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Effect of *Ligustrum lucidum* polysaccharide on immunity of immunosuppressed mice

Shi Jing Jing, Shi Bo, Miao Mingsan and Li Qingya

Henan University of Traditional Chinese Medicine, Zhengzhou, Henan 450 046, China.

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Abstract

The aim of this study was to observe the effects of *Ligustrum lucidum* polysaccharide on immune functions of hydrocortisone-induced immuno-suppressed mouse model. There were phagocytic function of mouse perito-neal macrophage, condition of formation of hemolysin and hemolytic plague as well as condition of mouse lymphocyte transformation. The results indicate that through comparison between high-dose, medium-dose, low-dose *L. lucidum* polysaccharide groups, lentinan group and model group, phagocytic rate and phagocytic index of peritoneal macrophage obviously improved, thus obviously enhancing formation of hemolysin and hemolytic plague, and obviously improving the transformation rate of peripheral blood lymphocyte. Therefore, *L. lucidum* polysaccharide could markedly improve the immune functions of hydrocortisone-induced immunosuppressed model mouse.

Introduction

Ligustrum lucidum is the dry mature fruit of *L. lucidum* Ait. belonging to oleaceae plant. Modern chemical studies have shown that *L. lucidum* mainly contains oleanolic acid and other triterpene fat-soluble active ingredients as well as polysaccharide and phospho-lipids, etc. Various chemical components have various pharmacologic effects such as enhancing immunity, protecting liver, anti-inflammation and anti-aging (Jin and Fan, 2008; Jin et al., 2011) .

In the study, it mainly reported effects of *L. lucidum* polysaccharide on immunity of immunosuppressed mouse models.

Materials and Methods

Animal

Kunming mice, SPF grade, were provided by Wuhan Biological Products Assay Institute with certification number of 0036868, with weight of 18-21 g for each, and at the age of 6 weeks, and half male and half female. The temperature was controlled at 20-25°C, while humidity was 50-60%. The animals were allowed to get free diets.

Reagent

L. lucidum polysaccharide was provided by Chemical Room of Henan University of Traditional Chinese Medicine, and it was obtained from the dry mature fruit of *L. lucidum*. It was obtained from the plant after decoction, alcohol precipitation, concentration, filter and drying. Lentinan tablet (main component was lentinus edodes mycelia polysaccharide) was produced by Zhejiang Apeloa Natural Medicine Co., Ltd. (appro-val number: GYBZ Z20026215, specification: substrate weight 0.1 g, batch number: 20070401). Hydrocortisone injection (produced by Shanxi Jinxin Shuanghe Pharma-ceutical Co., Ltd, batch number: 200704261) and hyto-hemagglutinin (produced by Shanghai Yihua Medical Technology Co., Ltd, batch number: 040701) were used.

Apparatus

Adjustable pipette (Shanghai Labsystems Analytical

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Instrument Co., Ltd), centrifugal machine (Shanghai Anting Scientific Instrument Plant, model: TDL-40B), biological microscope (Olympus Corp., model: Olympus BX-60) and transmission electron microscopy (Hitachi, model: H-7500) were used in this study.

Methods

Fifty mice (weight range: 18-21 g) were randomly divide into 5 groups with 10 in each group. During the 1st, 2nd and 3rd days, respectively subcutaneously injected hydrocortisone to each mouse (50 mg/kg, subcutaneous injection twice at each morning and afternoon). Then *L. lucidum* polysaccharide suspension was added with high dose, medium dose and low dose by intragastric administration (0.2 g/kg, 0.1 g/kg, 0.05 g/kg, 0.2 mL/10 g), lentinan tablet suspension (0.2 g/kg, 0.2 mL/10 g) and same-volume normal saline (with volume of 0.2 mL/10 g). Separately set 1 group as blank group without modeling but to only drench same-volume normal saline (0.2 mL/10 g)(Liu et al., 2011; Miao, 2001). The drug was administered once per day for continuously 7 days. On the morning at the 7th day for the experiment, intraperitoneally injection of chicken erythrocyte normal saline solution of 5% for 0.5 mL to each mouse, and then normally taken intragastric administration after 2 hours and later execute the mice after intragastric administration for 2 hours. Hanks solution (2.5 mL) was injected intraperitoneally into each mouse. The abdomen of the mouse was rubbed lightly to make the Hanks solution fully exposed to the enterocoelia. The abdomen of the mouse was cut open in layers, and a small hole was made in the peritoneum to suck peritoneal fluid for 2 mL with sucker to place in the test tube and mixed uniformly. Pipette was used to suck a little peritoneal fluid to drop at one end of marked glass slide. The size of fluid point was about 1.5 × 2 cm. Pave sterile gauze in disinfected enameled dish, and then placed the gauze in the glass slide after wetting by normal saline, and later placed in the incubator of 37°C to incubate for 30 min. Attached cells on the glass slide were washed out with normal saline. Later, dye with Wright's stain, washed with normal saline and then dried in the air. Observed the slide under the microscope and counted the phagocytic rate and phagocytic index of peritoneal macrophage to chicken erythrocyte.

