ISSN : 0974 - 7435 Volume 10 Issue 24

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(24), 2014 [14849-14853]

Effect of fuzheng paidu tablet on immune function of immunosuppressive mice induced by cyclophosphamide

Weiyun Xin, Ming Bai, Shuo Tian, Jiaojiao Jia, Mingsan Miao*
Henan University of Traditional Chinese Medicine, Zhengzhou 450008, (CHINA)
E-mail: miaomingsan@163.com

ABSTRACT

Objective: To explore the effect of Fuzheng Paidu tablet on cyclophosphamide(CY) induced immunosuppressed mice immune function. Methods: with mushrooms polysaccharide as positive control mice, inhibit the immune function of model induced by cyclophosphamide, observe the high, middle and low dose of Fuzheng Paidu tablet (Fuzheng Paidu-HD, Fuzheng Paidu-MD, Fuzheng Paidu-LD)on immunosuppressive mice induced by cyclophosphamide, the influence of macrophage phagocytic percentage and phagocytic index of hemolysin and hemolytic plaque forming and influence of lymphocyte transformation. Results: Cyclophosphamide immunosuppression model was successfully created. Compared with the model group, high, middle and low dose of Fuzheng Paidu tablet could significantly improve the inhibition of mouse peritoneal macrophage phagocytic percentage, phagocytic index of cyclophosphamide induced immunity, and could promote the formation of hemolysin and hemolysis and the transformation of lymphocyte ($P<0.01$), Fuzheng Paidu-HD had the strongest effect. Conclusion: Fuzheng Paidu tablet has good improvement effect on immune function of mice induced by cyclophosphamide low.

KEYWORDS

Fuzheng; Attenuated; Cyclophosphamide; Immunosuppression.

INTRODUCTION

Fuzheng Paidu tablet is made up of Xiyangshen, Huangqi, Lianqiao, licorice and other drugs, has the effects of Supplementing Qi and nourishing Yin, clearing heat and detoxification, applicable to the clinical treatment of Qi Yin deficiency, intrinsic heat toxin asymptomatic human immunodeficiency virus infection. The party had decoction for early treatment of AIDS patients, the effect is good, the related basic research, clinical observation and application, has obtained the approval of hospital preparations. AIDS (acquired immunodeficiency syndrome) by the AIDS virus (HIV) caused serious damage to the immune system of the whole body of the infectious diseases [1]. But at present, the asymptomatic HIV infection is still no drugs available, the AIDS virus infection of $CD4^+$ cells, immune function injury patients, AIDS patients and asymptomatic human immunodeficiency virus infection has the characteristics of low immunity [2], only the $CD4^+$ below 200 can use antiviral drugs, and before this AIDS patients can do nothing but only wait. Clinical on Fuzheng Paidu tablet is mainly used for asymptomatic HIV infection. In order to investigate the effect of Fuzheng Paidu tablet characteristics, to observe its effect on cyclophosphamide induced immunosuppressed mice immune function, this paper reports the effects of Fuzheng Paidu tablet on immune function induced by cyclophosphamide in mice model with low.

MATERIALS AND METHODOLOGY

MATERIALS

Instruments and reagents drugs::UV-2000 UV VIS spectrophotometer, unique (Shanghai) Instrument Co. Ltd; FA (N)/JA (N) series electronic balance, Shanghai Minqiao Precision Instrument Co Ltd; TDL-40B type centrifuge, Shanghai Anting scientific instrument factory production; BI-2000 medical image analyzer, Chengdu taimeng Technology Co., Ltd production.Rapid Wright's stain, Mike Technology Co., Ltd production; phytohemagglutinin (PHA), Shanghai Yihua Medical Technology Co.Ltd production; physiological saline, Zhengzhou chemical pharmaceutical factory production;Fuzheng Paidu tablet (Henan onitl Pharmaceutical Company Limited production); mushrooms polysaccharide tablet (Zhejiang Apeloa natural medicines limited production); cyclophosphamide injection (Jiangsu Hengrui Medicine Co., Ltd production).

Animals **:**Kunming mice of clean grade, provided by Wuhan, biological products inspection, Certificate No. 0036868, body weight 18~21g, 6 weeks old, half male and half female; guinea pig, the British Shorthair, ordinary level, provided by the Wuhan biological products inspection, Certificate No. 0036869.

