

## Research Article

### Effects of Different Doses of Aspirin on the Lymphocyte Activity in Mice Blood and Spleen

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**Abstract:** The experiment was conducted to evaluate the effect of Aspirin on animal immune function by studying the effects of different doses of Aspirin on the lymphocyte activity in mice blood and spleen. Forty eight Kunming mice (20±2g) were selected and assigned randomly to 4 groups to finish the experiment. The treatment contained low-dose (10mg/k g·bw), middle-dose (20 mg/k g·bw), high-dose (40 mg/k g·bw) Aspirin group and blank control group. The experimental stage was 20 days. On the 10 th and 20 th day in the trial, 6 mice were chosen randomly to be sampled and the indexes were determined. The activity of lymphocyte in low and middle dose groups were enhanced and the activity of lymphocyte in middle dose group was higher than that in low dose group, but there was no significant difference ( $p>0.05$ ). On the 20 th day in the trial, the activity of lymphocyte in spleen in middle dose group was significantly higher than that in control group ( $p<0.05$ ). On the 20 th day in the trial, the activity of lymphocyte in spleen in middle dose group was significantly higher than that in control group ( $p<0.05$ ). During the course of the trial, during the course of the trial, the activity of lymphocyte in blood and spleen in high dose group decreased, but there was no the activity of lymphocyte in blood and spleen in high dose group decreased, but there was no significant difference ( $p>0.05$ ). This indicated that proper dose of Aspirin could enhance the activity of lymphocyte in mice significant difference ( $p>0.05$ ). This indicated that proper dose of Aspirin could enhance the activity of lymphocyte in mice.

**Keywords:** Activity, aspirin, lymphocyte, mice

## INTRODUCTION

The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms and distinguish them from the organism's own healthy tissue. Antipyretic drugs can affect the body's immune response to vaccines from the information (Gross and Levandowski, 1994; Leon *et al.*, 2011).

Today, aspirin is one of the most widely used medications in the world, with an estimated 40,000 tones of it being consumed each year. Its basic role is antipyretic and analgesia and it can't produce drug dependence (Huo *et al.*, 2011). Another important role of aspirin is anti-inflammatory and anti-rheumatism; it is priority drug of treating rheumatism, rheumatoid arthritis. People have made a lot of researches before and the results were accordance. There are few studies about effects on the body immunity of aspirin (Zhang *et al.*, 2011).

According to materials, antipyretics can affect the body's immune response to the vaccine. The aspirin (Aspirin, ASP) as traditional and immemorial

antipyretic analgesics was object of study. Kunming mice were test animals. Effects on mice blood and spleen lymphocyte function of different doses of aspirin in the safe range were detected in vitro test. The purpose was to discuss whether Aspirin can regulate animal immunity and to determine the appropriate dose on immunity.

## MATERIALS AND METHODS

**Test animals:** Forty eight Kunming mice, weighing 20±2g, were purchased from the Qingdao veterinary supervision. Male and female were half, isolation raising, feeding, drinking water freedom.

**Reagent:** Aspirin (Sigma); Pokeweed (Phytolacca Americana, PWM) (Sigma); 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, MTT (Amresco); RPMI1640 medium (Gibco); Lymphocyte separation medium (TBD) (Tianjin Chuanye Biochemical Products Co., Ltd.).

Heparin, PBS, Trypan blue dye, *et al.* General reagents were all provided from veterinary drugs department of Liuhe.

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Table 1: Effects of Aspirin on the lymphocyte proliferation in mice

	Trial day	Control	Low dose group	Middle dose group	High dose group
lymphocyte proliferation in blood	10d	0.110±0.057 <sup>ab</sup>	0.136±0.056 <sup>a</sup>	0.146±0.076 <sup>a</sup>	0.083±0.043 <sup>b</sup>
	20d	0.121±0.070 <sup>a</sup>	0.134±0.082 <sup>a</sup>	0.146±0.057 <sup>a</sup>	0.111±0.073 <sup>a</sup>
lymphocyte proliferation in spleen	10 d	0.071±0.025 <sup>a</sup>	0.079±0.020 <sup>a</sup>	0.084±0.062 <sup>a</sup>	0.068±0.028 <sup>a</sup>
	20 d	0.070±0.043 <sup>b</sup>	0.078±0.043 <sup>ab</sup>	0.091±0.053 <sup>a</sup>	0.075±0.030 <sup>ab</sup>

Different letters indicated differences were significant ( $p < 0.05$ ); unmarked or the same letter, showed the difference was not significant ( $p > 0.05$ )

**Methods:** Forty eight mice (20±2g) whose body weight were similar were selected and divided into four groups: control group:

- Low-dose group (10 mg/kg/bw)
- Middle dose group (20 mg/kg/bw)
- High dose group (40 mg/kg/bw)
- Aspirin were administered once daily

There were half male and half female in every group. They were divided into cages to rise and were given a free choice access to diets and tap water. Mice were pre-raised into trial period after 3 days. Eyeball blood were collected in the trial period of 10 days, 20 days, with heparin, then mice were sacrificed by eagerly and spleens were collected aseptically. Each group procedures can be cross.

