

Effect of fuzheng guben granule on haematogenesis function in tumor bearing mice after chemotherapy

Objective: To observe the effect of Fuzheng Guben Granule on haematogenesis function in tumor bearing mice after chemotherapy.

Methods: Lewis lung carcinoma (LLC) model was established in 48 cases of c57bl/6 mice adapting to alternate light and dark, and were divided into such six groups as blank group, model group, chemotherapy group and Traditional Chinese Medicine (TCM) at Chen Shi (7-9 a.m) plus chemotherapy group (referred to as Chenshi group), TCM at Wushi (11 a.m. to 1p.m.) plus chemotherapy group (referred to as Wushi group) and TCM at Haishi (9 p.m. to 11 p.m.) plus chemotherapy group (referred to as Haishi group) by random number table, with 8 cases in each group. Five days after successful model establishment, the subjects were treated with Fuzheng Guben Granule and cisplatin chemotherapy different times. After 2 weeks, they were killed at the same time with the samples like blood collected for detection of related indexes.

Results: Compared with model group, white blood cells and red blood cells decreased significantly in chemotherapy group, Chenshi group and Wushi group, and the difference was statistically significant ($P < 0.05$). Compared with chemotherapy group, white blood cell count has rebounded in Chenshi, Wushi and Haishi groups with the difference being statistically significant ($P < 0.05$). Compared with Wushi group, white blood cell count rebounded more significantly in Haishi group of statistical significance ($P < 0.05$), but with no significant difference in between in red blood cell count ($P > 0.05$). Compared with model group, karyocyte count in marrow decreased significantly of statistical value ($P < 0.05$) in chemotherapy group, Chenshi group, Wushi group and Haishi group, in which the count decreased most significantly in chemotherapy group and has a varying degree of rebound in other three groups by contrast ($P < 0.05$). Compared with Chenshi group and Wushi group, the karyocyte count in marrow rebounded significantly in Haishi group of statistical value ($P < 0.05$). The levels of serum EPO and G-CSF decreased to varying degrees in all experimental groups, most significantly in chemotherapy group when compared with model group, and the difference was statistically significant ($P < 0.05$). Compared with chemotherapy group, the levels of serum EPO and G-CSF increased significantly in Haishi group of statistical value ($P < 0.05$). There were different degrees of serum EPO and G-CSF rebound in Chenshi, Wushi and Haishi groups, in which the rebound of G-CSF was more obvious in Haishi group than in Chenshi group of statistical significance, ($P < 0.05$), but with no significant change in serum EPO level ($P > 0.05$).

Conclusion: Fuzheng Guben Granule can improve chemotherapy induced bone marrow suppression in mice bearing Lewis lung cancer, and it produces varying effects on recovery of bone marrow suppression when different time is selected in its application, with the efficacy being most obvious at Haishi.

Nan Chen, Linwei Chen, Su Jiang, Zichuan Wang, Tao Liu*

School of Basic Medicine, Nanjing University of Chinese Medicine, Nanjing, 210023, PR China

*Author for correspondence: chennan3940@sina.com

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Introduction

Lung cancer is one of the most common human malignant tumors with highest morbidity and mortality in China. Most of patients have no obvious symptoms in the early stage, and more than 70% of those with symptoms are diagnosed to be in the late stage of the disease, leading to a low 5-year survival rate in patients [1]. Traditional Chinese medicine believes that [2] the occurrence and development of malignant tumors is mainly attributed to weakness of vital-qi, imbalance of Yin and Yang, disorder of the viscera function as well as causative agents, which further results in cementation of qi stagnation and blood stasis with phlegm-retention and toxin aggregation. Tumor growth will further cause loss of vital qi and then contributes to its development in turn. At present, chemotherapy is the main method for the treatment of advanced non-small cell lung cancer [3,4] but with unsatisfactory efficacy, it leads to obvious damages to normal cells of the body, including a decline in peripheral blood, bone marrow suppression and impaired immune function, which seriously affects the quality of life in patients. Today, efficacy enhancing and toxicity reducing has become a hot spot in oncology research, and traditional Chinese medicine plays an important and unique role in this field [5]. The method of "strengthening body resistance and eliminating evil" is one of the most frequent-use treatments in Chinese Medicine, Fuzheng Guben Granules applied in this study is an empirical formula from the treatment experience in advanced lung cancer in our hospital and has achieved good results in its early application. In this study, we conducted an experimental study on the model of mice bearing Lewis lung cancer to observe the effect of Fuzheng Guben Granule on Lewis lung cancer bearing mice after cisplatin chemotherapy and the effect of "time selected medication" on hematopoiesis function in mice.

