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Original article

Effects of Fuzheng Paidu tablet immunization on AIDS BALB/c mice

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ABSTRACT

Aim: To establish a Friend murine leukemia virus (FLV)-induced immunodeficient BALB/C mouse model and investigate the effects of Fuzheng Paidu tablets on the body weight, thymus, spleen, and $CD4^+$ and $CD8^+$ T lymphocytes of FLV-infected mice. FLV was passaged twice in BALB/c mice. The infected mice were divided into six groups of ten mice based on their weights. The groups included the normal control group; virus control group; AZT group; high- (2.8 g/kg), medium- (1.4 g/kg), and low-dose (0.7 g/kg) Fuzheng Paidu tablet groups; and Fuzheng Paidu decoction (10 g/kg) group. The mice were administered Fuzheng Paidu tablets via gavage for 21 days. The body weight and changes in the thymus, spleen, and $CD4^+$ and $CD8^+$ T lymphocytes of each mouse were measured.

Results: The splenic weight of the virus control group is significantly higher than that of the normal control group, with significant splenomegaly. In addition, the splenic inhibition indices of the AZT group and the high- and medium-dose Fuzheng Paidu tablet groups were approximately 93.80%, 37.80%, and 28.07%, respectively. Furthermore, the high and medium dose Fuzheng Paidu tablets could increase the thymus weights of the infected mice.

Conclusion: Fuzheng Paidu tablets could inhibit splenomegaly, lower the splenic indices, and increase the thymic weights and thymic indices of FLV-induced immunodeficient mice.

1. Introduction

The human immunodeficiency virus (HIV) attacks and destroys T4 lymphocytes, leading to the collapse of the immune system, loss of disease resistance, and eventually death. In traditional Chinese medicine, AIDS is categorized as an "epidemic", "hidden exogenous seasonal disease", "consumptive disease", and "five types of impairments caused by overstrain". Fuzheng Paidu tablets, which are primarily comprised of American ginseng, *Astragalus membranaceus*, *Lithospermum erythrorhizon*, *Forsythia suspensa*, and *Hedyotis diffusa Willd*, are used in clinical settings to treat both "Qi and Yin impairments" and "heat toxin endoretention" in patients with asymptomatic HIV. In this study, the Friend leukemia virus (FLV)-induced immunodeficient BALB/c mice model was used to investigate the effects of FLV on the body weight (Chen et al., 2016; Wei and Xu, 2015), thymus, spleen, and $CD4^+$ and $CD8^+$ T lymphocytes of BALB/c mice and study the effects of immunization with Fuzheng Paidu tablets on BALB/c mice during AIDS treatments (Liu and Zhang, 2015).

2. Material and methods

2.1. Drugs and reagents

The 0.37 g film-coated Fuzheng Paidu tablets (2009.5) and 1.8 g/ml Fuzheng Paidu solution used for decoction were obtained from Henan Aolite Pharmaceutical Co., Ltd. The positive control drug zidovudine (AZT) was obtained from Northeast Pharmaceuticals. The AZT was prepared with sterile distilled water and administered at a dose of 40 ml/kg body weight via gavage. The dosages were weight-adjusted weekly. APC anti-mouse CD4 (L3T4) (CloneGK1.8), FITC anti-mouse CD3e (p425-2e11), PE anti-mouse CD8a (Ly-2), and a 1 × RBC lysis buffer were purchased from eBioscience Ltd.

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2.2. Animals

Female SPF-grade BALB/c female mice weighing 13–15 g were provided by the Guangdong Medical Laboratory Animal Center (animal quality certification number: Yue 2008A023). The mice were bred at 23 ± 2 °C under 12-h light-dark cycles with illumination, 75 ± 5% humidity, and 12 air changes per hour. The feeding and management requirements recommended by the good laboratory practice (GLP) regulations were followed in all of the experiments herein using standard feed and water. The mice were used in the experiments after being fed for one week and meeting the indicator requirements.

