Chin J Integr Med 2009 Jun;15(3):184-188

ORIGINAL ARTICLE

Efficacy of Auxiliary Therapy with Danggui Buxue Decoction No.1 (当归补血汤 1号) in Treating Patients of Non-small Cell Lung Cancer at Peri-operational Stage

DU Qing-cong (杜庆聪), YANG Kui-zhong (杨奎忠), and SUN Xue-fei (孙雪飞)

ABSTRACT **Objective:** To observe the therapeutic efficacy of Danggui Buxue Decoction No.1 (当归补血汤1号, DB1) in treating patients of non-small cell lung cancer (NSCLC) at the peri-operational stage and its impact on the patients' immune function. **Methods:** Eighty-two NSCLC patients were randomly assigned to two groups equally, the control group and the test group, they were given conventional treatment, while to the test group, DB1 were given additionally. The observation was conducted by testing the changes of T-lymphocyte subsets, natural killer (NK) cell activity, serum levels of immunoglobulin (IgA, IgM, IgG), interleukin-2 (IL-2), tumor necrosis factor α (TNF-α), cytokeratin fragment 19 (CYFRA21-1) and carcinoembryonic antigen (CEA) in NSCLC patients before and after administration of DB1, and analyzing the patients' general condition. **Results:** The level of $CD3^+$, $CD4^+$, the ratio of $CD4^+$ and $CD8^+$, IgA, IgM, IgG and IL-2 decreased in patients with NSCLC on day 1 after operation, and the level of $CD8^+$ and TNF-α increased compared to pre-operation. While the levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$, NK cell activity, serum IgA, IgM, IgG, IL-2 began to elevate, $CD8^+$ and TNF-α levels began to decline in patients administered with DB1 on day 3 after the operation, earlier than patients who did not use the decoction. The level of CYFRA21-1 and CEA, was immediately decreased after operation in both groups. **Conclusions:** Applying DB1 to NSCLC patients at an early post-operational stage could alleviate the impairment and accelerate the recovery of immune function of patients to enhance their immunity. DB1 also shows an anti-tumor action to a certain degree.

KEY WORDS non-small cell lung cancer, peri-operational stage, immune function, Danggui Buxue Decoction No.1, immunotherapy

The pathogenesis and development of tumors are closely related with the immune function of the host, as the morbidity of the hosts is raised when their immune function is low or suppressed. The immune function of host would be suppressed along with the progressive development of the tumor. The two mutually act and impact, and work together to influence both the genesis and prognosis of tumors. For this reason, immunotherapy becomes an important approach for the treatment of tumor. In order to explore the conditions of immune function in patients of non-small cell lung cancer (NSCLC) and its relationship with the patients' clinical pathological characteristics, the rule of immune functional change at post-operational stage, the necessity of immunotherapy at the peri-operational stage, and the therapeutic efficacy of Danggui Buxue Decoction No.1 (当归补血汤 1号, DB1), this study was designed to summarize the changing rule of immune function in NSCLC patients at the peri-operational stage, observe the efficacy of auxiliary treatment with DB1, through testing the changes of cellular and humoral immune function at various time-intervals in the peri-operational stage, so as to provide a new way of thinking in integrative Chinese and Western medical treatment of NSCLC.

METHODS

General Materials

All the 82 subjects enrolled were inpatients of the thoracic department of the Wujing Shandong Zongdui Hospital, who were hospitalized from 2003 to 2007. The diagnosis of NSCLC was confirmed after operation by pathological examination. They were assigned by simple randomization method to two groups equally. The 41 patients in the test group consisted of 28 males and 13 females, with a mean age of 54.02 years. The 41 in the control group were 25 males and 16 females with a mean age of 52.56

The Fourteen Area, Wujing Shandong Zongdui Hospital, Jinan (250014), China
Correspondence to: Dr. DU Qing-cong, Tel：86-531-83197443, E-mail: dukingsoon@yahoo.com.cn
DOI: 10.1007/s11655-009-0184-y

years. The two groups were statistically insignificantly different in terms of sex, age, condition of disease and clinical pathological characteristics (P>0.05, Table 1).