Phagocytic rate (%): Number of macrophage phagocytizing chicken erythrocyte among 100 macrophages / 100 × 100%

Phagocytic index = Total number of chicken erythrocyte phagocytized by 100 macrophages / 100

Formation of hemolysin and hemolytic plaque

With regard to animal grouping, modeling and drug administration, it was same with above.

At the same time (day 1) chicken erythrocyte (0.2 mL) in normal saline suspension of 5% was injected intraperitoneally into each mouse for immunity. Blood was collected from the mouse's eyes 2 hours after intragastric administration on day 7. It was centrifuged, the serum was separated and then 1 mL of the solution was taken and diluted with normal saline as per 1:10 to mix uniformly with 0.5 mL chicken erythrocyte suspension of 5%. In addition, separately set the control tube without serum. After fully and uniformly mixing the suspension was placed into the incubator of 37°C to incubate for 30 min. The sample was then taken out to place in the ice water to stop reaction. The sample was centrifuged and the supernatant was separated for reading at ultraviolet and visible spectrophotometer with wavelength 540 nm.

Sacrificed the mouse and spleen was collected. Splenocyte were separated and diluted with the normal saline (5×10^6/mL). 0.5 mL spleen cell suspension, 0.5 mL chicken erythrocyte solution of 0.2% and 0.5 mL guinea pig serum as per 1:10 were mixed uniformly. Separately set the blank control group (without alexin but only adding same-volume normal saline), and kept into the incubator of 37°C for 1 hour. Then the supernatant was collected by centrifugation and absorbance was measured using ultraviolet and visible spectrophotometer at 413 nm.

Lymphocyte transformation

With regard to animal grouping, modeling and drug administration, it was same with above.

In addition, during the 1st~3rd days of experiment, phytohemagglutinin (10 mg/kg, 0.1 mL/10 g) was administered intramuscularly to each mouse. On day 7 morning, made intragastric administration firstly for 2 hours and then blood was drawn from each mouse through cutting the tail to produce blood smear, and adopt Wright's stain to dye, observed under high power lens. The percentage of lymphocyte transformation was the calculated.

Statistical analysis

SPSS for windows statistical software was used, and measuring data is denoted with mean ± SD and comparison of measuring data among groups is made through t-tests.

Results

Effect of *L. lucidum* polysaccharide on phagocytosis of peritoneal macrophages of hydrocortisone-induced immunosuppressed mouse results are shown in Table I. It can be shown that compared with blank group, phagocytic percentage and phagocytic index of mouse peritoneal macrophage under model group to chicken erythrocyte both were markedly decreased ($p<0.01$). These indicated that immunosuppressed mouse model

was made successfully. Compared with model group, lentinan group, high-dose, medium-dose and low-dose *L. lucidum* polysaccharide groups could markedly improve the phagocytic percentage and phagocytic index of immunosuppressed mouse peritoneal macrophage to chicken erythrocyte (p<0.01).

Table I

Effect of *L. lucidum* polysaccharide on phagocytosis of peritoneal macrophages in immunosuppressed mice

Group	*% Phagocytic*	*Phagocytic index*
Blank	48.8 ± 4.7[a]	0.57 ± 0.03[a]
Model	37.7 ± 3.6	0.48 ± 0.04
Lentinan	50.1 ± 5.1[a]	0.62 ± 0.05[a]
High-dose *L. lucidum* polysaccharide	51.3 ± 3.4[a]	0.62 ± 0.04[a]
Medium-dose *L. lucidum* polysaccharide	54.4 ± 4.1[a]	0.65 ± 0.05[a]
Low-dose *L. lucidum* polysaccharide	50.4 ± 3.9[a]	0.61 ± 0.04[a]

Data are mean ± SD; n=10; [a]p<0.01 through comparison with model group

When compared with the blank control group, the hemolysin value and absorbance value of hemolytic plaque of mice under model group both markedly decrease (p<0.01; Table II). Compared with the model group, lentinan group, high-dose, medium-dose and low-dose *L. lucidum* polysaccharide groups could markedly enhanced the formation of hemolysin and hemolytic plaque of immunosuppressed mouse (p<0.01), among which, effects under high-dose and medium-dose *L. lucidum* polysaccharide groups were obvious.

