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Schizontocidal effects of oral artesunate on *Plasmodium berghei* in mice and *P knowlesi* in monkeys

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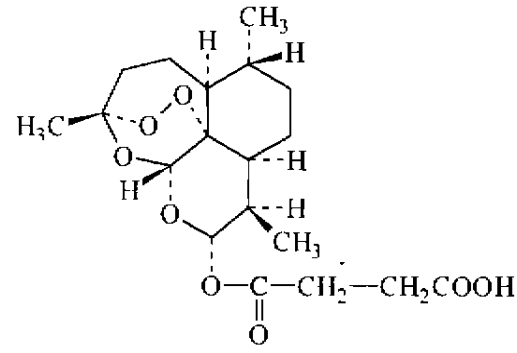
KEY WORDS artesunate; chloroquine; *Plasmodium berghei*; *Plasmodium knowles*; antimalarials

ABSTRACT

AIM: To study the blood schizontocidal effect of oral artesunate on *P berghei* in mice and *P knowlesi* in monkey. **METHODS**: Effects of artesunate and chloroquine were detected with "4-day test" and "28-day test" on *P berghei* in mice and "7-day test" on *P knowlesi* in *Macaca mudatta*. **RESULTS**: The suppressive efficacy of oral artesunate was inferior to chloroquine on *P berghei* K_{173} strain but the time for 50 % and 90 % reduction and the time of clearance of parasitemia was 10 - 15 h shorter than that of chloroquine. Its curative effect on RC/K_{173} line was markedly superior to that of chloroquine. Moreover, artesunate showed no cross-resistance with chloroquine, index of resistance I_{90} was only 1.4. At 31.6, 10.0, and 3.16 mg·kg^{-1}, artesunate and chloroquine oral administrations cured *P knowlesi* in all monkeys. Recrudescence did not occur in 105 d. **CONCLUSION**: The study of effects of oral artesunate in *P berghei*/mice and *P knowlesi*/*Macaca mulatta* model provided a useful index for clinical trial.

INTRODUCTION

Artesunate is one of the derivatives of artemisinin isolated from the Chinese herb *Artemisia annua* L. It is the hemisuccinate of dihydroartemisinine[1-3]. Artesunate iv was approved in China in 1986. It has the advantages of being highly effective and rapid in onset of action with low toxicity. It has no cross-resistance with chloroquine resistant lines[1,4]. Oral artesunate was far superior to artemisinin and matched iv artesunate[3]. Therefore it can be used in the treatment of critical cases. This study was to further evaluate the blood schizontocidal effect of oral artesunate dose on *P berghei*/mice and *P knowlesi*/*Macaca mulatta* system.



Artesunate

MATERIALS AND METHODS

Drug Artesunate powder produced by the First Guilin Pharmaceutical Factory (batch № 890606) was suspended in sterilized water with Tween-80 and diluted with water. Chloroquine phosphate powder was produced by Shanghai 14th Pharmaceutical Factory (batch № 821029) and diluted with water. Dose of chloroquine phosphate was expressed as base. Both drugs were administered by intragastric gavage (ig).

Mice Swiss-Kunming cross breed mice, ♀, Grade Ⅱ, Certificate № 01-3023, weight about 18 - 22 g were provided by Animal Center, Academy of Military Medical Sciences. Mice were divided into groups at random. The breeding room was air-conditioned (22 ℃ ± 2 ℃, relative humidity 65 %).

Monkeys Monkeys (*Macaca mulatta*) of either

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sex, weighing about 2.0 – 3.5 kg were supplied by Animal Center, Academy of Military Medical Sciences. They were transferred into the laboratory and caged individually 2 wk before the test.

Parasites *Plasmodium berghei* K_{173} strain was introduced from Department of Medical Protozology, London School of Hygiene and Tropical Medicine in 1983. RC/K_{173} line was highly chloroquine resistant (I_{90} = 110) which was developed from K_{173} strain by our department[5-7]. *Plasmodium knowlesi* Nuri strain was introduced from Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine in 1983. It was kept in liquid nitrogen. The parasites were revived by intravenous injection to a normal monkey as a parasite donor.

Determination of suppressive efficacy and cross-resistance level to chloroquine The "4-day test" was adopted in this experiment[5-9]. Using heparin as anticoagulant, on d_4 blood 10 mL was taken from retrobulbar phexus of mouse. Blood sample of each dose group was mixed well. Thin blood smears of pooled blood was made and fixed in methanol. Giemsa stain to calculate the parasitemia. The doses required to 50 % and 90 % reduction which were calculated by regression equation[12] were expressed as SD_{50} and SD_{90}, respectively. Index of resistances: I_{50} and I_{90} were calculated according to SD_{50}/SD_{90} against RC line divided by SD_{50}/SD_{90} against N strain. Test was performed 3 times, and $\bar{x} \pm s$ were calculated.