Table 1. Comparison of the General Materials between the Two Groups

Items	Test (41)	Control (41)
Sex (Male/Female)	28/13	25/16
Age		
⩽40 yr.	3	5
41-50 yr.	10	9
51-60 yr.	16	18
61-70 yr.	12	9
Average age (yr, $\bar{x} \pm s$)	54.02±9.96	52.56±12.72
Pathological type		
Squamous	24	21
Adeno	13	17
Large Cell	4	3
Grade of differentiation		
High	8	10
Moderate	20	16
No/Low	13	15
Diameter of tumor		
⩽4 cm	23	20
> 4 cm	18	21
Pathological stage		
Ⅰ	9	7
Ⅱ	11	15
Ⅲ	21	19

Treatment

Conventional treatment, including anti-inflammatory, symptomatic and supportive therapies, was applied on both groups in the same way, while to the test group, DB1 was additionally given. One dose of DB1 consisted of milkvetch root 30 g, Chinese angelica root 6 g, spatholobus stem 30 g, forsythia fruit 15 g, globethistle root 10 g, chuanxiong 10 g and white-stiff silkworm 10 g, which was administered in a single dose every day, excepting the day of operation, from three days before operation to the end of the 4th week after operation, with the decoction taken orally in two parts in the morning and evening.

Items and Methods of Observation

Immune function and lung cancer markers were detected at various time-intervals, i.e. before the operation (T0), on the 1st day (T1), 3rd day (T2), 5th day (T3), 7th day (T4), 10th day (T5), 2nd week (T6), 3rd week (T7) and 4th week (T8), respectively, after operation. The general condition and scoring of the patients was examined at T0, T6 and T8, respectively. Venous fasting blood sample drawn from the cubital median vein in the morning of the testing day and was anti-aggregated with 10 mL of heparin. Then, the peripheral blood mononuclear cells isolated by lymphocyte stratifying liquid were taken for detection immediately, while the serum was separated and stored at -80 ℃ for central detection.

Natural killer (NK) cell activity was detected by the radio-nuclide release method. T-lymphocyte subsets ($CD3^+$, $CD4^+$ and $CD8^+$) were detected by indirect immunofluorescent method and serum immunoglobulin (IgG, IgA and IgM) by the one-way immunodiffusion method. Serum interleukin 2 (IL-2) and tumor necrosis factor α (TNF-α) were detected by the double antibody sandwich enzyme linked immunosorbent assay (ELISA) with the test kits purchased from the Jingmei Bioengineering Co., Ltd., Shenzhen, China. The cytokeratin fragment 19 (CYFRA21-1) and carcinoembryonic antigen (CEA) were detected by the radio-immune method with the kits purchased from the Sanwei Biotechnique Engineering Company, Weifang Development Area, China production batch number 1345092.

Statistical Analysis

With SAS 6.2 statistical software, the comparison of enumeration data was conducted by χ^2 test, comparison between groups by t-test for mean value of two samples, comparison between pre- and post-treatment in the same group by paired t-test and comparison of mean values among multiple groups by variance analysis (F-test).

RESULTS

Changes of T-lymphocyte Subsets

As compared with T0, $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ ratio were lowered at T1 in both groups, but $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ began to rise at T2 in the test group, significantly earlier than that in the control group and showing significant difference (P<0.01). The $CD3^+$, $CD4^+/CD8^+$ ratio at T4 and T8, $CD4^+$ at T3 and T8 were higher, while the $CD8^+$ at T8 was lower in the test group than in the control group, all showing statistical significance (P<0.05, Table 2).

Changes of NK Cell and Immunoglobulins

The levels of immunoglobulins and NK cell decreased

Chin J Integr Med 2009 Jun;15(3):184-188

Table 2. Changes of T-lymphocyte Subsets between the Two Groups ($\bar{x} \pm s$)