**Preparation of lymphocyte suspension (Cui, 2004; Yang, 2003):** The peripheral blood were taken from anticoagulation with heparin saline. The appropriate amount of lymphocyte separation medium were added in a centrifuge tube, anticoagulant blood were covered lightly in lymphocyte separation medium surface, centrifuging 15 min by 1500r/min. The white turbid zone between the two liquid phases were sucked (including lymphocytes) as far as possible and transferred to another centrifuge tube, adding appropriate amount of PBS and washing twice. Bloods were diluted lymphocytes to  $1 \times 10^6$ /mL with RPMI 1640 complete medium. Cell viability was detected by trypan blue, live cell ratio should be greater than 95%.

**Preparation of lymphocyte suspension:** Spleen were sterile taken and were placed plates which were added 3mL PBS in advance. Blood and fat were washed, the spleen tissue were ground into cells suspension in the appropriate amount of PBS (P H7.4) by 200 mesh copper mesh. The appropriate amount of lymphocyte separation medium were added in a centrifuge tube, anticoagulant blood were covered lightly in lymphocyte separation medium surface, centrifuging 15 min by 1500 r/min. The white turbid zone between the two liquid phases were sucked (including lymphocytes) as far as possible and transferred to another centrifuge tube, adding appropriate amount of PBS and washing twice. Bloods were diluted lymphocytes to  $2 \times 10^6$ /mL with RPMI 1640 complete medium. Cell viability was detected by trypan blue, live cell ratio should be greater than 95%.

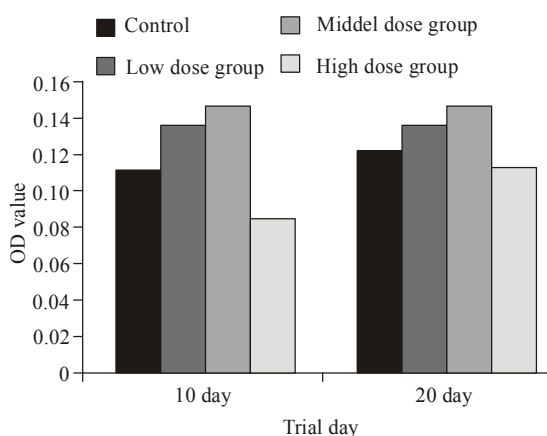


Fig. 1: Effects of aspirin on the lymphocyte proliferation in blood of mice

**Determination of immunocyte function:** Blood lymphocytes suspension, spleen lymphocytes suspension were separately prepared. Function determination: 96 hole cell culture plate was used, 50µL cell suspension were added in each hole, RPMI1640 complete medium were added in empty hole, 5 replicates were set each sample. Cell was cultivated 36h in CO<sub>2</sub>, temperature was 37°C and saturated humidity was 5%. 25 µL MTT each hole were added before culture termination, cell were incubated for 4h in 37°C. DMSO added to each well 100 µL, OD values was measured in the wavelength of 570 nm by ELISA; the results were showed the average of 5 holes.

All data were presented as the mean±SD ( $\bar{X} \pm SD$ ) by Microsoft Excel and analyzed in One-Way ANOVA by using SAS9.0.

## RESULTS AND ANALYSIS

Results of 10<sup>th</sup> day and 20<sup>th</sup> day after administration showed that blood and spleen lymphocyte activity of low dose group and middle dose group were all tend to rise. Cell activity of middle dose group was higher than ones of the low dose group, but the difference was not significant ( $p > 0.05$ ). On 20<sup>th</sup> day of the trial period, spleen lymphocyte activity of the middle dose group was significantly higher than that in the control group ( $p < 0.05$ ). Two measurements of blood and spleen lymphocyte activity of the high dose group were inhibited, but the difference was not significant ( $p > 0.05$ ) (Table 1, Fig. 1 and 2).