2.3. Experimental method

2.3.1. Friend virus and the passage method

The Friend murine leukemia virus was purchased from the American Type Culture Collection (ATCC). After performing a 1:10 diluted with PBS, the virus was intraperitoneally administered to the mice (BALB/c). After one passage, the median lethal dose was measured. Three weeks after infection, the mice were euthanized (Jing et al., 2011). Then, the spleens were extracted aseptically and weighed. The TID_{50} of the spleens exhibiting splenomegaly, defined herein as a splenic weight three standard deviations greater than the average splenic weight of the normal group, was calculated. The spleens of the infected mice were then stored at −80 °C.

2.3.2. Construction of the AIDS mouse model and group administration

The 70 BALB/c mice were divided into the six groups, including the normal control group; virus control group; AZT group; high- (2.8 g/kg), medium- (1.4 g/kg), and low-dose (0.7 g/kg) Fuzheng Paidu tablet groups; and Fuzheng Paidu decoction (10 g/kg) group. The 10% normal mouse spleen cell suspension was only administered to the mice in the normal control group. The mice in the remaining groups received intraperitoneal injections of 0.5 ml TID_{50} Friend-MuLV. The abdominal injection sites were disinfected with alcohol and iodine before administration. The infected mice were divided into six groups of ten based on their weights (Peng et al., 2006). Four hours after inoculation, the infected mice in the treatment groups were administered gavage treatments once each day for 21 days, while the virus control group was administered an equal volume of solvent. Then, 21 days after infection, the mice were euthanized and weighed. The spleen and thymus of each mouse was extracted aseptically and weighed using an electronic balance. The spleen index (mg/g) of each mouse was then calculated by dividing the splenic weight (mg) by the body weight (g). A splenic mice weight standard deviations greater than the average splenic weight of the normal control group was defined as splenomegaly. The splenomegaly inhibition rate was calculated as (the number of cases of splenomegaly in the drug treatment group - the number of cases of splenomegaly in the virus control group)/the number of cases of splenomegaly in the virus control group × 100]. Similarly, the thymic index (mg/g) was calculated by dividing the thymic weight (mg) by the body weight (g). After conventional HE staining, the morphological and histological changes in the spleen of each mouse were observed under a light microscope (Li et al., 2007).

Before euthanasia, a 50 μl blood sample was collected from each mouse via the eyeball extraction method and stored in a test tube containing an EDTA anticoagulant. The three antibodies with different fluorescent labels were diluted with distilled water to 0.125 μg/10 μl according to the provided instructions. Next, each 50 μl anticoagulant blood sample was added to a test tube containing $CD_3^+/CD_4^+/CD_8^+$ specific fluorescent mAb. The test tubes were then mixed thoroughly and incubated in the dark at room temperature for 20–30 min. Next, 250 μl hemolysin was added to each tube with a pipette. The test tubes were then kept in the dark at room temperature for 10 min to facilitate hemolysis. Once the solution became transparent, 1 ml cold PBS solution was added to each test tube to terminate the reaction. The test tubes were then centrifuged at 2000 rpm at room temperature for 5 min. The supernatant was discarded, and 1 ml PBS solution was added to the precipitant and mixed well. The resulting samples were fixed in 1.1 ml 1% paraformaldehyde and analyzed via flow cytometry.

2.3.3. Statistical analysis

All of the experimental data was represented as mean ± standard deviation ($\chi \pm s$). SPSS 11.0 for windows was used to perform the ANOVA analysis, in which P-values <0.05 were considered statistically significant (Jiang et al., 2010).

3. Results & discussion

The FLV-infected rats exhibited splenomegaly, thymic atrophy, elevated red and white blood cell counts, anti-viral antibodies, persistently low viral loads, immunosuppression, splenic helper dysfunction, suppressor and cytotoxic T cells, and decreased $CD4^+/CD8^+$ ratios and PHA responses. Since these symptoms are similar to the symptoms characteristic of human AIDS, this disease is called AIDS Mouse, or Murine Acquired Immunodeficiency Syndrome (MAIDS). Within approximately three weeks, the infected mice demonstrated severe weight loss, shine loss, reduced activity, and significant.

