Tan J, Yang RD, Zhao H, *et al*. Effect of moxibustion on T lymphocyte subsets in peripheral blood of rats with gastric cancer. J Acupunct Tuina Sci, 2019, 17(3): 161-167
DOI: https://doi.org/10.1007/s11726-019-1107-x

Basic Study

Effect of moxibustion on T lymphocyte subsets in peripheral blood of rats with gastric cancer

艾灸对胃荷瘤大鼠外周血中T淋巴细胞亚群的影响

Tan Jing (谭静), Yang Ren-da (阳仁达), Zhao Huan (赵欢), Peng Zhuo-jun (彭卓隽), Ouyang Li-zhi (欧阳里知), Lin Ya-ping (林亚平)
Hunan University of Chinese Medicine, Changsha 410208, China

Abstract

Objective: To observe the effect of moxibustion on T lymphocyte subsets in peripheral blood of rats with gastric cancer.

Methods: Sixty healthy Sprague-Dawley (SD) rats were adaptively fed for one week. By the random number table method, 10 rats were randomly selected as a blank group, and 12 rats were randomly selected to simulate the tumor transplantation process; after transplantation, 10 rats were randomly selected as a sham operation group. The remaining 38 rats were used to prepare gastric cancer models by gastric transplantation of the Walker-256 tumor tissue; 8 rats were randomly selected to verify the successful modeling after 7 d; the remaining 30 rats were randomly divided into a model group, a moxibustion group and an infrared group by the random number table method, with 10 rats in each group. From the first day of enrollment, the rats in the moxibustion group received mild moxibustion at Zhongwan (CV 12), Guanyuan (CV 4) and bilateral Zusanli (ST 36) (the first group) and bilateral Pishu (BL 20) and Weishu (BL 21) (the second group), and the two groups of acupoints were alternated every other day. The rats in the infrared group received infrared radiation on the stomach area and the area on the back between the T_{12}-T_{13} spinous processes, the two areas were alternated every other day. Rats in the moxibustion group and the infrared group were treated for 20 min each time, once a day for 21 d. Rats in the blank group, the sham operation group, and the model group were simultaneously grasped and fixed, and no other treatment was performed. After 21 d of intervention, the rats in each group were fasted for 12 h, and blood was collected from the orbits. The numbers of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$ T lymphocytes in peripheral blood were determined by flow cytometry, and the ratio of $CD3^+CD4^+/CD3^+CD8^+$ was calculated. The rats were sacrificed and the thymus was dissected under sterile conditions to calculate the thymus index.

Results: Compared with the blank group, the thymus index, peripheral blood $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD3^+CD4^+/CD3^+CD8^+$ ratio in the sham operation group did not change significantly (all $P>0.05$). Compared with the blank group, the thymus index of the model group was increased ($P<0.05$), the $CD3^+$ and $CD3^+CD8^+$ T lymphocytes were increased (both $P<0.01$), and the $CD3^+CD4^+/CD3^+CD8^+$ ratio was decreased ($P<0.05$). Compared with the model group, the thymus index of the moxibustion group was increased ($P<0.01$), and $CD3^+$, CD3+CD^{4+} and $CD3^+CD8^+$ T lymphocytes and the ratio of $CD3^+CD4^+/CD3^+CD8^+$ in peripheral blood were increased (all $P<0.05$). Compared with the infrared group, the thymus index of the moxibustion group was significantly increased ($P<0.05$), the $CD3^+$ and $CD3^+CD4^+$ T lymphocytes in the peripheral blood were significantly increased (both $P<0.01$), and the $CD3^+CD8^+$ was increased ($P<0.05$).

Conclusion: Moxibustion can significantly increase the thymus index of gastric cancer-bearing rats and activate $CD3^+CD4^+$ and $CD3^+CD8^+$ T lymphocytes in peripheral blood.

Keywords: Acupuncture-moxibustion Therapy; Moxibustion Therapy; Moxa Stick Moxibustion; Neoplasms; Stomach; T-lymphocytes; Immunity, Cellular; Rats

