Effects of Shenmai Zhusheye on Qi and Yin deficiency non-small cell lung cancer patients.

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Abstract

Background: The effects of Shenmai Zhusheye on the expression of B7, CD28 and CTL-4 (CD152) in Qi and Yin Deficiency non-small cell lung cancer patient is surveyed in this study.

Methods: 150 patients of Qi and Yin Deficiency non-small cell lung cancer are randomized into two groups with 75 patients in each group, the patients in control group is given symptomatic treatment, and the patients in treatment group is given symptomatic treatment plus Shenmai Zhusheye. Pre-treatment and post-treatment blood routine examination results, hepatic and renal function, and expression of CD4, CD8, CD28, CTLA-4 (CD152), B7(CD80, CD86) of the two group is compared respectively.

Results: No significant difference of blood routine examination results and hepatic and renal function between the two groups is observed after 15 d of treatment. Immune index between the two groups are comparable and no significant difference before treatment is observed; While post-treatment CD4, CD8, CD28 and CD152 of the two groups showed statistical difference (P<0.05); all results except CB86 show intra-group difference (P<0.05) in the treatment group and all results except CD28 show intra-group difference (P<0.05) in the control group.

Conclusions: Immune function of patient of deficiency of both QI and YIN type non-small cell lung cancer is improved by Shenmai Zhusheye.

Introduction

Shenmai Zhusheye is a traditional Chinese medicine preparations prepared from Radix Ginseng Rubra and Radix Ophiopogonis with the effects of supplementing QI to prevent collapse, nourishing YIN and promoting the production of body fluid, and activating pulse. It is widely used as clinical adjuvant treatment for cardiovascular disease and various chronic diseases [1-6], and some therapeutic effect is achieved. Besides, Shenmai Zhusheye shows preferable effect in treating tumor, especially in improving immune function of tumor patient with solid evidence [7-9]. Then what is the mechanism behind the effects of Shenmai Zhusheye in improving immune index including CD4, CD8, and NK cell? This paper regards this question as an entry-point and discusses the CD28 factor with the effect of activating CD4 and CD8.

Materials and Methods

General information

150 patients of Qi and Yin Deficiency non-small cell lung cancer in our hospital from December 2010 to June 2014 are observed in this project. The inpatients are randomized into treatment group and control group, 75 patients are in treatment group including 12 female, 63 male, 39 squamous carcinoma, 26 adenocarcinoma and 10 non-small cell lung cancer patients between 49-89 y old with average age of (64.01 ± 8.98). 75 inpatients are in control group including 16 female, 59 male, 34 squamous carcinoma, 34 adenocarcinoma and 7 non-small cell lung cancer patients between 43-88 y old with average age of (66.69 ± 8.46). There is no statistically significant difference in gender, pathology and age between the two groups.

Therapy

The patients in control group were given symptomatic treatment, and the patients in treatment group were given symptomatic treatment plus Shenmai Zhusheye. Immunity adjusting medicine (e.g. ubenimex, thymosin, traditional

Chinese medicine or Chinese patent medicine such as Shenqi Koufuye) and therapy are not used in the symptomatic treatment.

Observational index

The expressions of CD4, CD8, CD28, CTLA-4 (CD152), B7 (CD80, CD86) in PB (peripheral blood) are determined through FCM (flow cytometry) and statistical analysis is performed.

Assay method

Principle: Stain the cells to be tested and prepare them into a single cell suspension. Press the cell suspension to be tested into flow chamber under certain pressure, the cell-free equilibration buffer is ejected from the sheath tube under high pressure, and the sheath tube is at an angle to the sample flow to be measured so that the sheath fluid can circulate around the sample at high speed to generate a cycloidal flow, and the cells to be measured are arranged in a single row under the coating of the sheath fluid, and pass through the laser irradiation area (the vertical intersection of the liquid column and the incident laser beam is referred to as the detection area). Fluorescent cells are irradiated with intense fluorescence to emit light and produce light scattering. The fluorescence signal is collected by an optical system (lens, light bar, filter and photodetector, etc.) in a direction of 90 degrees perpendicular to the laser beam. The fluorescence and lateral angle scattered photodetector are photomultiplier tubes, and the forward angle scattering photodetector is a photodiode. The cells were labeled with fluorescent antibody, and the proportion of lymphocytes and immunophenotype of peripheral blood was used to prepare the samples.