Group	Case	Time	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Control	41	T0	57.37 ± 5.32	31.12 ± 4.42	30.53 ± 4.38	1.02 ± 0.25
		T1	52.01 ± 4.52	28.13 ± 4.95	31.86 ± 3.44	0.89 ± 0.12
		T2	49.83 ± 2.56	26.47 ± 4.18	30.42 ± 3.97	0.87 ± 0.26
		T3	51.39 ± 3.94	29.32 ± 3.73	29.85 ± 4.62	0.97 ± 0.25
		T4	53.82 ± 5.64	32.56 ± 4.98	29.27 ± 3.65	1.09 ± 0.37
		T5	55.61 ± 6.57	35.81 ± 5.31	27.56 ± 3.64	1.29 ± 0.28
		T6	58.47 ± 7.77	41.83 ± 4.75*	25.64 ± 3.51	1.63 ± 0.29
		T7	63.65 ± 5.34*	49.76 ± 6.17	25.05 ± 3.47	1.98 ± 0.32*
		T8	64.73 ± 4.23	50.55 ± 5.00	24.16 ± 3.45	2.08 ± 0.35
Test	41	T0	58.21 ± 4.97	30.93 ± 5.63	29.97 ± 3.72	1.03 ± 0.21
		T1	51.31 ± 3.56	27.31 ± 4.62	30.63 ± 4.18	0.89 ± 0.28
		T2	52.87 ± 5.36	30.45 ± 6.13	28.45 ± 3.29	1.07 ± 0.29
		T3	58.41 ± 6.74	37.69 ± 5.07△	27.33 ± 2.94	1.35 ± 0.18
		T4	62.33 ± 7.72△	41.37 ± 3.53*	26.47 ± 3.08	1.55 ± 0.19△
		T5	68.54 ± 7.96*	45.44 ± 4.12	24.91 ± 4.43	1.82 ± 0.23*
		T6	70.89 ± 8.47	51.77 ± 5.43	23.86 ± 3.28	2.15 ± 0.34
		T7	72.45 ± 7.58	55.82 ± 4.67	22.73 ± 3.58*	2.46 ± 0.30
		T8	73.12 ± 6.42△	56.12 ± 4.38△	21.97 ± 3.76△	2.55 ± 0.27△

Notes: *P<0.05, compared with the same group at T0; △P<0.05, compared with the control group at the same time point

in both groups at T1, but they began to rise at T2 in the test group, significantly earlier than that in the control group, and shows significant statistical meaning as compared with those before operation (P<0.01). Comparisons between groups show that the NK at T3 and T8, IgA and IgG at T5 and T8, and IgM at T8 in the test group were significantly higher than those in the control group (P<0.05, Table 3).

Changes of TNF-α and IL-2

At T1, the level of TNF-α got elevated and IL-2

Table 3. Changes of NK Cell and Immunoglobulins between the Two Groups ($\bar{x} \pm s$)

Group	Case	Time	NK cell (%)	IgG (g/L)	IgA (g/L)	IgM (g/L)
Control	41	T0	16.95 ± 3.68	10.11 ± 2.35	1.55 ± 0.63	1.06 ± 0.41
		T1	14.85 ± 2.78	7.63 ± 2.81	1.33 ± 0.47	0.84 ± 0.24
		T2	13.94 ± 2.24	7.36 ± 2.73	1.26 ± 0.37	0.80 ± 0.19
		T3	15.49 ± 3.76	8.38 ± 3.01	1.44 ± 0.33	0.94 ± 0.42
		T4	17.98 ± 3.48	10.05 ± 2.63	1.64 ± 0.61	1.00 ± 0.31
		T5	18.82 ± 3.65	12.12 ± 2.45	1.88 ± 0.32	1.10 ± 0.28
		T6	23.25 ± 4.53	14.24 ± 2.81*	2.03 ± 0.46	1.26 ± 0.24
		T7	25.64 ± 5.23*	15.26 ± 2.64	2.32 ± 0.54*	2.11 ± 0.46*
		T8	26.68 ± 4.62	15.76 ± 3.80	2.35 ± 0.41	2.15 ± 0.29
Test	41	T0	17.01 ± 2.99	9.93 ± 2.15	1.54 ± 0.58	1.07 ± 0.38
		T1	14.37 ± 3.01	7.82 ± 2.32	1.32 ± 0.87	0.83 ± 0.21
		T2	17.25 ± 2.56	9.71 ± 3.14	1.41 ± 0.76	0.95 ± 0.25
		T3	22.67 ± 3.17△	10.43 ± 2.33	1.68 ± 0.53	1.03 ± 0.34
		T4	25.47 ± 2.65*	12.73 ± 2.75*	1.95 ± 0.27*	1.28 ± 0.47
		T5	28.36 ± 3.54	15.22 ± 2.16△	2.56 ± 0.34△	1.55 ± 0.26*
		T6	29.71 ± 4.02	17.06 ± 2.36	2.81 ± 0.45	2.09 ± 0.28
		T7	34.69 ± 4.13	18.32 ± 3.24	3.25 ± 0.74	3.17 ± 0.45
		T8	35.16 ± 3.87△	18.67 ± 3.15△	3.37 ± 0.35△	3.62 ± 0.39△

Notes: *P<0.01, compared with the same group at T0; △P<0.05, compared with the control group at the same time point

Table 4. Changes of Cytokines and Tumor Marders between the Two Groups (ng/L, $\bar{x} \pm s$)

