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Effects of artesunate on immune function in mice

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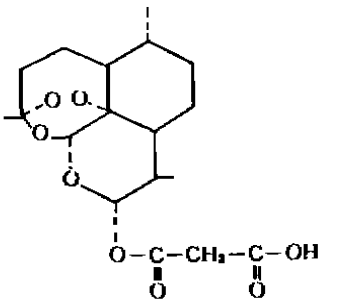
AIM: To study the effects of artesunate (dihydroartemisinine-12-α-succinate, Art) on immune function in mice. **METHODS**: Hemolysin concentration was determined by colorimetric method. Serum IgG and C3 contents were measured by single immunodiffusion method. Percentage of lymphocyte transformation, phagocytosis percentage and phagocytic index were counted under microscope. **RESULTS**: Art im 75 mg kg^{-1} bid × 7 d decreased the humolysin-forming capacity and levels of serum IgG of mice sensitized with sheep red blood cell. The serum complement 3 level rose remarkably, when Art was given im to *Plasmodium berghei*-infected mice. Art enhanced the PHA-induced lymphocyte transformation rate (*in vivo*) in mice and increased the weight of spleen but reduced that of thymus in mice. Art elevated the DNFB-induced delayed-type hypersensitivity. Art im 75 mg kg^{-1} bid × 5 d reduced the percentage of phagocytosis of peritoneal macrophages and the phagocytic index. **CONCLUSION**: Art suppressed the humoral immune responses but enhanceed the cell-mediated immunity.

KEY WORDS artesunate; hemolysins; IgG; complement 3; lymphocyte transformation; immunization; delayed hypersensitivity; phagocytosis

Artesunate (Art), a derivative of artemisinin, is a hemisuccinate of dihydroqinghaosu (reduced qinghaosu). It is a new antimalarial agent with high effectiveness yet low toxicity. Clinical studies showed that Art was superior to qinghaosu in treating cerebral malaria and in chloroquine-resistant cases[1]. There had been only a few reports on the immuno-pharmacological effects of Art[1,2], in spite of a lot of reports on such effects of qinghaosu and artemether[4—10]. To investigate the immuno-pharmacologic actions of Art, several methods were used for determining the effects of Art on immune function in mice in this paper.



Artesunate

MATERIALS AND METHODS

Mice ICR mice of either sex, aged 5 — 6 wk were bred from the Animal Breeding Unit of our Institute.

Drugs Art was supplied by Guilin Pharmaceutical Factory. The drug was dissolved in sterile peanut oil at the concentration of 15 g L^{-1}. The dose was 75 mg kg^{-1} bid im for 5 — 7 d. The control mice were given im peanut oil of the same volume.

Serum hemolysin assay ICR mice weighing 23±*s* 4 g, were randomly divided into 2 groups of comparable body weight and sex ratio. Mice in experimental group were given im Art 75 mg kg^{-1} bid × 7 d, while the control group was given peanut oil only. All mice were sensitized by sheep red blood cell (0.2 mL 20 %

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SRBC suspension, ip) once on d 2 of medication. Five days after being sensitized by SRBC, mice were bled and serum samples was diluted with normal saline to 1 : 400. Hemolysin concentration was determined by colorimetric method[11].

Serum IgG level determination Serum IgG was measured in an antibody-agar plate using single immunodiffussion method[12].

Lymphocytes transformation (*in vivo*) test ICR mice, weighing 20±*s* 2 g, were randomly divided into 2 groups. Art was given im for 7 d. At the same time, on d 4, d 5, and d 6, lymphocytes transformation were induced by PHA (6 mg kg^{-1} × 3 d im). Peripheral blood smears were prepared for Wright and Giemsa staining. Percentage of lymphocyte transformation was counted in 100 lymphocytes under microscope (10 × 100). On d 8 the mice were sacrificed. The spleen and thymus were weighed.

Delayed hypersensitivity assay ICR mice (20±*s* 2 g) were divided into Art (im 75 mg kg^{-1} bid × 7 d) group and the control group. On d 0 each mouse was sensitized by dinitrofluorobenzene (50 μL 1 % DNFB, sc). On d 7, 1 % DNFB was spread on the right ear as an attack. On d 8, all the mice were killed. The left and right ear pinnae (φ = 8 mm) were weighed separately. The degree of DTH was shown by the difference of the weight of the 2 ears.