Table II

Effect of *L. lucidum* polysaccharide on formation of hemolysin and hemolytic plaque in immunosuppressed mice

Group	*Formation of hemolysin (A)*	*Formation of hemolytic plaque (A)*
Blank	0.13 ± 0.01[a]	0.36 ± 0.03[a]
Model	0.07 ± 0.02	0.25 ± 0.03
Lentinan	0.12 ± 0.02[a]	0.41 ± 0.03[a]
High-dose *L. lucidum* polysaccharide	0.13 ± 0.02[a]	0.42 ± 0.04[a]
Medium-dose *L. lucidum* polysaccharide	0.13 ± 0.02[a]	0.42 ± 0.03[a]
Low-dose *L. lucidum* polysaccharide	0.12 ± 0.01[a]	0.39 ± 0.04[a]

Data are mean ± SD; n=10; [a]p<0.01 through comparison with model group

From Table III, it can be seen that compared with the blank control group, lymphocytic transformation rate of mouse under the model group obviously decreased (p<0.01). Compared with model group, lentinan tablet group and high-dose, medium-dose and low-dose *L. lucidum* polysaccharide groups might obviously enhanced the lymphocytic transformation rate of immunosuppressed mouse (p<0.01).

Table III

Effect of *L. lucidum* polysaccharide on lymphocyte transformation in immunosuppressed mice

Group	*Lymphocyte transformation rate*
Blank	40.4 ± 4.9[a]
Model	32.5 ± 5.8
Lentinan	52.9 ± 8.1[a]
High-dose *L. lucidum* polysaccharide	57.8 ± 6.3[a]
Medium-dose *L. lucidum* polysaccharide	55.2 ± 7.1[a]
Low-dose *L. lucidum* polysaccharide	51.8 ± 6.3[a]

Data are mean ± SD; n=10; [a]p<0.01 through comparison with model group

Discussion

Hydrocortisone, being the long-acting preparation of glucocorticoid, has inhibiting the effect on many links during body immunity. It might damage lymphocyte and plasmocyte of sensitive animals, inhibits phagocytosis and treatment of macrophage to antigen, and might inhibit cellular immunity and humoral immunity of the body. It mainly acts on cellular immunity system (Arinsburg et al., 2012; Li et al., 2014; Peng et al., 2014; Peng et al., 2014; Tan et al., 2012). The mice might appear with less activity, poor mental status and reduced body mass after injection of hydrocortisone for 3 days. It can be observed after dissection that the thymus, spleen and other immune organs of the mouse may obviously shrink, obviously different from that of mice under normal group (Li et al., 2014; Peng et al., 2014; Peng et al., 2014). It can be assumed that immune functions of animals under model group markedly decrease, and immunosuppressed mouse model is made successfully.

As one of the important components in body immune system, Macrophage may directly evaluate its only functions and indirectly evaluate immune response ability through detecting the phagocytic function of foreign particles. The experiment, concerning effects of *L. lucidum* polysaccharide on phagocytic function of immunosuppressed mouse peritoneal macrophage may reflect the effect on non-specific immunological competence of immunosuppressed model mouse.

Respectively through hemolysin measuring method taking chicken erythrocyte as the immunogen in mouse hemolysin formation experiment and through hemolytic plague measuring method taking chicken erythrocyte as the immunogen in mouse hemolytic plague formation experiment, it observed the effect of *Ligustrum lucidum* polysaccharide on humoral immune function of immunosuppressed model mouse, thus reflecting the humoral immunity status of the body. Through intramuscular injection of phytohemagglutinin to activate lymphocyte in the body of immunosuppressed mouse, observation of effects of drugs to be determined on lymphocyte transformation and lympho -cyte transformation rate, it may reflect the cellular immune level of the body (Li and Jian, 2006; Tao and Chen, 2004; Zhang and Wang, 2008) .

Modern pharmacological studies have shown that *L. lucidum* has effects such as improving body immunity functions, anti-inflammation and anti-bacteria, rising white blood cell, anti-aging and anti-fatigue, improving immunity of cancer patients and anticancer etc. (Zhang and Huang, 2006).

Conclusion

L. lucidum polysaccharide may improve the immunologic functions of immunosuppressed animals, providing certain pharmacological basis for development of *L. lucidum* to anti-neoplastic and anticancer drugs used together with chemotherapeutic drugs.

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Conflict of Interest

The authors declare that they have no competing interest.

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Author Info
Li Qingya (Principal contact)
e-mail: liqingya12007034@163.com

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