Materials and methods

Animals and tumor strains

The experimental animals were 48 male SPF grade C57BL/6 inbred mice aged from 4 to 6 weeks and weighing 19~23 g and they were purchased from Nanjing Junke Biological Engineering Co. Ltd. The strains of mice bearing Lewis lung cancer were purchased from Shanghai Xinyu Biological Technology Co, Ltd.

Test drugs and reagents

Fuzheng Fuben Granules, purchased from Kaiyuan Pharmaceutical Co., Ltd. in Shanxi, consisted of *Astragalus mongholicus*, *epimedium*, *Ligustrum lucidum*, *Polygonum multiflorum*, *Rehmannia glutinosa*, madder, *Rhizoma Polygonatum* and ginseng. It was synthesized by the method of soaking, boiling and water bath with the content of crude drug as 1 g/ml, administered by perfusion according to the average dose (10g/60kg). P4394-cisplatin was provided by Shanghai Suo Bao Biological Technology Co., which was configured to the solution of 0.25 mg/ml with physiological saline for experiment. Granulocyte colony-stimulating factor (G-CSF) and the content of serum erythropoietin (EPO) were determined by sandwich ELISA with double-antibodies with the kit provided by Shanghai Xinyu Biotechnology Co. Ltd.; and the full-automatic enzyme-linked immunometric meter was purchased from Shanghai Jinggong Industrial Co, Ltd.

Modeling and grouping

A total of 60 male C57BL/6 inbred mice were fed adaptively for 1 week followed by establishing mouse model as follow. The mice cultured for 14 days was killed with cervical vertebra removal, and then immersed in 75% alcohol for 10 min for disinfection. The tumor tissue was extracted under sterile conditions, and necrosis tissue and fibrous tissue were removed to sterile containers. Then it was cut into fragments, lightly grinded into tissue homogenate with 4 ml normal saline added and filtered by 200-mesh sieve to make single cell suspension with trypan blue staining used to count the alive cells under light microscope. The cell concentration was adjusted to 1×10^7 /ml with normal saline. The above suspension (0.2 ml) was taken by 1 ml syringe and except those in blank group, every mouse was inoculated subcutaneously in the armpit of right forelimb. After model establishment, the mice were randomly divided according to random number table into blank group, model group, chemotherapy group and TCM at Chen Shi (7-9 a. m) plus chemotherapy group (referred to as Chenshi group), TCM at Wushi (11 a.m. to 1p.m.) plus chemotherapy group (referred to as Wushi group) and TCM at Haishi (9 p.m. to 11 p.m.) plus chemotherapy group (referred to as Haishi group), with 8 mice in each group.

Administration time and methods

Mice were given administration in armpit after five days of successful modeling. Those in blank group were given no treatment, those in Shenshi, Wushi and Haishi groups were treated with Fuzheng Guben Granules once a day and intraperitoneal injection of cisplatin for chemotherapy every 2 days, those in chemotherapy group were injected with cisplatin at 9: 00 a.m every 2 days and with same amount of normal saline everyday, those in control group were injected with same amount of normal saline for 2 weeks. Time was selected as follow from the Traditional Twelve Two-Hour Periods in traditional Chinese medicine: Chenshi (the time responding to stomach meridian: 7:00-9:00), Wushi (the time responding to heart meridian: 11:00-13:00) and Haishi (the time responding to SAN JIAO meridian: 21: 00-23:00).