METHODOLOGY

Effect of inhibition of phagocytosis of mouse peritoneal macrophages induced by cyclophosphamide immune

Took 60 Kunming mice, weighing 18~21g, half male and female, were randomly divided into 6 groups, 10 rats in each group. Divided into blank group, model group, mushrooms polysaccharide group, Fuzheng Paidu tablet in high dose group, middle dose group, low dose group (Fuzheng Paidu-HD, Fuzheng Paidu-MD, Fuzheng Paidu-LD). 5 of them were made mice induced by CY immunosuppression model, in the administration of first, second and three days, were injected with 80mg/kg of CY(4mg/ml, 0.2ml/10g), on the first day, model 5 group were fed high,middle and low dose of Fuzheng Paidu tablet suspension (0.14g/ml, 0.07g/ml, 0.035g/ml, intragastric volume 0.2ml/10g), mushrooms polysaccharide tablets suspension (0.01g/ml, intragastric volume is 0.2ml/10g) and saline of the same volume (volume of perfusion for 0.2ml/10g). Another 1 groups as control group, normal saline of the same volume only (0.2ml/10g). To the medicine 1 times a day, continuous administration for 7 days. The morning of the seventh day of each rat were injected 5% chicken red blood cell (CRBC) in physiological saline solution 0.5ml, on the seventh day after intragastric administration with 2H, to chicken red blood cells after 4h, the mice were sacrificed by cervical dislocation.Intraperitoneal injection of Han's liquid 2.5ml, gently massage the abdomen of mice, the mice abdominal skin cut, cut a small hole in the peritoneum, absorb the mix the peritoneal fluid 2ml in a test tube, with Straw, learn a little of peritoneal fluid drops on the slide, liquid about the size of 1.5cm× 2cm. The slides on the auxiliary sugar porcelain plate wet gauze, 37℃incubation of 30min, physiological saline flush to attached cells, Wright's stain, water washing to dry, phagocytosis of peritoneal macrophages of mice were observed under microscope, and calculate the phagocytic percentage and phagocytic index[3]. Phagocytosis of peritoneal macrophages of mice to observe the situation of CRBC in the BI-2000 medical image analyzer to calculate, and phagocytic percentage and phagocytic index, the results in table 1.

Effect of inhibiting hemolysin and hemolytic plaque forming cyclophosphamide induced immunity

Animal grouping, making model and administration were the same as before, CRBC physiological saline groups were on the first day of each rat was induced by intraperitoneal injection of 5% suspension 0.2ml immune, on the seventh day after administration of mouse 2h, orbital blood, centrifugation, serum diluted with physiological saline, 1:100, 1ml and 5% the CRBC suspension 0.5ml blending, other control tubes without serum, suspension and mixing, in the constant temperature of 37℃incubation 30min, suspension reaction, in ice water for centrifugation, supernatant on UV-2000 ultraviolet visible spectrophotometer 540nm colorimetry, measuring each group of hemolysin formation [4].

Mice were sacrificed by cervical dislocation after blood, dissected out the spleen, removing the spleen on adhesion of adipose tissue, with adhesion dry with filter paper spleen blood, 2 mice in each group together with spleen homogenate,

saline regulating the spleen cell suspension concentration is 5×106 n /mL, spleen suspension solution 0.5mL, and CRBC 0.5ml 0.2% and 1:10, 0.5mL in a test tube in guinea pig serum, mixing, blank another without complement and add the same volume of physiological saline to take care of, the incubation of 1H, 37℃thermostat centrifugal supernatant on UV-2000, UV visible spectrophotometer 413nm colorimetric test groups, the formation of hemolytic plaques. The results are shown in table 2.

Effect of inhibition of lymphocyte transformation in mice induced by cyclophosphamide immune

Animal numbers, grouping, and doses were administered as before, in the administration of the former 3D, per rat per day are combined with intramuscular injection of PHA8mg/kg, in the 1 final 2h after administration, mice tails blood smear, Switzerland, dye staining, oil microscopic observation, computation of the peripheral lymphocyte transformation rate [5].