【摘要】目的：观察艾灸对胃荷瘤大鼠外周血中 T 淋巴细胞亚群的影响。**方法：**将 60 只健康 Sprague-Dawley (SD) 大鼠适应性喂养一周后，按随机数字表法随机选出 10 只作为空白组，同时随机选出 12 只通过手术模拟瘤体移植过程，成功后，随机将 10 只作为假手术组。剩余 38 只采用 Walker-256 瘤组织胃部移植制备胃荷瘤模型，7 d 后随机选取 8 只验证造模成功，剩余 30 只用随机数字表法依次分入模型组、艾灸组和红外组，每组 10 只。自入组第 1 日起，艾灸组接受温和灸中脘、关元和双侧足三里(第一组)以及双侧脾俞和胃俞(第二组)治疗，两组穴位隔日交替使用。红外组接受红外线照射胃脘部及背部 T_{12}-T_{13} 棘突间两个区域，两个区域隔日交替使用。艾灸组、红外组每次干预 20 min，每日 1 次，连续 21 d。空白组、假手术组和模型组同时间抓取、固定，不予其他处理。干预 21 d 后，各组大鼠禁食不禁水 12 h，眼眶采血，流式细胞仪测定外周血中 $CD3^+$、$CD3^+CD4^+$和 $CD3^+CD8^+$ T 淋巴细胞

Author: Tan Jing, doctoral degree candidate, lecturer
Corresponding Author: Lin Ya-ping, M.M., professor, doctoral supervisor.
E-mail: 421192639@qq.com

含量，计算$CD3^+CD4^+/CD3^+CD8^+$比值。处死动物，无菌条件下剖取胸腺，计算胸腺指数。**结果**：与空白组比较，假手术组大鼠胸腺指数、外周血中$CD3^+$、$CD3^+CD4^+$、$CD3^+CD8^+$及$CD3^+CD4^+/CD3^+CD8^+$比值均无显著变化(均$P>0.05$)。与空白组比较，模型组大鼠胸腺指数增高($P<0.05$)，外周血中$CD3^+$、$CD3^+CD8^+$ T淋巴细胞增多(均$P<0.01$)，$CD3^+CD4^+/CD3^+CD8^+$比值下降($P<0.05$)。与模型组比较，艾灸组大鼠胸腺指数增高($P<0.01$)，外周血中$CD3^+$、$CD3^+CD4^+$和$CD3^+CD8^+$ T淋巴细胞以及$CD3^+CD4^+/CD3^+CD8^+$比值均增加(均$P<0.05$)。与红外组比较，艾灸组大鼠胸腺指数明显增高($P<0.05$)，外周血中$CD3^+$和$CD3^+CD4^+$ T淋巴细胞显著增加(均$P<0.01$)，$CD3^+CD8^+$稍有增加($P<0.05$)。**结论**：艾灸可明显提高胃荷瘤大鼠胸腺指数，激活外周血中$CD3^+CD4^+$和$CD3^+CD8^+$ T淋巴细胞。

【关键词】针灸疗法；灸法；艾条灸；肿瘤；胃；T淋巴细胞；细胞免疫；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Gastric cancer (GC) is a malignant tumor derived from gastric epithelial cells mainly characterized by abdominal discomfort, pain, vomiting, etc., and relatively poor prognosis. Due to bad food habits and other reasons, the incidence and mortality of GC in China are far higher than the global average, causing a serious burden on families and society[1-2]. Although surgery plus radiotherapy and chemotherapy is still the main treatment method of GC, other anti-cancer methods such as immunotherapy and thermotherapy are continuously explored and generalized. Studies have shown that[3], high temperature environment can change cell membrane permeability and fluidity, and promote cell apoptosis by destroying the cytoskeleton and disturbing the metabolism. Because the tumor tissue is rich in blood vessels and poor in heat dissipation, it is easier to absorb energy to get warmer and apoptosis. At the same time, with the increase of body temperature, the body's immune function, such as the number and activity of lymphocytes, is activated, which greatly enhances the immune clearance of tumor cells[4], the most suitable temperature is 40-42 ℃. With the development of science and technology, the therapeutic mechanism of traditional moxibustion has been continuously revealed. Modern research has confirmed that moxibustion can activate the meridian system in the body, through comprehensive thermal stimulation to achieve prevention and treatment of diseases[5]. Although moxibustion has been widely used in the symptomatic treatment of various adverse reactions after surgery, radiotherapy and chemotherapy in cancer patients[6-7], the tumor suppressive effect of moxibustion itself has not been explored in depth[8]. Studies have shown that when moxibustion (mild moxibustion) is applied, the skin temperature is around 42 ℃ and the human body feels comfortable, which can enhance the immune function of the body[6]. Our previous study showed that when mild moxibustion was applied to gastric cancer-bearing rats using a 7 mm diameter moxa stick, the test rats did not escape and struggle, and the skin temperature was around 42 ℃. After 21 d of moxibustion treatment, the number of natural killer (NK) cells in the peripheral blood of the tumor-bearing rats was increased significantly[9]. The following experiment was conducted for the purpose of exploring if moxibustion also affects other immune cells such as T lymphocyte subsets.