Specimen preparation and index detection

The samples were collected from each group of mice 2 days after administration; they were drawn blood from eyeball as blood samples, which were poured to a tube for EDTA anticoagulation and 2 ml EP tube respectively. The blood in the 2 ml EP tube was centrifuged after the cold storage at 3000r/min for 15 min followed by extraction of serum placed in the upper -20°C refrigerator prepared test. The blood in the EDTA anticoagulant tube was rapidly used for the count of red blood cells and white blood cells. After the mice were killed, the bone marrow specimens were prepared from the complete left femur of mice. The prepared marrow cell liquid was put into the 5 ml EP tube to be measured. The determination of the serum granulocyte colony stimulating factor (G-CSF) and erythropoietin (EPO) in mice was conducted strictly according to the kit instructions.

Statistical methods

SPSS 22 software was used for statistical analysis, the measurement data were expressed by mean \pm standard deviation ($\bar{x} \pm s$), single factor analysis of variance was used to compare among groups and Student-Newman-Keuls (S-N-K) test was used between two groups, $P < 0.05$ suggested that there was statistically significant difference.

Results

The general condition of mice

There was no death case in 48 experimental

mice. At the beginning of the experiment, there was no significant difference among groups in general states of eating, drinking and activity. Four days after the model establishment, the scleroma, 2-5 mm in diameter, was seen in each group except the blank group, and the rate of tumor formation was 100%. With the administration time and the tumor growth, the activities of eating, drinking and skin cleaning in mice were all reduced in model group, chemotherapy group, Chenshi group, Wushi group and Haishi group in which the mice were apathetic, slow to weakness and with no glory fur. While those in blank group had no obvious change in general condition of feeding and drinking.

Comparison of peripheral white blood cell and red blood cell in mice

Compared with model group, white blood cells and red blood cells decreased significantly in chemotherapy group, Chenshi group and Wushi group, and the difference was statistically significant ($P < 0.05$). Compared with chemotherapy group, white blood cell count has rebounded in Chenshi, Wushi and Haishi groups with the difference being statistically significant ($P < 0.05$). Compared with Wushi group, white blood cell count rebounded more significantly in Haishi group of statistical significance ($P < 0.05$), but with no significant difference in between in red blood cell count ($P > 0.05$), as shown in **TABLE 1**.

Comparison of karyocyte count in marrow of mice

Compared with model group, karyocyte count in marrow decreased significantly of statistical value ($P < 0.05$) in chemotherapy group, Chenshi group, Wushi group and Haishi group, in which the count decreased most significantly in chemotherapy group, and has a varying degree of rebound in other three groups by contrast ($P < 0.05$). Compared with Chenshi group and Wushi group, the karyocyte count in marrow rebounded significantly in Haishi group of statistical value ($P < 0.05$), as shown in **TABLE 2**.

Comparison of EPO and G-CSF levels in serum of mice

The levels of serum EPO and G-CSF decreased to varying degrees in all experimental groups, most significantly in chemotherapy group when compared with model group, and the difference was statistically significant

($P < 0.05$). Compared with chemotherapy group, the levels of serum EPO and G-CSF increased significantly in Haishi group of statistical value ($P < 0.05$). There were different degrees of serum EPO and G-CSF rebound in Chenshi, Wushi and Haishi groups, in which the rebound of G-CSF was more obvious in Haishi group than in Chenshi group of statistical significance, ($P < 0.05$), but with no significant change in serum EPO level ($P > 0.05$) as shown in **TABLE 3**.