RESULT AND DISSCUSS

Effect of inhibition of phagocytosis of mouse peritoneal macrophages induced by CY immune

Table1 Effect of Fuzheng Paidu tablet on CY induced inhibition of phagocytosis of mouse peritoneal macrophages immune ($\bar{x} \pm s$)

Groups	n	Phagocytic percentage(%)	Phagocytic index
Blank group	10	49.9±2.2	0.57±0.028
Model group	10	38.6±2.1	0.44±0.028
Mushrooms polysaccharide	10	48.9±1.8	0.55±0.030
Fuzheng Paidu-HD	10	50.6±1.8	0.61±0.032
Fuzheng Paidu-MD	10	51.2±2.2	0.61±0.032
Fuzheng Paidu-LD	10	48.8±2.1	0.58±0.032

Note: compared with the model group, ***P*<0.01

As can be seen from the table 1, compared with the blank group, model group mice peritoneal macrophages on the phagocytic percentage and phagocytic index were significantly decreased (*P*<0.01), illustrating the successful establishment of CY immunosuppressed mice model. Compared with the model group, mushrooms polysaccharide tablet group and Fuzheng Paidu tablet high, middle and low dose group could significantly increase the CY induced immunosuppressed mice peritoneal macrophages on the phagocytic percentage and phagocytic index (*P*<0.01).

Effect of inhibiting hemolysin and hemolytic plaque forming CY induced immunity

Table 2 Effect of Fuzheng Paidu tablet on CY induced inhibition of hemolysin and hemolytic plaque forming immune ($\bar{x} \pm s$)

Groups	n	The formation of hemolysin(OD)	The formation of hemolytic plaque(OD)
Blank group	10	0.133±0.013	0.348±0.037
Model group	10	0.072±0.018	0.250±0.030
Mushrooms polysaccharide	10	0.126±0.017	0.399±0.031
Fuzheng Paidu-HD	10	0.132±0.017	0.422±0.036
Fuzheng Paidu-MD	10	0.130±0.014	0.433±0.038
Fuzheng Paidu-LD	10	0.122±0.016	0.404±0.048

Note: compared with the model group, ***P*<0.01

As can be seen from the table 2, compared with the blank group, the model group, hemolysin value decreased significantly (*P*<0.01), the absorbance of haemolytic plaque values decreased significantly (*P*<0.01), illustrating the successful establishment of CY immunosuppression model. Compared with the model group, mushrooms polysaccharide tablet group and Fuzheng Paidu tablet in high,middle and low dose group, ccould significantly promote the formation of hemolysin immune suppression, the absorbance value increased significantly (*P*<0.01); could obviously promote the

formation of immune suppressed mice hemolytic empty spot, the absorbance value increased significantly (P<0.01), and Fuzheng Paidu tablet in high and middle dose group effect obviously.

Effect of inhibition of lymphocyte transformation in mice induced byCY immune

Table 3 Effect of Fuzheng Paidu tablet on CY induced inhibition of lymphocyte transformation in mice by immunization ($\bar{X} \pm S$)

Groups	n	Lymphocyte transformation rate(%)
Blank group	10	42.2±5.9
Model group	10	32.5±5.8
Mushrooms polysaccharide	10	52.1±6.1
Fuzheng Paidu-HD	10	57.2±7.7
Fuzheng Paidu-MD	10	54.0±6.5
Fuzheng Paidu-LD	10	52.2±5.1

Note: compared with the model group, **P<0.01

As can be seen from the table 3, compared with the blank group, the model group mice lymphocyte transformation rate decreased significantly (P<0.01), illustrating the successful establishment of CY immunosuppression model. Compared with the model group, mushrooms polysaccharide tablet group and Fuzheng Paidu tablet in low dose group, middle and high, could significantly promote the immunosuppressive mice lymphocyte transformation rate (P<0.01); and Fuzheng Paidu tablet in high, middle dose group effect obviously.

CONCLUSIONS

AIDS is the human immunodeficiency virus (HIV) caused by chronic infectious disease, which can lead to a very high mortality rate of human body immunity is low so that the human body is easy to produce a variety of diseases, has the characteristics of long duration, the condition is complex, since the first discovery in twentieth Century 80th, in the world scope the rapid dissemination and spread, has become the the major problems that threaten public health and hinder the development of the society.

CY is the most commonly used for the treatment of malignant tumors of alkylating agent on behalf of the drug, is also a kind of strong immune inhibitor [6]. Therefore, this experiment used animal model of low immune function of CY. This experiment shown that : Fuzheng Paidu tablet could enhance immune suppressed mice celiac macrophage phagocytic function, could promote the transformation of immunosuppressed mice induced by CY immune lymphocytes, enhance immune function. Fuzheng Paidu tablet on CY induced immunosuppressed mice could increase the content of serum hemolysin immune, enhancing immune function, at the same time, Fuzheng Paidu tablet increased immune suppression in mice serum content of total complement.