1 Materials and Methods

1.1 Laboratory animals and grouping

Six healthy SPF Sprague-Dawley (SD) rats (male, weighing 160-180 g) were used to prepare subcutaneous solid tumors; 60 healthy SPF SD rats (male, weighing 200-240 g) were used for group experiments.

Grouping method: Sixty healthy Sprague-Dawley (SD) rats were adaptively fed for one week. By random number table method, 10 rats were randomly selected as a blank group, and 12 rats were randomly selected to simulate the tumor transplantation process; after transplantation, 10 rats were randomly selected as a sham operation group. The remaining 38 rats were used to prepare gastric cancer models by gastric transplantation of the Walker-256 tumor tissues; 8 rats were randomly selected to verify the successful modeling after 7 d; the remaining 30 rats were randomly divided into a model group, a moxibustion group and an infrared group by random number table method, with 10 rats in each group.

The experimental animals were provided by the China Food and Drug Control Research Institute [qualification number: SCXK (Beijing) 2014-0013], and kept in cages at the Animal Experimental Center of Cancer Hospital Chinese Academy of Medical Sciences. The feeding temperature and the humidity were 20-25 ℃ and 50%-70%, respectively. Rats were treated following the relevant ethical regulations throughout the experiments.

1.2 Main reagents and instruments

Walker-256 cells (Cancer Hospital Chinese Academy of Medical Sciences, China); lymphocyte separation solution and red blood cell lysis solution (Huamei Bio, China); rat CD3-APC, CD4-PE and CD8a-PE-Cyanine 7 (eBioscience, USA); monoclonal antibody (Sigma, USA); LSRII flow cytometer (Becton Dickinson, USA); LD5-10B low speed centrifuge (Beijing Jingli Centrifuge Co., Ltd., China); 6002B infrared therapeutic apparatus (Xuzhou Tianfei Electronic Equipment Co., Ltd., China); digital surface thermometer (Zhejiang Bosiou, China); moxa

stick (7 mm in diameter) (Changsha Aiyi Technology Co., Ltd., China); homemade small animal moxibustion frame.

1.3 Model preparation method

Animal models were prepared by orthotopic transplantation of tumor tissues based on the references[10-12]. The brief steps are as follows: Three rats (weighing 160-180 g) were intraperitoneally injected with 6.0×10^6 Walker-256 cells. After the ascites was formed, the rats were sacrificed, Walker-256 cells in ascites were collected and adjusted to 0.5×10^7 cells/mL, then 1.0×10^5 cells were injected subcutaneously into the fore- and hind limbs of three rats with a body weight of 160-180 g. After 7 d, subcutaneous solid tumors with a size of about 1.5 cm×1.5 cm×1.0 cm were formed at the injection sites. The rats were sacrificed and fresh fish-like tumor tissues with a size of 0.5 cm×0.5 cm×0.5 cm were sutured at the junction of the skin and gland areas of the arcus major ventriculi. Laparotomy was performed after 7 d of tumor transplantation. A tumor tissue with a diameter of 1 cm at the original transplantation site showing a smooth surface and clear boundary, no adhesion and organ invasion indicated successful modeling.

1.4 Intervention method

1.4.1 Blank group

Normally reared, grasped and fixed in the moxibustion group and the infrared group without other treatment.

1.4.2 Sham operation group

The laparotomy, stomach wall stimulation, and abdomen closure were performed during the modeling process, and the tumor tissue was not transplanted. The grasping and fixing were performed without other treatment used in the moxibustion group and the infrared group.

1.4.3 Model group

Rats in the model group received modeling. Grasping and fixing were performed without other process used in the moxibustion group and the infrared group.

1.4.4 Moxibustion group

From the first day of enrollment, the rats received mild moxibustion. Two groups of acupoints were applied, the first group was Zhongwan (CV 12), Guanyuan (CV 4) and bilateral Zusanli (ST 36), and the second group was bilateral Pishu (BL 20) and Weishu (BL 21). The acupoint location was determined referring to *Experimental Acupuncture Science*[13]. The two groups of acupoints were alternately applied with moxibustion every other day. When applying moxibustion, the moxa stick of 7 mm in diameter was fixed on the homemade moxibustion rack and ignited vertically above the acupoint. During the moxibustion, the surface temperature was measured using a surface thermometer, the height was adjusted according to the temperature degree, and the ash was removed in time to ensure that the skin temperature was maintained at (42±1) ℃. Moxibustion was conducted for 20 min each time, once a day for 21 d.