3.1. Splenomegaly

The body weight and splenic weight of each mouse were measured after 21 days of infection. The body weights of the infected mice are decreased ($P < 0.01$), demonstrating the accuracy of the established model. In addition, the body weights of the other groups are increased significantly compared to the virus control group ($P < 0.01$). However, the infected mice exhibited significantly slower weight gain than the normal control group. Compared to the normal control group, the splenic index of the virus control group is increased significantly, indicating significant splenomegaly. Furthermore, compared to the virus control group, the splenic indices of the AZT group and the high- and medium-dose Fuzheng Paidu tablet groups are decreased significantly ($P < 0.05$). The splenomegaly inhibition rates of the AZT group and the high- and medium-dose Fuzheng Paidu tablet groups were approximately 93.80%, 37.80%, and 28.07%, respectively (see Table 1).

3.2. Splenic histopathology results

The spleens of the mice in the normal control group appeared normal, with evenly distributed red and white pulps.

The spleens obtained from the mice in the virus control group were slightly enlarged and constricted by their capsules. The splenic sections appeared dark red and soft. The red pulps were infiltrated with diffuse myeloblasts, suppressing the white pulps. Tumor cells were distributed throughout the splenic capsules. Some of these tumor cells were atypic, with large and dark-stained nuclei.splenic structures of the AZT group were clearer than those of the virus control group, with no myeloblast infiltration. However, some abnormal lymphoid cells were observed under the splenic capsules, with dark-stained nuclei and pathologic mitosis in some areas.

The splenic lesions found in the Fuzheng Paidu tablet groups exhibited varying degrees of relief. The splenic structures of these groups were clearer than those of the virus control group, with no

Table 1
Splenic weight changes ($\chi \pm s$) in the mice after 21 days of infection.

Group	Dose (g/kg)	Case (n)	Initial body weight (g)	Final body weight (g)	Spleen index (mg/g)	Splenomegaly inhibition rate (%)
Virus control group	–	8	13.58 ± 1.25	15.65 ± 3.47	102.60 ± 34.62##	–
Normal control group	–	10	14.75 ± 1.33	18.98 ± 3.21##	5.53 ± 0.49**	–
AZT group	0.1	9	13.68 ± 1.19	17.17 ± 1.23##	6.36 ± 1.03**	93.80
High-dose Fuzheng Paidu tablet group	2.8	8	14.16 ± 1.36	17.12 ± 2.54#	63.80 ± 23.99*	37.80
Medium-dose Fuzheng Paidu tablet group	1.4	9	13.52 ± 1.43	16.78 ± 3.11#	73.80 ± 32.44*	28.07
Low-dose Fuzheng Paidu tablet group	0.7	8	13.27 ± 1.46	16.46 ± 3.25##	94.50 ± 57.34	7.80
Fuzheng Paidub decoction group	10.0	9	13.21 ± 1.72	15.44 ± 2.69#	83.63 ± 33.95	18.48

** Compared to the virus control group: $P < 0.01$.
* Compared to the virus control group: $P < 0.05$.
# Compared to the normal control group: $P < 0.05$.
Compared to the normal control group: $P < 0.01$.

myeloblast infiltration. The spleens obtained from the low-dose Fuzheng Paidu tablet group exhibited a wider distribution of tumor cells, with large, dark-stained, atypic nuclei and pathologic mitosis (see Fig. 1).

3.3. Thymic changes

After 21 days of infection, the thymic index of each mouse was measured. The thymic index of the virus control group is decreased significantly compared to the normal control group ($P < 0.01$), demonstrating the accuracy of the developed model. The thymic indices of the AZT group and the high- and medium-dose Fuzheng Paidu tablet groups are increased significantly compared to the virus control group ($P < 0.05$). In contrast, the thymic indices of the low-dose Fuzheng Paidu tablet group and decoction group are decreased significantly compared to the normal control group ($P < 0.05$). The thymic weight of the virus control group is significantly smaller than that of the normal control group, indicating thymic suppression. Therefore, all of the treatment groups exhibited improved thymic weights.

3.4. Effect on the cellular immune function of the FLV-mice

The effects of the treatments on the $CD4^+$ and $CD8^+$ T lymphocytes of the FLV-mice were determined. The $CD4^+$ lymphocytes of the normal control group, AZT group, and high- and medium-dose tablet groups are all increased significantly compared to the virus control group ($P < 0.01$). In addition, the $CD8^+$ lymphocytes and $CD4^+/CD8^+$ ratios of the normal control group, AZT group, and high- and medium-dose tablet groups are all increased significantly compared to the virus control group ($P < 0.05$).