Phagocytosis of peritoneal macrophages ICR mice (27 ± *s* 2 g), were randomly allotted into 2 groups, the Art (im 75 mg kg^{-1} bid × 5 d) group and the control group. On d 3 of administration, peritoneal macrophages were induced by ip 1 mL of 2 % soluble starch. On d 5, 2 h after administration of Art, 0.5 mL of 5 % chicken red blood cells (CRBC) was injected ip into each mouse and the mice were killed 4 h later. The peritoneal macrophages were washed by Hanks' solution. The fluid of abdominal cavity was collected to make a smear for each. The smears were incubated at 37 ℃ for 30 min in a wet box, washed by physiological saline solution, then stained by Wright-Giemsa staining after quick drying. The phagocytosis percentage and phagocytosis index were calculated as indicators for measuring the phagocytosis.

Complement 3 (C 3) determination ICR mice (26±*s* 2 g, ♀ and ♂) were divided into 3 groups. On d 1 of experiment, the mice were inoculated with 5 × 10^6 infected RBC (*Plasmodium berghei*) ip, except the normal control group. During d 3—7 the treated group was given im Art 75 mg kg^{-1} bid × 5 d, while the normal control group and malarial group were im peanut oil. On d 8, the mice were bled to obtain serum. Serum C 3 contents were measured with rabbit anti-mouse C 3 antibody by single immunodiffusion method. The rabbit anti-mouse C 3 antibody was prepared in rabbits immunized with inulin-C 3 (mice) complexes[13].

Statistical analysis The significance of the differences obtained was evaluated by *t* test.

RESULTS

Effect of Art on humoral immune function in mice Art was im to mice at a dose of 75 mg kg^{-1} bid for 7 d. Results showed that Art markedly reduced the serum hemolysin content in SRBC-sensitized mice ($P<0.01$). Moreover, the levels of serum IgG in mice sensitized with SRBC were markedly lowered ($P<0.05$, Tab 1).

Tab 1. Effects of artesunate 75 mg kg^{-1} (im bid for 5—7 d) on mouse immunoresponses. Number of mice in parentheses. $\bar{x}\pm s$.
$^bP<0.05$, $^cP<0.01$ *vs* control.

	Control	Artesunate
50 % Hemolytic concentration	761±62 (17)	64±30 (15)^c
Serum IgG, g L^{-1}	13.3±3.1 (18)	10.6±4.2 (17)^b
Lymphocyte transformation	0.18±0.07 (17)	0.24±0.01 (16)^b
DNFB DTH, mg	13±3 (16)	17±3 (14)^c
Phagocytosis	0.43±0.44 (11)	0.28±0.06 (11)^c
Phagocytic index	0.94±0.19 (11)	0.54±0.26 (11)^c
Thymus index, g kg^{-1}	5.43±0.96 (17)	4.43±0.44 (16)^c
Spleen index, g kg^{-1}	9.41±1.37 (17)	14.84±4.31 (16)^c

Effects of Art on the rate of PHA-

induced lymphocyte transformation in mice Art was *im* to mice at a dose of 75 mg kg^{-1} bid for 7 d. Results showed that Art enhanced the rate of PHA-induced lymphocyte transformation in mice ($P<0.05$). Besides, Art reduced the weight of thymus in PHA-treated mice ($P<0.01$) while increased the weight of spleen ($P<0.01$) *vs* control (Tab 1).

Effects of Art on DNFB-induced delayed type hypersensitivity (DTH) in mice Art (75 mg kg^{-1} bid × 7 d) elevated the DNFB-induced DTH on mice ($P<0.01$). Results suggested that Art can enhance the cellular immune responses (Tab 1).

Effects of Art on phagocytosis of peritoneal macrophages in mice On d 5, the results showed that Art (75 mg kg^{-1} bid × 5 d) suppressed phagocytosis of peritoneal macrophage in mice. The phagocytic rate and index were both lower in the treated than in the control group ($P<0.01$, Tab 1).

Effects of Art on serum complement 3 levels in mice Results showed that serum C 3 levels in *Plasmodium berghei*-infected mice were markedly lower than those in the normal mice ($P<0.01$). Art 75 mg kg^{-1} bid for 5 d im to malarial mice caused a marked increase of serum C 3 level ($P<0.01$, Tab 2).