1.4.5 Infrared group

Infrared irradiation was performed from the day after enrollment. Irradiation sites included the stomach area and the area on the back between the T_{12}-T_{13} spinous processes, and the two areas were alternated every other day. For the infrared, the wavelength was 0.4-3.0 μm, the intensity was grade 1, and the spot diameter was 3 cm. During the irradiation, the surface temperature was measured using a surface thermometer, and the irradiation height was adjusted according to the temperature degree to ensure that the skin temperature was maintained at (42±1) ℃. Moxibustion was conducted for 20 min each time, once a day for 21 d.

1.5 Observed items and detection methods

1.5.1 Peripheral blood T cell subset detection

The next day after the intervention, the rats in each group were fasted for 12 h, and 2 mL blood was collected from the orbits for flow cytometry detection. At the time of detection, 100 μL of peripheral anticoagulation blood within 24 h was added to the bottom of the flow cytometry tube; appropriate amount of CD3 APC, CD4 PE, CD8 PE-Cy7 fluorescently labeled antibodies were added; and incubated at room temperature for 20 min in the dark after mixing; 2 mL of red blood cell lysate was added and mixed, and red blood cells were lysed at room temperature for 10 min; centrifuged at 2 000 r/min for 5 min, discarded the supernatant, added 2 mL PBS, mixed at 2 000 r/min for 5 min and discarded the supernatant; added 500 μL PBS and mixed, and applied to the cytometer to obtain the analysis by FACS Diva6.0 software.

1.5.2 Thymus indexes

After blood collection, the rats were sacrificed, the thoracic cavities were dissected in a sterile environment, the thymuses were removed; after washed with PBS, the thymuses were blotted dry with sterile gauze, and the weights were measured with an electronic precision balance. Thymus index = Thymus weight (mg) ÷ Rat body weight (g).

1.6 Statistical methods

All data were processed using SPSS 20.0 software. All measurement data were tested for normality and homogeneity of variance. When normality was satisfied, the data were expressed as mean ± standard deviation ($\bar{x}\pm s$). For comparison among multiple groups, one-way ANOVA was used, when the variance was equal, the least significant difference (LSD) was used; when the variance was not equal, the Dunnett T3 method was

used. The difference was statistically significant at $P<0.05$.

2 Results

2.1 Comparisons of thymus index

Compared with the blank group, there was no significant difference in thymus index in the sham operation group ($P>0.05$), while the thymus index of the model group was significantly increased ($P<0.01$). Compared with the model group, the thymus index of the moxibustion group was significantly increased ($P<0.01$), and the thymus index of the infrared group was increased ($P<0.05$). Compared with the infrared group, the thymus index of the moxibustion group was significantly increased ($P<0.05$). The details are shown in Table 1.

Table 1. Comparisons of thymus index of rats in each group

Group	*n*	Thymus index
Blank	10	1.1906±0.0859
Sham operation	10	1.1892±0.0990
Model	10	1.5178±0.1820[1)]
Moxibustion	10	1.8684±0.2165[2)3)]
Infrared	10	1.6390±0.2199

Note: Compared with the blank group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$; compared with the infrared group, 3) $P<0.05$

2.2 Comparisons of lymphocyte subsets

Compared with the blank group, the percentages of $CD3^+$ and $CD3^+CD8^+$ T lymphocytes in the model group were significantly increased (both $P<0.01$), and the ratio of $CD3^+CD4^+/CD3^+CD8^+$ was decreased ($P<0.05$). Compared with the model group, the $CD3^+CD8^+$ and the ratio of $CD3^+CD4^+/CD3^+CD8^+$ in the moxibustion group were increased (both $P<0.05$), and $CD3^+$ and $CD3^+CD4^+$ were significantly increased (both $P<0.01$). Compared with the infrared group, the percentages of $CD3^+$ and $CD3^+CD4^+$ in the moxibustion group were significantly increased (both $P<0.01$), and $CD3^+CD8^+$ was also increased ($P<0.05$). Please see Figure 1-Figure 5.

3 Discussion

GC is one of the diseases that threaten human health. According to statistics, the number of cancer deaths in the world is as high as 8 million per year, and it is increasing at a rate of 3% every year[14]. In China, due to the bad diet habits and living environment, the incidence and mortality of gastric cancer are much higher than the global average. More than half a century of research has confirmed that the immune status of the body is closely related to the occurrence and development of tumors. On the one hand, the weakening of immune surveillance and immune clearance promotes tumorigenesis. On the other hand, tumorigenesis weakens the immune function of the body through various ways, and ultimately constitutes an important part of the malignant causal transformation chain in the process of tumor development. Therefore, improving the body's immune function, promoting immune killing of tumors, and reducing tumor immune escape are the keys to tumor management[15].