Compared to the normal control group, the virus control group exhibit significantly decreased $CD4^+$ and $CD8^+$ levels, $CD4^+/CD8^+$ ratios, and degrees of immunosuppression. However, the $CD4^+/CD8^+$ ratios of the AZT group and high- and medium-dose Fuzheng Paidu tablet groups were elevated compared to the virus control

Table 2
Thymic weight changes ($\chi \pm s$) in the mice after 21 days of infection.

Group	Dose (g/kg)	Thymus wet weight (mg)	Thymus index (mg/g)
Virus control group	–	17.11 ± 10.1	1.09 ± 0.31##
Normal control group	–	47.2 ± 9.63	2.48 ± 0.42*
AZT group	0.1	39.51 ± 11.14	2.30 ± 0.68*
High-dose group	2.8	40.34 ± 12.21	2.35 ± 0.38*
Medium-dose group	1.4	36.60 ± 10.63	2.18 ± 0.26*
Low-dose group	0.7	22.22 ± 8.28	1.34 ± 0.39#
Fuzheng Paidu decoction group	10	28.12 ± 13.06	1.82 ± 0.44#

* Compared to the virus control group: $P < 0.05$.
# Compared to the normal control group: $P < 0.05$.
Compared to the normal control group: $P < 0.01$.

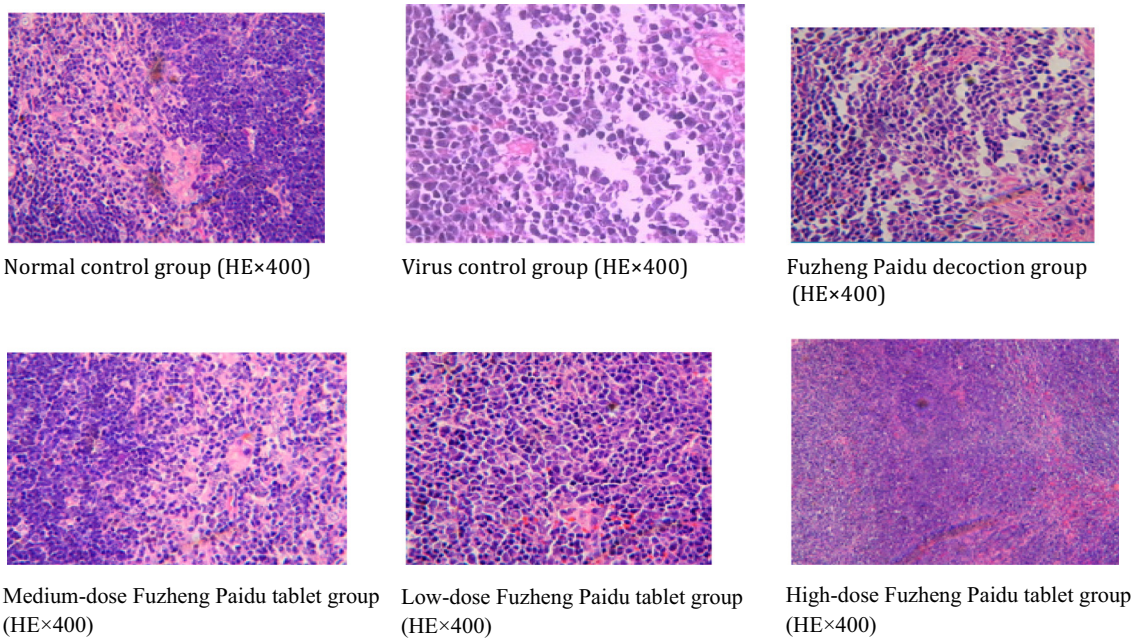

Fig. 1. Splenic histopathology results.

Table 3
Effects on the $CD4^+$ and $CD8^+$ T lymphocytes of the FLV-mice.