Tab 2. Effect of artesunate (im bid × 5 d) on serum complement 3 (C3) level in mice. $\bar{x}\pm s$. $^{*}P<0.01$ *vs* **malaria mice.**

	Dose, mg kg^{-1}	Mice	Serum C3, g L^{-1}
Control	—	17	$1.97\pm0.35^{*}$
Mal	—	17	1.25 ± 0.28
Mal+Art	75	17	$2.36\pm0.80^{*}$

DISCUSSION

According to pharmacokinetic studies, Art was widely distributed in tissues and eliminated fairly rapidly. In this paper, Art was *im* bid in order to prolong its plasma level. The above data demonstrated that Art reduced the levels of serum IgG and hemolysin-forming capacity of SRBC-sensitized mice. The data suggested that Art possessed suppressive effects on the humoral immunity. In addition, when Art was im to *Plasmodium berghei*-infected mice, serum C 3 level was elevated. Clinical trials showed that serum IgM and IgG levels were increased but serum C 3 contents were reduced in malarial patients. Sometimes these patients developed immune complex diseases. Thus it was considered that Art had the effect of inhibiting the humoral immunity and regulating the C3, which was beneficial to clear up the immune complexes in patients suffering from malaria or autoimmune and immune complex disease. Moreover, *in vivo* results indicated that Art enhanced the PHA-induced lymphocyte transformation and the weight of spleen in mice. Art could also elevate the DNFB-induced delayed type hypersensitivity in mice. These data suggested that Art possessed a stimulating effect on cell-mediated immunity. On the other hand, it was also observed that Art suppressed phagocytosis of the macrophages and reduceed the weight of thymus in mice.

Other reports indicated that Art enhanced the humoral immunity as well as non-specific immune functions. The experiments *in vitro* showed that Art inhibited lymphocyte proliferation induced by T cell mitogens[3]. To sum up, the immunopharmacologic effects of Art were rather complicated. The different dosages and different method of assay may exhibit different effects on the immune function. Therefore, immunopharmacologic actions of Art deserve further investigation.

According to previous reports[4-6] and the results of our study, the immunological effects of Art were quite similar to those of

artemisinin in mice.

REFERENCES

1 China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials. Antimalarial efficacy and mode of action of Qinghaosu and its derivatives in experimental models.
J Trad Chin Med 1982; 2: 17—24.

2 Huang GJ, Zhao Y. Experimental studies of the effect of sodium artesunate on the immune functions of mice.
J Trad Chin Med 1983; 3: 171—6.

3 Shen M, Ge HL, He YQ. Immuno-suppressive action of artemisinin. Sci Sin (B) 1983; (10): 928—34.

4 Qian RS, Li ZL, Yu JL, Ma DJ. The immunologic and antiviral effect of qinghaosu.
J Trad Chin Med 1981; 22: 463—6.

5 Lin PY, Pan JQ, Feng ZM.
Effect of artemisinin on serum IgG in mice.
Chin Trad Herb Drugs 1985; 16: 66—9.

6 Lin PY, Pan JQ, Feng ZM, Zhang D, Yang WL. Immunopharmacologic activity of artemisinin (qinghaosu).
Asia Pac J Pharmacol 1988; 3: 197—200.

7 Lin PY, Pan JQ, Feng ZM. The effects of artemether on serum IgG and spleen weight in mice.
Acta Pharm Sin 1985; 20: 211—3.

8 Li XY, Liang HZ. Effects of artemether on red blood cell immunity in malaria.
Acta Pharmacol Sin 1986; 7: 471—5.

9 Gu YX, Tsui YF, Wu BA, Shi XC, Teng XH. Effect of artemether on peripheral lymphocytes in beagle dogs.
Chin J Pharmacol Toxicol 1988; 2: 42—5.

10 Zhang D, Lin PY, Pan JQ, Yang WL.
Effect of artemether on immunological functions in mice.
Chin Pharmacol Bull 1989; 5: 37—9.

11 Xu XY, Li Y, Xu J. A modified humoral immune assay method, a method of hemolysin determination.
Acta Pharm Sin 1979; 14: 443—6.

12 Lin PY, Pan JQ.
A study of experimental model of serum IgG of mice.
Guangzhou Med J 1981; 12 (6): 28—32.

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青蒿琥酯对小鼠免疫功能的影响

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目的：研究青蒿琥酯对小鼠免疫功能的影响. **方法**：溶血素含量用分光光度法测定，血清IgG和C3含量用单向免疫扩散法测定，淋巴细胞转化率、巨噬细胞吞噬百分率和吞噬指数镜检计数. **结果**：青蒿琥酯 im 75 mg kg^{-1} bid for 5—7 d 能降低 SRBC 致敏小鼠血清溶血素和 IgG 的含量，抑制抗体生成，但增加疟鼠补体 C3的含量. 青蒿琥酯能促进 PHA 诱导的小鼠体内淋巴细胞转化，能提高 DNFB 所致的迟发型超敏反应，并减少腹腔巨噬细胞的吞噬百分率和吞噬指数. **结论**：青蒿琥酯对体液免疫有抑制作用，但对细胞免疫有促进作用.

关键词 青蒿琥酯；溶血素类；免疫球蛋白G；补体成分3；淋巴细胞转化；免疫法；迟发型超敏反应；吞噬作用