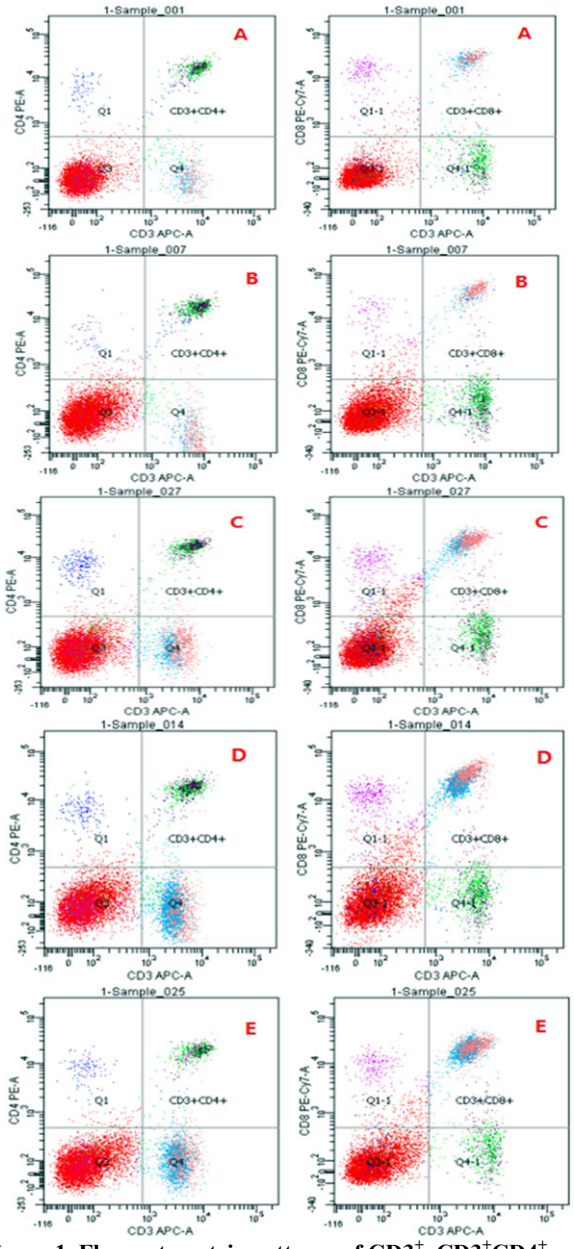

Figure 1. Flow cytometric patterns of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells in peripheral blood of rats in each group

Note: A=Blank group; B=Sham operation group; C=Model group; D=Moxibustion group; E=Infrared group

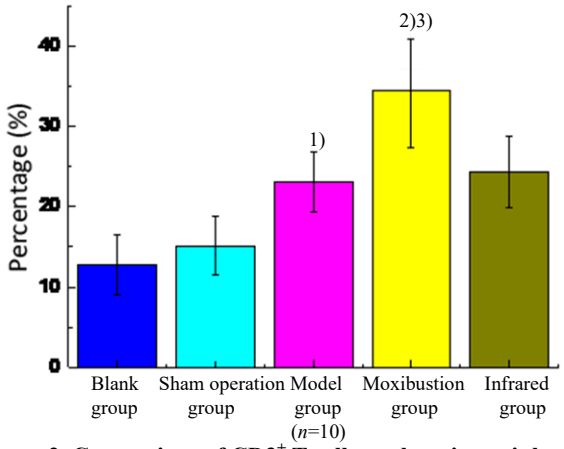

Figure 2. Comparison of $CD3^+$ T cell numbers in peripheral blood of rats in each group

Note: Compared with the blank group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$; compared with the infrared group, 3) $P<0.01$

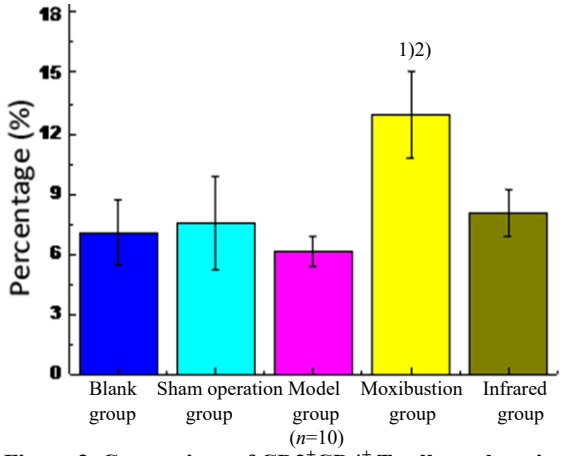

Figure 3. Comparison of $CD3^+CD4^+$ T cell numbers in peripheral blood of rats in each group