Reagent: 1. manufacturers: Beckman Coulter, BD. 2. stability: preserve in refgrirator at 2-8°C. 3. composition: CD45 PerCP, CD3 APC, CD80 FITC, CD86 PE, CD28 FITC, CD152 PE. 4. auxiliary agents: 610 diluent.

Specimen preparation: 1. take two tubes, label them with specimen number 1a and 1b respectively; 2. add 20 µl of CD45 PerCP, CD3 APC, CD80 FITC and CD86 PE antibodies each into tube 1a, add 20 µl of CD45 PerCP, CD3 APC, CD28 FITC, and CD152 PE antibody each into tube 1b; 3. mix the blood samples evenly; add 40 µl of blood samples into each tube and mix; 4. keep the sample in dark place at room temperature for 15 min; 5. dilute red cell lysate with 9 folds of deionized water; 6. add 300 µl of diluted red cell lysate into each tube, mix evenly; and 7. keep in dark place at room temperature for 15 min.

Statistical analysis

Statistical analysis was performed using SPSS15.0 statistical software and DPS software. Measurement data is showed as x̄ ± S, begin with normal distribution and variance homogeneity test. On condition that measurement data shows normal distribution and equal variance, perform inter-group comparison using Q test and pre and post treatment comparison through t-test. Perform statistical analysis on measurement data with χ^2 test, rank sum test is carried out in case of unequal variances. Ranked data from multi-sample is compared in pairs through rank sum of DPS classification data statistics. $P<0.05$ is considered to be statistically significant.

Results

Changes in blood routine examination and liver and kidney function before and after treatment

No intra- or inter-group difference is observed before and after treatment (Table 1).

Changes of CD4, CD8, CD28, CD80, CD80 and CD86 before and after treatment

There was no significant difference between the two groups before treatment. Comparing the two groups after treatment, statistical significant difference is observed in CD4, CD8, CD28, and CD152 ($P<0.05$), all results except CB86 show intra-group difference ($P<0.05$) in the treatment group and all results except CD28 show intra-group difference ($P<0.05$) in the control group (Table 2).

Table 1. *Before and after treatment security index observation of the two groups.*

	Treatment group		Control group	
	Prior treatment	Post treatment	Prior treatment	Post treatment
WBC	7.7 ± 8.07	6.6 ± 1.60	6.9 ± 1.71	6.8 ± 1.28
HB	93.04 ± 5.05	94.24 ± 3.54	94.51 ± 3.46	95.41 ± 3.67
PLT	248.78 ± 3.76	249.58 ± 1.88	244.47 ± 1.07	239.06 ± 8.39
ALT	20.41 ± 7.75	20.61 ± 1.77	19.3 ± 9.06	22.5 ± 8.13
AST	22.92 ± 3.00	23.82 ± 0.28	21.4 ± 6.80	22.3 ± 6.73
Cr	81.41 ± 8.30	80.11 ± 7.84	77.22 ± 1.17	75.81 ± 7.32

No intra- or inter-group difference is observed before and after treatment between the two groups, P>0.05.