Summary: Compared with model group, white blood cells and red blood cells decreased significantly in chemotherapy group, Chenshi group and Wushi group, and the difference was statistically significant ($P < 0.05$); compared with chemotherapy group, white blood cell count has rebounded in Chenshi, Wushi and Haishi groups with the difference being statistically significant ($P < 0.05$); compared with Wushi group,

white blood cell count rebounded more significantly in Haishi group of statistical significance ($P < 0.05$), but with no significant difference in between in red blood cell count ($P > 0.05$); compared with model group, karyocyte count in marrow decreased significantly of statistical value ($P < 0.05$) in chemotherapy group, Chenshi group, Wushi group and Haishi group, in which the count decreased most significantly in chemotherapy group, and has a varying degree of rebound in other three groups by contrast ($P < 0.05$); compared with Chenshi group and Wushi group, the karyocyte count in marrow rebounded significantly in Haishi group of statistical value ($P < 0.05$); the levels of serum EPO and G-CSF decreased to varying degrees in all experimental groups, most significantly in chemotherapy group when compared with model group, and the difference was statistically significant

Table 1 Comparison of peripheral white blood cell and red blood cell in the experimental groups ($\bar{x} \pm s$)

Groups	n	White blood cell count (10^6 cells/ml)	Red blood cell count (10^9 cells/ml)
Blank group	8	6.17 \pm 0.54	7.87 \pm 0.47
Model group	8	5.54 \pm 0.48	6.66 \pm 0.28
Chemotherapy group	8	3.16 \pm 0.69*	5.43 \pm 0.27*
Chenshi group	8	4.24 \pm 0.73* Δ	6.12 \pm 0.24* Δ
Wushi group	8	4.04 \pm 0.65* Δ	6.27 \pm 0.25* Δ
Haishi group	8	4.84 \pm 0.70 Δ°	6.42 \pm 0.35 Δ

Note: compared with model group, * $P < 0.05$; compared with chemotherapy group: $\Delta P < 0.05$, compared with Wushi group, $^\circ P < 0.05$

Table 2 Comparison of karyocyte count in marrow of mice in experiment groups ($\bar{x} \pm s$)

Groups	n	Karyocyte count in marrow (10^6 cells/ml)
Blank group	8	14.05 \pm 1.27
Model group	8	11.65 \pm 1.16
Chemotherapy group	8	5.54 \pm 1.08*
Chenshi group	8	8.66 \pm 1.13* Δ
Wushi group	8	8.75 \pm 1.09* Δ
Haishi group	8	9.27 \pm 1.12* Δ°

Note: compared with model group, * $P < 0.05$; compared with chemotherapy group: $\Delta P < 0.05$, compared with Wushi group, $^\circ P < 0.05$

Table 3 Comparison of EPO and G-CSF levels in serum of mice in the experimental groups ($\bar{x} \pm s$)

Groups	n	EPO level (IU/L)	G-CSF level (ng/L)
Blank group	8	30.34 \pm 4.78	1007.40 \pm 46.23
Model group	8	26.26 \pm 3.55	743.29 \pm 40.17
Chemotherapy group	8	17.81 \pm 3.34*	527.49 \pm 30.46*
Chenshi group	8	21.73 \pm 3.12	612.18 \pm 51.06*
Wushi group	8	20.34 \pm 3.32	643.04 \pm 54.76
Haishi group	8	24.46 \pm 3.45 Δ	742.38 \pm 32.51 Δ°

Note: compared with model group, * $P < 0.05$; compared with chemotherapy group: $\Delta P < 0.05$, compared with Wushi group, $^\circ P < 0.05$

($P < 0.05$); compared with chemotherapy group, the levels of serum EPO and G-CSF increased significantly in Haishi group of statistical value ($P < 0.05$); and there were different degrees of serum EPO and G-CSF rebound in Chenshi, Wushi and Haishi groups, in which the rebound of G-CSF was more obvious in Haishi group than in Chenshi group of statistical significance, ($P < 0.05$), but with no significant change in serum EPO level ($P > 0.05$). These results suggest that Fuzheng Guben Granules can improve chemotherapy induced bone marrow suppression in mice bearing Lewis lung cancer and produces varying effects on recovery of bone marrow suppression when different time is selected in its application, with the efficacy being most obvious at Haishi.

