($P < 0.01$). Correlation analysis showed positive correlation between Foxp3$^+$ Treg and HA ($P < 0.05$). After treatment, the incidence of side effects in two groups was significantly decreased. And there was significant difference between two groups ($P < 0.05$).

Conclusions: Combined chemotherapy and radiotherapy with Yupingfeng treatment can decrease the levels of Foxp3$^+$ Treg and HA in nasopharyngeal carcinoma patients. Yupingfeng can also effectively reduce the side effect due to radiation and chemotherapy.

1. Introduction

The morbidity of nasopharyngeal carcinoma (NPC) is highest in South China. Immune system can kill tumor cells via immune response. However, immune escape often occurs, leading to tumor spread and metastasis, which is common in NPC. CD4$^+$ CD25$^+$ Treg cells can inhibit antitumor immune reaction [1], and Foxp3 is the most reliable marker. It is proved that Foxp3$^+$ T of CD4$^+$CD25$^+$ has immune regulatory function [2–6]. Hyaluronic acid is a receptor of main extracellular matrix, it plays an important role in mesenchyme transferring, invasion, growth and progression of tumor. Besides, it also provides molecular foundation for biological effects such as information transfer, morphological control, movement and proliferation of cells, *etc* [7]. It is reported that Yupingfeng granule has antitumor effect and can decrease side effect due to radiotherapy and chemotherapy by boosting immunity. But the effect of Yupingfeng granule on NPC has not been reported. This study aims to explore the effect of Yupingfeng granule on Foxp3$^+$ Treg and HA.

2. Materials and methods

2.1. Objects selection

A total of 58 NPC cases were selected, who were admitted from February 2010 to December 2013, including 36 males and 22 females, aged 23–85 years old, with average age as (51.5 ± 6.3) years old. All patients had complete clinical data, which is conformed to NPC diagnosis standard by Practical Medical Oncology [2], and none of them received radiotherapy or chemotherapy. Based on clinical stage standard made by Radiation Oncology Branch of Chinese Medical Association in 2008 [3], these patients consisted of 44 cases with squamous carcinoma, 11 cases with anaplastic carcinoma, 3

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cases with adenocarcinoma; 14 cases at stage I, 12 cases at stage II, 12 cases at stage III, 10 cases at stage IVa, 10 cases at stage IVb; 16 cases without lymphatic metastasis, and 32 with lymphatic metastasis. All patients were randomly divided into two groups, and there was no significant difference in age, gender, pathological type, stage or metastasis status ($P > 0.05$). And all signed informed consent.

2.2. Treatment and detection

All patients had radiotherapy and chemotherapy [8], while patients also received oral administration of Yupingfeng granule, 10 g/time, 3 times per day for 2 courses.

ELISA of Foxp3$^+$ Treg and HA was provided by Yiding Biological Company in Hainan. Ten milliliter venous blood of all patients was extracted before treatment and after treatment, respectively. The blood was centrifugated for 10 min at 1 500 rp/min. Upper layer of serum was obtained and preserved at −80 °C. HA level was detected by radioimmunoassay, and Foxp3$^+$ Treg was detected by flow cytometry. Positive cell percentage was recorded and analyzed by Cellquest software.

2.3. Statistical analysis

All date was expressed as mean ± SD, and analyzed by t test, χ^2 and Spearman analysis. $P < 0.05$ was considered as significant difference.

3. Results

3.1. HA levels

After treatment, HA levels in both groups were significantly decreased ($P < 0.05$), and the decrease in treatment group was more significant ($P < 0.01$) (Table 1).

3.2. Foxp3$^+$ Treg levels

After treatment, Foxp3$^+$ Treg levels in both groups were significantly decreased ($P < 0.05$), and the decrease in treatment group was more significant ($P < 0.01$) (Table 2).

3.3. Correlation analysis

Pearson analysis showed that there was positive correlation between HA and Foxp3$^+$ Treg ($P < 0.05$).

3.4. Side effect incidence

The main side effects included decreased leucopenia, decreased platelet, damaged hepatic function, gastrointestinal reaction, and oral mucosa reaction. There were significant differences in side effects between two groups, and between before and after treatment ($P < 0.05$) (Table 3).

Table 1
HA levels (mean ± sd).

Groups	Before treatment	1 course after treatment	2 courses after treatment
Treatment group	187.13 ± 31.4	132.73 ± 25.8[a,b]	125.29 ± 21.4[a,b]
Control group	189.42 ± 32.1	154.16 ± 29.7[a]	141.17 ± 29.5[a]

[a] Compared with before treatment, $P < 0.05$. [b] Compared with control group, $P < 0.01$.

Table 2
Foxp3$^+$ Treg level (mean ± sd) (%).

Groups	Before treatment	1 course after treatment	2 courses after treatment
Treatment group	19.7 ± 6.12	12.5 ± 4.19[a,b]	7.35 ± 1.48[a,b]
Control group	19.3 ± 5.91	15.8 ± 4.74[a]	11.5 ± 3.27[a]

[a] Compared with before treatment, $P < 0.05$. [b] Compared with control group, $P < 0.01$.

Table 3
Side effect incidence [n (%)].