Table 2. *Before and after treatment observation of the two groups.*

	Treatment group		Control group	
	Prior treatment	Post treatment	Prior treatment	Post treatment
CD4	40.3 ± 5.14	45.3 ± 4.37	41.5 ± 5.79	41.4 ± 5.72[1]
CD8	24.7 ± 6.97	20.9 ± 6.45	26.2 ± 7.50	25.8 ± 7.44[1]
CD28	6.3 ± 3.90	7.7 ± 4.46	6.2 ± 4.26	6.2 ± 4.341[2]
CD152	2.8 ± 3.20	1.8 ± 2.46	2.3 ± 2.84	3.3 ± 3.33[1]
CD80	2.2 ± 2.37	2.6 ± 2.55	3.1 ± 3.35	3.3 ± 3.34
CD86	1.5 ± 2.06	1.7 ± 2.662	1.4 ± 2.07	1.5 ± 2.12

Discussion

The anti-tumor immunity of human body is mainly T lymphocyte-mediated cellular immunity, and activated T lymphocytes play an important role in anti-tumor immunity. T cell activation requires dual signal stimulation: the first signal is provided by the T cell antigen receptor binding to the antigen peptide-MHC molecule complex on the Antigen Presenting Cell (APC), the second signal is offered by APC co-stimulatory molecule after binding with T cells corresponding ligands. Only with the dual signal co-stimulus can the specific T cells be effectively activated, the lack of second signal can lead to T cell clonal apoptosis, the tumor cells can go through immune escape. B7: CD28/CTLA-4 is a relatively actively studied membrane molecule in recent years, it belongs to the immunoglobulin superfamily, which is highly homologous and derived from the same ancestral gene. B7 family includes B7-1(CD80), B7-2 (CD86) and B7-3, which are transmembrane glycoproteins with relative molecular mass of 50-70 kD. B7 is an activated antigen of lymphocytes that is expressed in activated B cells and is not expressed in resting B cells. One of the corresponding receptors for B7 is CD28 molecule, a homodimer with a relative molecular mass of 44 kD. Another receptor of B7 is CTLA-4, which is 31% amino acids identical, mainly expressed on T cells and plasma cell surface. At present, CD28 and CTLA-4 are a pair of important costimulatory molecules with positive and negative regulatory function. B7/CD28 signal transduction pathway is dominant when APC expresses high level B7 molecules in activated B cells and DCs. B cells are activated to secrete IL-2 and other cytokines and then proliferate and differentiate into effector cells, activating a large number of T cells and expressing CTLA4 and CD28, while CTLA4 and CD28 competitively binds to B7 molecules, inhibiting T cells change from G1 phase into S phase and inhibiting the activity of IL-2 transcription factor, and reducing or stopping T cell responses [10]. CD28 and T cell antigen receptor (TCR) signals promote cell activation synergistically and possibly regulates T cell activation threshold by reducing the number of TCRs [11]. *In vitro*, the absence of CD28 molecules during T cell activation can lead to cell death or the formation of immune tolerance to T cells.

Shenmai Koufuye is an intravenous injection developed from the ancient prescriptions of the "Shengmai San" composing by Radix Ginseng Rubra and Radix Ophiopogonis with the main active ingredients of ginsenosides, flavone of Radix Ophiopogonis and trace ginseng polysaccharides and so on. Studies have shown that ginsenosides [12,13] and some components of Radix Ophiopogonis [14,15] have certain anti-tumor and immune regulating effects. This study showed that SHENMAI KOUFUYE can increase CD4 and reduce CD8 of QI and YIN deficiency non-small cell patients with statistical significance (P<0.05) comparing to the control group, which is consistent with current clinical reports [7-9]. Meanwhile, it can increase CD28 and CD80 and reduce CTLA-4 (CD125) with statistical significance (P<0.05) comparing to the control group (P<0.05). It is shown that Shenmai Koufuye has certain immune regulation effect for Qi and Yin Deficiency non-small cell lung cancer patients.

In summary, the immune function regulating effect of Shenmai Koufuye in Qi and Yin Deficiency non-small cell lung cancer patients has been confirmed. Later we can make further research about its effect on survival time, recurrence and metastasis etc. to provide more evidence for its clinical application.

Disclosure of Conflict of Interest

The authors have declared that no competing interests exist.

Acknowledgements

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