Note: Compared with the model group, 1) $P<0.01$; compared with the infrared group, 2) $P<0.01$

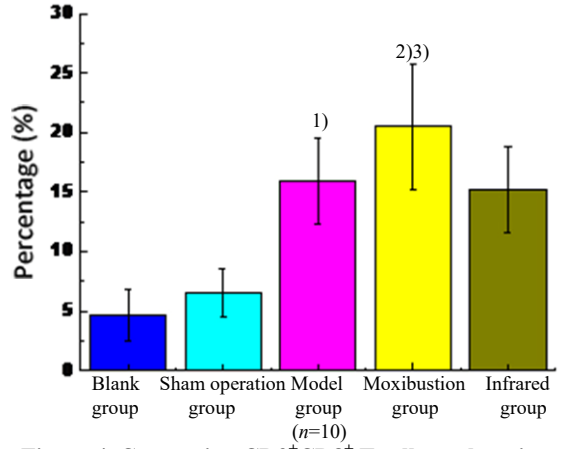

Figure 4. Comparing $CD3^+CD8^+$ T cell numbers in peripheral blood of rats in each group

Note: Compared with the blank group, 1) $P<0.01$; compared with the model group, 2) $P<0.05$; compared with the infrared group, 3) $P<0.05$

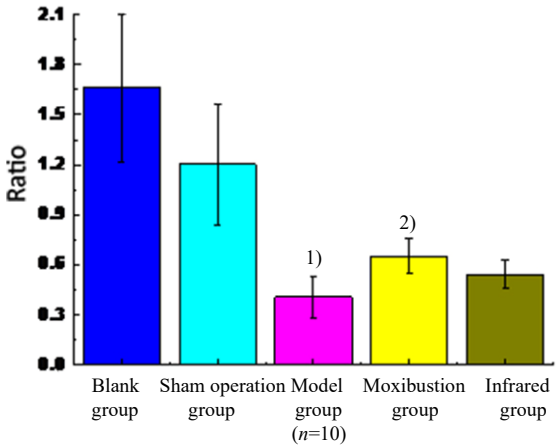

Figure 5. Comparing $CD3^+CD4^+/CD3^+CD8^+$ ratio in peripheral blood of rats in each group

Note: Compared with the blank group, 1) $P<0.01$; compared with the model group, 2) $P<0.05$

In recent years, researchers have used physical factors to produce heat in the body to 'kill' cancer cells based on the characteristics that 'cancer cells are more sensitive to high temperatures than normal cells', making it a new cancer treatment method after tumor immunotherapy. Tumor thermotherapy not only has direct cytotoxic effects, but also enhances the body's immunity, enhances the efficacy of radiotherapy and chemotherapy, and improves the quality of patient life. Studies have confirmed that the tumor temperature is 5-10 ℃ higher than the normal tissues during thermotherapy[16]. On one hand, high temperature increases cell membrane permeability and inactivates enzymes to promote programmed cell death[17]. On the other hand, high temperature accelerates blood circulation, increases vascular pressure, causes rupture of blood vessels, and collapses the tumor[18].

Moxibustion is an important part of traditional Chinese therapy. Chinese medicine believes that the warm characteristics of moxibustion are suitable for the symptoms of 'deficiency', 'cold', 'phlegm', 'stagnation' in cancer. A large number of literatures have confirmed that moxibustion has been widely used in the control and management of symptoms in cancer patients, such as reducing the side effects of postoperative cancer, radiotherapy and chemotherapy, improving the quality of life, etc., especially in improving the immune function of patients[19-21]. Modern research believes that warm stimulation is the main role of moxibustion, which is derived from the infrared and red light released by the burning of the moxa leaves. Compared with infrared thermotherapy, the heat of moxibustion can activate the sensory layers of the skin and regulate the whole body through the neuro-endocrine-immune network[22].

The neuro-endocrine-immune network of the human body is a complex regulatory system in which T lymphocytes are an important player in the network. T lymphocytes originate from lymphoid stem cells in the bone marrow. After being differentiated and matured in the thymus, they are distributed to the immune organs and tissues of the whole body through lymphatic and blood circulation, and play an important role in cell and humoral immunity. T lymphocytes express a variety of membrane proteins, such as CD3, CD4 and CD8 molecules, which are involved in T cell recognition, activation and proliferation. In the early stage of differentiation, T lymphocytes do not express CD4 molecules and CD8 molecules, called double- negative T cells (CD4-CD8-T), and do not have any function. Subsequently, CD4-CD8-T cells are selected to form double positive T lymphocytes ($CD4^+CD8^+$ T) via the β-chain and α-chain precursors of the T cell receptor (TCR), but still do not have any function. $CD4^+CD8^+$ T cells are then selectively differentiated into one of the $CD4^+$ T or $CD8^+$ T by major histocompatibility complex (MHC) class I and class II molecules (there is still no activity at this time). Then, the above cells are double stimulated by CD3 and T lymphocyte TCR, and the activation signal generated is transmitted to the inside of the T lymphocytes to become immunologically active $CD3^+CD4^+$ T lymphocytes and $CD4^+CD8^+$ T lymphocytes.