At present the international effect of antiviral treatment of AIDS evaluation index is the number of viral load determination and $CD4^+$ cell detection, adverse clinical commonly used cytotoxic drugs are usually accompanied by some, and the adverse reactions of chemotherapy response usually associated with mutual superposition, aggravated the injury on a patient's body [7]. Traditional Chinese medicine has its unique theoretical system and treatment method, for different disease period, different syndromes of AIDS patients according to the principle of dialectical treatment, with prescription syndrome. Therefore, Chinese medicine treatment of AIDS has important research value, and also be faced with both opportunities and challenges.

Fuzheng Paidu tablet is a compound preparation of traditional Chinese medicine, ginseng, astragalus, mainly composed of Xiyangshen, Huangqi, Baizhu, Fangfeng, Nvzhenzi, Shanzhuyu, Nanshashen, Zicao, Lianqiao, Baihuasheshecao, Gancao, and so on. The Xiyangshen power designed supplementing qi and nourishing Yin, to support healthy as monarch drug; Huangqi, Baizhu and Fangfeng, as the traditional Yuping Feng San Fang Qi solid form, function, and can realize health and resist external evil; Nvzhenzi and Shanzhuyu nourishing liver and kidney, Nanshashen Yangying Qingfei, help Xiyangshen Qi Yin, a total of ministerial drug; Zicao, Lianqiao,Baihuasheshecao clearance in vivo is deficiency of the toxin to Zuo medicine; Gancao to reconcile the various drugs to make. The drug combination, played a total of nourishing vital qi, attack evil Qudu, with specimen and cure effect, apply to Qi and yin deficiency, intrinsic heat toxin asymptomatic human immunodeficiency virus infection. Modern pharmacological research show that Xiyangshen, Huangqi,

Gancao can better improve the body immunity; Lianqiao and Baihuasheshecao all can inhibit human immunodeficiency virus, antagonistic activity. The party had decoction for early treatment of AIDS patients, the effect is good, the related basic research, clinical observation and application, has obtained the approval of the hospital preparation [2].

Chinese medicine treatment of AIDS in alleviating the symptoms, improving the immune function of patients, improve the quality of life and other aspects of the role, has been part of clinical verification. Therefore, to explore a suitable for the situation of AIDS treatment Chinese also has China characteristics, in order to spend the lowest, best effect, toxic and side effects of the minimum objective Fuzheng Paidu tablet, but also for the development of immune enhancing agent to provide the theory basis, in order to improve China's AIDS anti viral treatment level, to provide scientific basis for reasonable treatment scheme.

ACKNOWLEDGEMENT

The research work is supported by Genuine medicinal materials deep processing in Henan Province Engineering Laboratory (2012-1431-10); science and technology innovation team in University (2012IRTSTHN011); Zhengzhou city science and technology innovation team plan (131PCXTD612); technical center efficacy rating (Education Science and Technology (2012) 78-12); Henan University of Traditional Chinese Medicine of traditional Chinese medicine and geoherbalism of deep processing of Collaborative Innovation Center (2012) 188-2.

REFERENCES

[1] D. Chen, J. Liu, D. H. Liu., Development trend of China's AIDS epidemic forecasting and control measures, Chinese public health management, 29, 3(2013).

[2] B. Peng , M. S. Miao , H. J. Guo , et al., Effect of Fuzheng Paidu tablet on the immune function of mice with immunosuppression induced by cyclophosphamide, Chinese Journal of Clinical Rehabilitation ,10, 127(2006).

[3] M. S. Miao, W.Y. Xin, M. Bai, L.Guo., Effects of Kangbingdu Oral Liquid on immunological function of normal mice, Pharmacology and clinical, 8, 21(2014).

[4] Z. K. Hu, H. L. Liu, J. Y. Qiao, et al., Effects of Radix Ranunculi Ternati Polysaccharide on Immunological Function of the Immunosuppressional Model Mice Induced by Cyclophosphamide, China Modern Applied Pharmacy, 27, 90(2010).

[5] L. N. Wang, Q. Wang, M. S. Miao., FCPS cyclophosphamide-induced immune suppression of immune function in mice, Journal of Traditional Chinese Medicine, 4, 677(2010).

[6] Y.Motoyoshi, K. Kaminoda, O. Saitoh, et al., Different mechanisms for anti-tumor effects of low-and high-dose cyclophosphamide, Oncol Rep, 16, 141-146(2006).

[7] S. Q. Wang, L. H. Zhang, Y. Li, et al., Effects of Shenhuang Capsule on immunofunction of cyclophosphamide-immuno-depressed mice, The modern clinical medicine, 28, 507(2013).