Group	Case	Time	IL-2	TNF-α	CYFRA21-1	CEA
Control	41	T0	10.19±3.13	447.93±100.62	29.08±28.74	73.02±53.50
		T1	7.49±2.31	573.24±71.32	28.32±28.13	72.03±48.94
		T2	6.17±2.98	507.94±68.45	25.64±20.24	67.34±40.94
		T3	8.34±3.21	413.26±62.54	21.35±17.52	50.26±30.81*
		T4	9.22±2.48	230.74±50.41*	17.78±10.67*	37.48±18.57
		T5	12.08±3.54	123.77±21.36	13.68±8.75	23.42±12.65
		T6	15.68±3.09*	44.30±11.66	8.53±4.26	18.51±8.47
		T7	17.97±2.43	21.97±8.57	6.57±3.14	18.3±7.01
		T8	19.64±3.85	20.34±9.58	5.98±2.35	17.2±8.12
Test	41	T0	11.01±2.97	456.98±98.73	30.17±29.39	75.23±60.31
		T1	7.58±2.14	570.31±67.32	28.41±27.14	70.78±51.42
		T2	11.25±2.67	497.55±70.33	20.63±15.19*	59.48±30.21*
		T3	13.14±3.25	320.14±50.44*	14.25±13.78	33.12±21.57
		T4	15.72±2.84*	154.67±30.66	7.98±4.65△	17.86±13.25△
		T5	19.83±3.14△	80.37±10.98△	3.42±2.13	10.54±5.62
		T6	21.34±3.55	63.75±9.67	1.73±1.35	5.76±4.79
		T7	22.54±2.95	64.25±11.14	1.54±0.79	3.16±2.54
		T8	28.84±1.19△	63.24±9.24△	1.23±0.64△	3.24±2.02△

Notes: *P<0.01, compared with the same group before operation; △P<0.05, compared with the control group at the same time point

lowered in both groups. They began to restore at T2 in the test group, and with IL-2 at T5 and T8 higher, TNF-α at T8 and T5 were lower in the test group than those in the control group significantly (P<0.05, Table 4).

Changes of Tumor Markers

The two tumor markers, CYFRA21-1 and CEA was decreased immediately after operation in both groups, but the test group did show quicker lowering in speed and was higher in amplitude than that of the control group, with significant difference as compared with those before operation (P<0.01). The levels of the two tumor markers at T4 and T8 were significantly lower in the test group than those in the control group (P<0.05, Table 4).

DISCUSSION

The genesis and development of tumors are closely related with the immune function of the host. It has been known that the immune function is always decreased in patients with tumors to different extents[1], which is caused mainly by a large amount of immune-inhibiting factors produced or secreted by tumor tissues themselves to arrest the differentiation and maturation of $OKT4^+$ cells, induce the production of $OKT8^+$ cells and results in the lowering of NK cell activity and abnormality and proportional de-equilibrium of T-lymphocyte subsets[2]. Thanks to the factors of anesthesia, blood transfusion and operation, etc., when the tumor was removed, the immune function of the patients was still in suppressed, that is, in the "post-operative immune suppression aggravated stage".

Traditional Chinese medicine (TCM) holds that lung cancer comes into being from a deficiency of healthy energy and an excess of evil-pathogens. The relationship between healthy energy and evil-pathogens should be well-handled when one applies the TCM theory for treatment of lung cancer. Danggui Buxue Decoction (当归补血汤) is a classic TCM recipe the recipe is composed of only two Chinese herbal drugs, milkvetch root and Chinese angelica root, and has been commonly used to treat syndromes like inner impairing by tiredness, qi-insufficiency with blood deficiency, yang exhausting outside, etc. Patients bearing tumors frequently present the syndrome of qi-blood deficiency with residue stasis-toxin. Particularly dominant is qi-blood deficiency, which was caused by long-term evil-toxin invasion injuring healthy energy and transforming to stasis and surgical operational trauma on qi-blood. For this reason, the treatment of tumor should be supplementing qi, nourishing blood, removing toxic substances and dissolving stasis. DB1 is formulated aiming at the pathogenesis of NSCLC,

and is based on the Danggui Buxue Decoction. It has spatholobus stem, chuanxiong, forsythia fruit, globethistle root, and white-stiff silkworm added into it.