$CD3^+CD4^+$ T cells, also known as T helper (Th) cells, are mainly used to recognize soluble antigens secreted by tumor cells with the help of antigen-presenting cells, and then release various cytokines to promote activation of B cells, macrophages, and NK cells to promote the killing of tumor cells[23]. $CD4^+CD8^+$ T lymphocytes are also called cytotoxic T (Tc) cells. Mature cytotoxic T cells are contacted with target cells after antigen stimulation, release perforin and embed in the target cell membrane to form multimers. The extracellular fluid enters the target cells through the tubular structure, resulting in increased intracellular pressure and cell lysis. In addition, Tc cells also secrete granzymes, enter the target cells from small holes, and induce apoptosis of target cells. Since Tc cells are not damaged by themselves during the process of killing target cells, they can repeatedly act on target cells and have a strong tumor killing effect[24].

Modern research has confirmed that moxibustion has unique advantages in regulating the immune system, enhancing the function of immune organs, regulating immune cells and immune factors in both directions, improving the ability of immune surveillance and immune defense, and correcting the immune function of disorders[25]. Some scholars used evidence-based medicine to evaluate the effect of moxibustion on the immune function of patients with malignant tumors. It was found that moxibustion could improve the immune function of tumor patients to a certain extent, but more researches are needed[26]. In this study, compared with the blank group, there were no differences in all indexes of the sham-operated rats, but the thymus index and other indicators in the model group were different, indicating that these changes were caused by gastric tumors, but not caused by surgery. Since the tumor-bearing animals used to be healthy SD rats, the samples were collected 21 d after the model was established, that was, early tumor-bearing. During this period, the tumor tissue could induce the activation of the autoimmune system in the tumor-bearing animals, so the numbers of T cells and Tc cells in the model group were increased. Compared with the model group, moxibustion could further increase the numbers of T cells and Tc cells, and significantly increase Th cells (P<0.01). Studies have shown that, the initial anti-tumor immune response is mainly involved in Tc and NK cells, but Th cells have important memory functions in tumor immunity, so the maintenance of anti-tumor effect requires Th cells[27]. The results of this experiment suggest that moxibustion can activate immune cells in many aspects, and may produce a more obvious advantage in the subsequent anti-tumor effect. In addition, after activation, Th cells can further differentiate into Th1 and Th2 cells under the action of different cytokines, and further play a role in tumor immunity by secreting various cytokines such as interferon-γ (IFN-γ) and interleukin-2 (IL-2). What cytokines are released by moxibustion? In which immune links will these cytokines work? These need to be further explored.

Conflict of Interest

There was no potential conflict of interest in this article.

Acknowledgments

This work was supported by National Natural Science Foundation of China (国家自然科学基金项目, No. 81574077) and the Education Department of Hunan Province (湖南省教育厅优秀青年项目, No. 18B257).

Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

Received: 10 October 2018/Accepted: 30 October 2018

References

[1] Li M, Wan X, Wang Y, Sun Y, Yang G, Wang L. Time trends of esophageal and gastric cancer mortality in China, 1991-2009: an age-period-cohort analysis. Sci Rep, 2017, 7(1): 6797.

[2] Zong L, Abe M, Seto Y, Ji J. The challenge of screening for early gastric cancer in China. Lancet, 2016, 388(10060): 2606.

[3] Wang XL, Sun RM. Mechanism and clinical study of tumor hyperthermia. Keji Zixun, 2016, 14(25): 181-182.