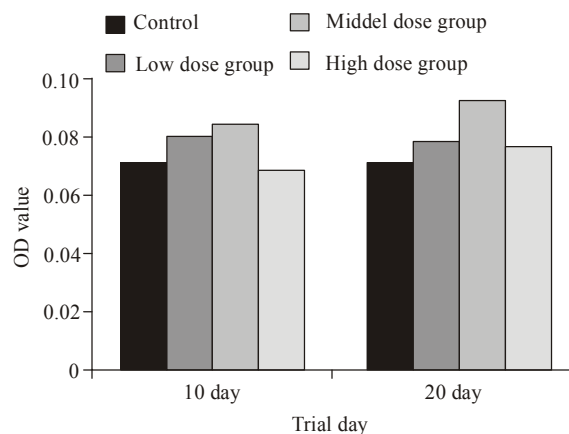


Fig. 2: Effects of aspirin on the lymphocyte proliferation in spleen of mice

## DISCUSSION

Lymphocytes are a class of immune cells, including immunocompetent cells (T cells and B cells), killer cells (killer cell, K cell) and natural killer cells (natural killer cell, NK cell), *et al.* Lymphocytes are widely distributed in vivo and quantity is much, they are existed in all organizations in addition to the central nervous system. Peripheral blood and spleen are the place which two major lymphocyte exist and proliferate and play roles of immunomodulatory. So the peripheral blood and spleen lymphocytes were as the detection indicators, effects on mice' immunity of aspirin was studied.

Lymphocyte transformation rate is an important indicator for the evaluation of cellular immune function. The process is lymphocyte and antigen or mitogen combine, cell metabolism and form change in succession. Mainly show that cell surface charge change for a short period of time, after a few hours intracellular enzyme activate, leading to the start of DNA synthesis, resulting in a series of changes, such as cells becoming largen, cytoplasm enlarging, vacuolus appearing, prominent nucleoli, loose chromatin, lymphocytes transforming into the mother cell. Lymphocyte proliferation reaction can be counted by morphological observation, increase of DNA synthesis in cell is detected by  $^3\text{H}$ -thymidine incorporation assay, accordingly irritative reactive of lymphocytes and functional status are estimated. The morphological method is simple, but the interpretation of the results are influenced by subjective factors, repeatability and reliability are poor.  $^3\text{H}$ -thymidine incorporation is more objective, accurate, but it need the radioactive detection equipment, measure is too much trouble. Study of Mosmann (1983) showed that the tetrazolium salt (MTT) assay was an ideal method of testing lymphocyte function. The principle is the activity of succinate dehydrogenase is existed in living cells, particularly in proliferating cells, this enzyme maybe restore MTT (the pale yellow oxazole nitrogen salt) into hepatic crystallize material (purple formazan) and

coloration. Its optical density (Optical Density, OD) can reflect the cell proliferation. This method have no use for special equipment and instruments, the operation is relatively simple. The choice of mitogen is the more important aspect in MTT assay. *Phytolacca Americana* (PWM) can induce lymphoid cell transformation, Lipo Poly Saccharides (LPS) can cause B cell transformation, Concanavalin A (Con A) can cause T cell mitogenic (Chen, 2004). Effects on lymphocyte activity of aspirin are initial surveyed, so PWM was chosen to mitogen.

Aspirin has a very wide range of uses, its most basic role is antipyretic and analgesic and does not produce drug dependence. Another important role of aspirin is anti-inflammatory, anti-rheumatic and is choice drug of the treatment of rheumatism, rheumatoid arthritis. Results of many researches is accordance. But there is little study about effects on body immunity of aspirin and there is no unified conclusion, it still in opinions vary. Crout *et al.* (1975) showed that aspirin can inhibit lymphocyte proliferation. In contrast, Smith *et al.* (1975) found that aspirin did not inhibit the lymphocyte proliferation. Graham (2008) considered aspirin could weaken antibody response. Panush studied that clinical antipyretics doses could enhance lymphocyte immune responses in vitro experimental, while high doses had roles of inhibition (Panush, 1978; Panush and Ossakow, 1979) and it is consistent with Graham's conclusions.

Mice was experimental animals, three different doses of aspirin test group were designed, mouse blood and spleen lymphocyte transformation were detected by MTT. Results showed that blood and spleen lymphocyte activity of mice of low-dose (10 mg/kg) and moderate doses (20 mg/kg) were enhanced, cell activity of middle dose group was higher than ones of the low-dose group, but the difference was not significant ( $p > 0.05$ ). In the 20<sup>th</sup> days in the trial period, the spleen lymphocyte activity was significantly higher than that in the control group ( $p < 0.05$ ). While mice blood and spleen lymphocyte activity of the high-dose group (40 mg/kg) was decreased, but the difference was not significant ( $p > 0.05$ ). This result was accordance with studies of Panush and Graham. However, aspirin can affect humoral immunity or cellular immunity, it needs to be further explored.

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