Results of the term tests on immune function and tumor markers in this study show that the global variational trend of all the indexes in the two groups were similar, but the suppression of cellular and humoral immune function at early post-operational stage in the test group was much milder than that in the control group. Although immune suppression appeared on day 3 in both groups, the immune indices began to rise on day 3 in the test group and got restored quicker, reaching normal range earlier by 1-2 weeks than those in the control group, with the inter-group difference of all indices showing statistical significance. Anti-tumor immunity is mainly cellular immunity, and the elevation of such indexes as NK cell, $CD3^+$ and $CD4^+$ should be favorable to improve the immune suppressive manner of tumor patients, raise the immune capability for tumor cell recognizing and killing, so as to control the growth and metastasis of tumor[3].

Schiller JH, et al[4] used TNF-α combined with IL-2 to treat NSCLC patients and obtained good effects, suggesting the two cytokines have a coordinative anti-tumor effect, which is possibly related with the IL-2 induced tumor cell superficial expression of TNF-α receptor[5]. The results of this study show that cellular immunity was significantly enhanced after DB1 treatment, displaying serum TNF-α and IL-2 levels higher than those in the control healthy persons, illustrating that DB1 plays a positive regulatory action on immunity.

Gaast A, et al[6] has reported that the relapse of tumor is definitely associated with the concentration of CYFRA21-1 in the patients' serum, the persistent high CYFRA21-1 level at the post-operation stage forecasts the high possibility of tumor relapse. Some other studies indicate that CYFRA21-1 is meaningful for the diagnosis and therapeutic efficacy of NSCLC. For example, the lowering of CYFRA21-1 could appear after surgical operation or chemotherapy, but when tumor was conservatively removed, it would inconspicuously be revealed. Therefore, CYFRA21-1 determination could not only be applied in the diagnosis of NSCLC, but also be used independently for monitoring treatment, evaluating therapeutic effectiveness and predicting the prognosis of tumors[7,8]. This study shows that the lowering of CYFRA221-1 and CEA after the operation was quicker in the test group than in the control group, and they could be lowered to normal level at the 2nd week in the test group. This didn't happen in the control group, suggesting that DB1 has a definite anti-tumor effect.

Thus, it could be seen that DB1 could markedly improve the immune suppression aggravation that occurs in early post-operative stages in NSCLC patients, continuously enhance their immunity and afford more obvious improvement. It also has an anti-tumor action and could accelerate hematopoiesis, protect the function of essential organs, promote post-operation recovery, improve physical condition and TCM syndromes of patients and thus, elevate the patients' living quality and their confidence in rehabilitation.

REFERENCES

1. Hirschowitz EA, Foody T, Hidalgo GE, Yannelli JR. Immunization of NSCLC patients with antigen-pulsed immature autologous dendritic cells. Lung Cancer 2007; 57:365-372.
2. Abiko T, Kawamura M, Izumi Y, Oyama T, Saito Y, Kobayashi K. Prediction of anti-tumour effect of thermochemotherapy with *in vitro* thermochemosensitivity testing for non-small cell lung cancer. Int J Hyperthermia 2007;23:267-275.
3. Ghiringhelli F, Menard C, Martin F, Zitvogel L. The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. Immunol Reviews 2006;214: 229-238.
4. Schiller JH, Morgan-Ihrig C, Levitt ML. Concomitant administration of interleukin-2 plus tumor necrosis factor α in advanced non-small cell lung Cancer. Am J Clin Oncol 1995;18:47-51.
5. OUM Ji-Hyun, HAN Juhyun, MYUNG Heejoon, HLEB Marija, SHARM urendra, PARK Jungchan. Molecular mechanism of NFAT family proteins for differential regulation of the IL-2 and TNF-α promoters. Molecules Cells 2002;13: 77-84.
6. Van der Gaast A, Schoenmakers CH, Kok TC, Blijenberg BG, Splinter TA. Evaluation of a new tumour marker in patients with non-small-cell lung cancer: CYFRA21-1. Br J Cancer 1994;69: 525-528.
7. Pujol JL, Grenier J, Daurès JP, Daver A, Pujol H, Michel FB. Serum fragment of cytokeration subunit 19 measured by CYFRA21-1. Immunoradiometric assay as a marker of lung cancer. Cancer Res 1993;53:61-66.
8. Yuan SD, Ding JR, Wang SX, Cui J, Zhou JQ, Lou ZL, et al. The monitoring function of CYFRA21-1 in the treatment of non-small-cell lung cancer. Chin J Mod Med (Chin) 1997;7:9-12.

(Received August 11, 2008)
Edited by TAO Bo