Discussion

The treatment of malignant tumors with Chinese medicine is conducted mainly in the principle of "strengthening the body resistance to eliminate pathogenic factors", and in patients with lung cancer the body itself may have immunosuppression, so it is required to enhance the immunity of patients and improve the anti-tumor ability of body during treatment [6,7]. Fuzheng Guben granules were collected in State Food and Drug Administration (WS-5250 (B-0250) -2002), mainly composed of such Chinese herbs as *Astragalus mongholicus*, *epimedium*, *Ligustrum lucidum*, *Polygonum multiflorum*, *Rehmannia glutinosa*, madder, *Rhizoma polygonatum* and ginseng, in which *Astragalus mongholicus* is put in an important position with its effects of invigorating vital energy and blood. The combination of above herbs has the efficacy of tonifying qi and yin, blood-cooling and toxin-relieving. Fuzheng Guben granule is mainly, through the combined medication, applied to radiotherapy and chemotherapy in patients with lung cancer, esophageal cancer or gastric cancer who are suffering from Qi-Yin deficiency with heat-toxin syndrome [8-10].

Time science in TCM pays attention to medication time as well as compatibility and incompatibility of activity, supports combination of prescriptions and advocates drugs taken separately in a regular manner. Fuzheng Guben granule can reduce the chemotherapy induced bone marrow suppression in this study, which

is demonstrated by the fact that the count of white blood cells, red blood cells and nucleated cells in bone marrow all has different degrees of rebound in Chenshi, Wushi and Haishi groups. This suggests that the prescription of Fuzheng Guben granule can help to reduce bone marrow caused by chemotherapy drugs and protect marrow hematopoietic function. According to the study results, the above indexes rebounded most obviously in Haishi group of statistical significance, indicating that Fuzheng Guben granule prescription has different effects on peripheral blood and marrow hematopoiesis at varying time of drug taken. And the difference of white blood cells, red blood cells and marrow nucleated cells in rebound degree is considered to relate to the cycle of cell proliferation.

Blood cell is derived from bone marrow hematopoietic stem cells (BMHSC), and some hemopoietic growth factors such as EPO and G-CSF, GM-CSF and IL-3 acting on hematopoietic precursors can promote BMHSC proliferation and differentiation as well as maturation of various immature cells [11,12]. Study has found that [13] some Chinese herbal medicines can improve the expression of hematopoietic growth factors such as EPO and G-CSF in human serum, and then promote the hematopoiesis of bone marrow. *Astragalus mongholicus* is one of herbs commonly used for hematopoiesis repair after bone marrow suppression with its main effective ingredient as *Astragalus polysaccharides*, which is proved [14] to enhance the immune function of patients with tumor, improve their quality of life and prolong their survival time. Another research has revealed that [15] *astragalus polysaccharides* could repair hematopoietic function of mice with bone marrow suppression induced by cyclophosphamide and significantly increase the number of bone marrow cells as well as peripheral blood cells in experimental mice with a certain dose-effect relationship. In this study, the contents of EPO and G-CSF in chemotherapy group decreased, suggesting that chemotherapy can reduce the expression of hematopoietic growth factors of EPO and G-CSF in hematopoietic growth factors, and Fuzheng Guben granule can affect the expression of EPO and G-CSF in serum, demonstrated by the rebound of EPO and G-CSF in Chenshi, Wushi and Haishi groups. Besides, the experimental

results also showed that rebound of G-CSF content is most obvious in Haishi group, illustrating that medication at varying time periods has different effects on body hematopoietic function, and chronomedicine in medical application is aimed to figure out the optimum time when drugs have largest lethality to tumor cells and least damages to normal tissues in chemotherapy as well as the best time to promotion of tissue recovery after chemotherapy treatment. We think that it may be related to the effect of time-selected medication on promotion of hematopoietic factor release, and the sensitivity of bone marrow progenitors to hematopoietic factors is varying with time. The specific mechanism needs to be further studied.

In conclusion, Fuzheng Guben Granule can improve chemotherapy induced bone marrow suppression in mice bearing Lewis lung cancer, and it produces varying effects on recovery of bone marrow suppression when different time is selected in its application, with the efficacy being most obvious at Haishi.

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