Side effect	Treatment group		Control group	
	1 course after treatment	2 courses after treatment	1 course after treatment	2 courses after treatment
Leucopenia	16 (64)[b]	9 (36)[a,b]	17 (74)	15 (65)[a]
Platelet	10 (40)[b]	4 (16)[a,b]	14 (61)	12 (52)[#]
Hepatic function	3 (12)[b]	2 (8)[a,b]	5 (22)	4 (17)[#]
Gastrointestinal reaction	12 (48)[b]	5 (20)[a,b]	17 (74)	14 (61)[#]
Oral mucosa reaction	21 (84)[b]	10 (40)[a,b]	22 (96)	20 (87)[#]

[a] Compared with 1 course before treatment, $P < 0.05$. [b] Compared with control group, $P < 0.01$.

4. Discussion

Radiotherapy, chemotherapy combined with drugs to reduce side effect is main treatment for NPC [9,10]. The efficacy is fine in most cases, but the life quality of patients is remarkably influenced. In addition, some cases show uncertain curative effect, even have lymphatic metastasis, which is related with injured or weakened immunity system.

HA is an unbranched polysaccharides, mucopolysaccharide, and is an important composition of extracellular matrix. It is main ingredient of synovial fluid, and provides viscoelasticity to the fluid. It can protect cells, promote wound healing, and reduce scar. Besides, it can specifically combine with cell membrane surface receptor, and play a regulatory role in tissue production, tumor invasion and metastasis *etc* [11,12]. Lymphatic metastasis is the main metastasis type of NPC. HA can degrade oligosaccharides of hyaluronic acid, then destroy the junction between oligosaccharides of hyaluronic acid and zonula occludens-1. Treg cell can maintain stability of immune system, and Foxp3 is its specific mark. It is reported that when positive percentage of Treg cells is increased, immune escape often occurs, which can inhibit antitumor immune response. In our study, we find that most cases are at advanced stage or have metastasis. And we also find that before treatment, the levels of HA and Foxp3$^+$ Treg have been increased and been in stress state.

Yupingfeng granule consists of *Astragalus membranaceus*, atractylodes and *Pastinaca sativa*. It can tonifying qi, consolidating superficies and arresting sweating. Pharmacological researches also proved that Yupingfeng granule has extensive

immunomodulatory effect, and is used in treating allergic reaction and immunocompromised patients. Xu *et al* reported that children with acute anaphylactoid purpura showed remarkably lymphocyte dysfunction, indicating significant differentiation and hyperfunction of $CD3^+$, $CD8^+$, $CD19^+$ [13]. It is also reported that Yupingfeng granule can increase T cells in spleen, and help recruit T cells in tumor tissues [14].

This study shows that after treatment, HA and $Foxp3^+$ Treg are decreased. And the longer the course is, more significant this decrease is. The incidence of side effect is also lower in treatment group. Yupingfeng granule can play a role in antitumor treatment via immunoregulation, and can improve the survival rate and life quality.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, et al. Immunologic tolerance maintained by CD25+; CD4+ regulatory T cells their common role in controlling autoimmunity tumor immunity and transplantation tolerance. *Immunol Rev* 2001; **182**(1): 18-32.

[2] Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; **155**(3): 1151-1164.

[3] Von Boehmer H. Mechanisms of suppression by suppresser T cell. *Nat Immunol* 2005; **6**(4): 338-344.

[4] Nishikawa H, Jager E, Ritter G. CD4+ CD25+ regulatory T cells control the induction of antigen-specific CIM+ helper T cell responses in cancer patients. *Blood* 2005; **106**(3): 1008-1011.

[5] Sakaguchi S. Naturally arising FoxP3 expressing CD4+ CD25+ regulatory T cells in immunological tolerance to self and non-self. *Nat Inmmunol* 2005; **6**(4): 345-352.

[6] Zhang RX, Tu CX, Zhang XJ, Liu M, Zhang YY, Wu ML, et al. Effect of *N*-acetylglucosamine on hyaluronic acid synthesis of skin fibroblast. *Chin Dermatol J* 2009; **42**(5): 355-357.

[7] Sun DY, Li X, Wang HD, Kong XY, Mou AN. Relationship between HA and lymphatic metastasis. *Chin Tumor* 2011; **20**(5): 391-394.

[8] Fan CG, Han YQ, Chen YP, Wu XW. Effect of concurrent radiochemotherapy on children and adolescents with nasopharyngeal carcinoma: 72 cases report. *Mod Tumor* 2011; **19**(6): 1107-1109.

[9] Pan SM, Chen ZR, Qiu QS, Guo MJ, Situ HJ. Effect of nedaplatinon advanced local nasopharyngeal carcinoma. *Mod Tumor* 2011; **19**(7): 1327-1329.

[10] Cheng X, Chen GJ, Li FM, Zhang QH. Relationship between lymphatic metastasis and prognosis in nasopharyngeal carcinoma patients. *Heilongjiang Med* 2011; **35**(7): 483-486.

[11] Li JH, Hao J, Ge LH, Tang XF, Xing RD. Expression of hyaluronic acid in oral squamous cell carcinoma. *Beijing Stomatol* 2007; **15**(6): 310-312.

[12] Zhng QM, Liao XB, Tang WP, Mo MY. Effect of hyaluronic acid on proliferation of nasopharyngeal carcinoma cell and growth of transplantation tumor in nude mouse. *Prac Tumor J* 1999; **14**(1): 14-17.

[13] Xu ZB, Zeng P, Zeng HS. Effect of Yupingfeng granule on lymphocyte subsets of children with anaphylactoid purpura. *Chin Med J* 2013; **28**(2): 513-516.

[14] Zhan LR, Yao F, Jiang GR. Effect of Yupingfeng granule on immunoregulation of tumor-bearing mice with Hepa1-6 liver cancer. *J Southeast Univ* 2014; **33**(1): 34-39.