[4] Yu JA, Wang BB. Latest research progress of solid tumor hyperthermia. Liaoning Zhongyiyao Daxue Xuebao, 2017, 19(1): 221-224.
[5] Guo Y. Experimental Acupuncture Science. Beijing: China Press of Traditional Chinese Medicine, 2016: 110.
[6] Lu L, Fu WB, Liu Y, Qu YC, Wang J. Meta analysis on moxibustion for QLQ-C30 before and after chemotherapy in patients with malignant tumor. Yixue Yanjiusheng Xuebao, 2016, 29(1): 75-82.
[7] Shi KJ. Meta-analysis of Moxibustion for Prevention and Treatment of Leukopenia after Chemotherapy for Malignant Tumors. Beijing: Master Thesis of Beijing University of Chinese Medicine, 2018: 34-35.
[8] Hou XF, Ni GX. Research progress in moxibustion for malignant tumors. Shijie Zhongxiyi Jiehe Zazhi, 2014, 9(1): 101-104.
[9] Ouyang LZ, Tan J, Zhao H, Peng ZJ, Dai LW, Chen YP, Li TL, Lin YP. Effect of moxibustion on CD3-CD161$^+$ NK and CD3$^+$CD161$^+$ NKT in peripheral blood of tumor bearing gastric carcinoma rats. Hunan Zhongyiyao Daxue Xuebao, 2018, 38(5): 541-545.
[10] Sui YZ, Xia YJ, Wang XJ, Kan SH, Kong FZ. Construction of implanted gastric cancer model of SD rats and comparison of inducing-cancer rates with different construction ways. Zhongguo Linchuang Yanjiu, 2014, 27(2): 135-137.
[11] Zhang JR, Wang DR. Experimental animal model of gastric cancer: an overview of current research. Zhongguo Putong Waike Zazhi, 2013, 22(6): 771-775.
[12] Yuan YT. Establishment of Animal Model of Gastric Carcinoma *in Situ* with Lymph Node Metastasis. Nanjing: Master Thesis of Nanjing University, 2016.
[13] Yu SG, Guo Y. Experimental Acupuncture Science. Shanghai: Shanghai Scientific & Technical Publishers, 2009: 150.
[14] Waldum HL, Sagatun L, Mjønes P. Gastrin and gastric cancer. Front Endocrinol (Lausanne), 2017, 8: 1.
[15] Gao XJ, Ba Y. Research progress of immunotherapy for gastric cancer. Zhongguo Zhongliu Linchuang, 2018, 45(3): 152-156.
[16] Lin HC. Advances in research on tumor hyperthermia mechanism and clinical application. Linchuang Yiyao Wenxian Dianzi Zazhi, 2018, 5(16): 197-198.
[17] Fan YF, Qin Y, Li DG, Kerr D. Retrospective clinical study of advanced pancreatic cancer treated with chemotherapy and abdominal hyperthermia. J Glob Oncol, 2018, 4: 1-4.
[18] Maluta S, Kolff MW. Role of hyperthermia in breast cancer locoregional recurrence: a review. Breast Care (Basel), 2015, 10(6): 408-412.
[19] Mo T, Tian H, Yue SB, Fan ZN, Zhang ZL. Clinical observation of acupoint moxibustion on leukocytopenia caused by tumor chemotherapy. Shijie Zhongyiyao, 2016, 11(10): 2120-2122.
[20] Li L, Wang P, He W, Yuan HL, Jiao YW, Yang QH, Zhang CJ, Si HB. The effect of acupoint moxibustion on the treatment of nausea and vomiting in patients with end stage tumor. Shizhen Guoyi Guoyao, 2016, 27(6): 1417-1418.
[21] Chen L, Yao LG, Kong TD, Wang LY, Duan FF, Zhang KK. Clinical observation on moxibustion in prevention and treatment of bone marrow suppression caused by chemotherapy in patients with malignant tumor. Zhongguo Jixu Yixue Jiaoyu, 2016, 8(10): 174-175.
[22] Guo Y. Experimental Acupuncture Science. Beijing: China Press of Traditional Chinese Medicine, 2016: 103.
[23] Tong MH, Shao J, Chen YH, Xing Y. Change on CD3$^+$, CD4$^+$ and CD8$^+$ T lymphocyte levels in peripheral blood of patients with gastric cancer. Jianyan Yixue, 2012, 27(6): 445-447.
[24] Zhang J. Expression and Clinical Significance of miR-195 and Tumor Infiltrating Lymphocytes CD3$^+$CD4$^+$CD8$^+$ in Gastric Carcinoma. Zhenjiang: Master Thesis of Jiangsu University, 2016: 233-236.
[25] Zhan ZJ, Wang J. The influence of moxibustion on human body's immune function. Zhongyi Xuebao, 2016, 31(3): 449-452.
[26] Tao SC, Li JH, Xu YY. Meta analysis for moxibustion on the immune function of patients with malignant tumor. Zhongguo Linchuang Baojian Zazhi, 2018, 21(4): 509-513.
[27] Klink M. Interaction of immune and cancer cells. Anticancer Res, 2014, 34(6): 203-206.

Translator: Yang Yan-ping (杨燕萍)