Group	Dose (g/k)	CD_4^+ (%)	CD_8^+ (%)	CD_4^+/CD_8^+
Virus control group	–	25.40 ± 12.1	12.95 ± 5.78	1.96 ± 0.13
Normal control group	–	53.32 ± 14.72**	18.15 ± 3.24*	2.93 ± 0.14*
AZT group	0.1	55.41 ± 9.67**	18.42 ± 1.59	3.00 ± 0.28*
High-doset group	2.8	35.24 ± 5.18**	16.28 ± 1.69*	2.16 ± 0.42*
Medium-dose group	1.4	28.09 ± 3.69**	13.22 ± 1.73*	2.12 ± 0.19*
Low-dose group	0.7	33.85 ± 10.9	19.2 ± 5.61	1.76 ± 0.21
Fuzheng Paidu decoction group	10	39.76 ± 13.27	19.9 ± 2.60	1.99 ± 0.35

* Compared to the virus control group: $P < 0.05$.
** Compared to the virus control group: $P < 0.01$.

group, indicating that the Fuzheng Paidu tablet could enhance the cellular immune function of AIDS BALB/c-infected mice (see Tables 2 and 3).

4. Conclusions

The "epidemic toxin" of AIDS attacks internal organs, primarily the spleen. The spleen is the postnatal basis, or the source of Qi-blood. Therefore, damage to the spleen results in Qi-blood loss and Yin-Yang disruption. The pathogenesis of AIDS is complicated, but primarily involves the spleen. Qi-blood deficiency and Yin-Yang disruption increases the susceptibility of the body to exogenous pathogens and leads to the production of phlegm, morbid fluid (Jiang et al., 2010), and other bodily fluids. In general, the asymptomatic stage of AIDS is described as a reduction in vital energy manifested by symptoms such as chronic diarrhea, wheezing (Xie et al., 2016; Liu et al., 2012), and chronic fever. The infectious symptoms of AIDS appear as the viral load increases. The "epidemic toxin" of AIDS and the "epidemic toxin" of traditional Chinese medicine are different in terms of transmission, cause, and pathological changes. AIDS is also different from the traditional "consumptive disease" of traditional Chinese medicine (Jiang et al., 2012).

The results of this experiment demonstrated that the infected mice exhibited a reduction in body weight compared to the normal control group (Liu et al., 2016; Zhang, 2012). The mice in all of the treatment groups exhibited different levels of weight gain. The splenic weight of the virus control group increased significantly compared to the normal control group. In addition, the splenic inhibition indices of the AZT group and the high- and medium-dose Fuzheng Paidu tablet groups were approximately 93.80%, 37.80%, and 28.07%, respectively. The splenic histopathology results demonstrated that the splenic lesions in the Fuzheng Paidu tablet groups exhibited varying degrees of relief. Moreover Wang et al. (2012) and Zhang et al. (2008), the splenic structures of the treatment groups were clearer than those of the virus control group, with no myeloblast infiltration. The spleens of the low-dose Fuzheng Paidu tablet group exhibited a wider distribution of tumor cells, with large, dark-stained atypic nuclei and pathologic mitosis. The thymic index results demonstrated that the thymic weight of the virus control group decreased significantly compared to the normal control group. All of the treatment groups exhibited improved thymic weights (He, 2011), Furthermore, the virus control group exhibited significantly decreased $CD4^+$ and $CD8^+$ levels, $CD4^+/CD8^+$ ratios, and degrees of immunosuppression compared to the normal control group. However, the $CD4^+/CD8^+$ ratios of the AZT group and the high- and medium-dose Fuzheng Paidu tablet groups were elevated compared to the virus control group, indicating that Fuzheng Paidu tablets could enhance the cellular immune function of AIDS BALB/c infected mice.

According to the results of this experiment, the splenic weights of FLV-mice can be used to reflect FLV-facilitated immune damage. In addition, the splenic index can be used as an intuitive and reliable indicator of that damage. The thymus, one of the central immune organs, serves as a location for immune cell growth [11], development, and maturation. Fuzheng Paidu tablets could be used to significantly increase the splenic and thymic indices of FLV-mice. Although the low-dose treatments did not function as well as the AZT treatments, they still affected the progression of AIDS in the BALB/c infected mice (Tao et al., 2016; Feng et al., 2016; Basso and Marini, 2015).

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