Parasitemia clearance[6,9] The doses of artesunate and chloroquine were 8 and 16 times, respectively, of their SD_{90}, given twice at an interval of 6 h. Blood smear was made every 6 h. The reductive rate of parasitemia in each smear was calculated. By regression equation, the regression coefficient (b) and time required for the 50 %/90 % reduction were calculated.

Curative efficacy The "28-day test" was employed[9,10]. There were 5 dose groups (2, 4, 6, 8, and 10 times of SD_{90}) for artesunate and chloroquine. Each group consisted of 20 mice, 1×10^7 N strain parasited RBC were inoculated ip per mouse. After 2 h, the drugs were given ig once daily for 5 d. Blood smears were made every 3 – 4 d thereafter till 28 d. The mouse was considered cured if no parasite was found during this period.

Schizontocidal efficacy of oral artesunate on *P knowlesi* The "7-day test" was conducted[10,11]. Three doses of artesunate and chloroquine were given ig 31.6, 10.0, and 3.16 mg·kg^{-1}. Each group consisted of 3 monkeys. For each monkey 5×10^9 parasited RBC were injected iv. When parasitemia attained 3.5 % – 6.0 %, the drugs were administered ig once daily for 7 d. After first dose thin and thick smears were made every 6 h, 3 times after parasitemia become negative. Smears were examined till 105 d. Using linear regression equatorial the time for 50 % and 90 % reduction and regression coefficient was calculated to evaluate the rapidity of parasite clearance of the drugs.

Curative efficacy was evaluated according to the following criteria Ineffective (I): before and after treatment no significant difference in degrees of parasitemia or continuously parasitemia increases noted; Slight suppression (SS): parasitemia was suppressed temporarily and increased thereafter; Marked suppression (MS): parasitemia became negative for >2 d, but within 30 d recrudescence occurred; Complete suppression (CS): recrudescence occurred in 30 – 105 d after first negative parasitemia; Cure (C): no recrudescence in 105 d after the first negative parasitemia.

RESULTS

Blood schizontocidal efficacy of oral artesunate on *P berghei* K_{173} N/RC strain in mice

Suppressive efficacy and index of resistance The blood schizontocidal efficacy of artesunate ig was inferior to chloroquine on N strain line but its suppressive efficacy on RC/K_{173} line was markedly superior to chloroquine. Artesunate did not show cross-resistance with chloroquine. I_{50} and I_{90} of artesunate were 1.0 and 1.4, while I_{50} and I_{90} of chloroquine were 45.8 and >101.7 (Tab 1).

Parasitemia clearance of *P berghei* K_{173} N strain The time for parasite clearance of 16 times of SD_{90} of artesunate were 13 and 9 h shorter than those of chloroquine at the time for 50 % and 90 % reduction of parasitemia. The time for clearance of parasitemia for artesunate was 14 h shorter than equivalent chloroquine dose. Equally 8 times of SD_{90} of artesunate was 13 and 10 h shorter than chloroquine in the times for 50 % and 90 % reduction of parasitemia

Tab 1. Suppressive effects of artesunate and chloroquine ig on *P berghei* K_{173} N strain and *P berghei* RC/K_{173} line. $\bar{x} \pm s$.

	K_{173} N strain		K_{173} N line			
	SD_{50}	SD_{90}	SD_{50}	SD_{90}	I_{50}	I_{90}
	mg·kg^{-1} × 4 d		mg·kg^{-1} × 4 d			
Artesunate	6.33 ± 0.23	23 ± 7	6.4 ± 0.4	32 ± 19	1.0	1.4
Chloroquine	1.4 ± 0.4	3.54 ± 0.22	63 ± 11	>360	45.8	>101.7

and the time of clearance of parasitemia was 15 h shorter than that of chloroquine (Tab 2).

Tab 2. Time of parasitemia clearance of artesunate and chloroquine ig *P berghei* K_{173} strain. $n = 10$ mice. $\bar{x} \pm s$.

Dose/mg·kg^{-1}		Time of SD_{90}	% Infected RBC before treatment	Time for 50 % reduction/h	Time for 90 % reduction/h	Time of parasitemia clearance/h
Artesunate	374.4	16	57.3	14.0	26.4	40.4
	187.2	8	56.2	18.0	28.1	42.6
Chloroquine	56.6	16	57.4	27.1	35.1	54.0
Phosphate	28.3	8	54.7	30.6	38.0	57.6

Curative efficacy Curative efficacy of artesunate and chloroquine oral on *P berghei* K_{173} N strain was shown in Tab 3. The mice administered orally with 6, 8, and 10 times of artesunate SD_{90} were cured, while those received 2 and 4 times of their SD_{90} were not cured, but those received chloroquine 7.1 mg·kg^{-1} were completely cured (Tab 3).

Tab 3. Curative effects of artesunate and chloroquine ig on *P berghei* K_{173} strain.

Dose/mg·kg^{-1}		Time of SD_{50}	Cured/total mice
Artesunate	234.0	10	20/20
	187.2	8	20/20
	140.4	6	20/20
	93.6	4	11/20
	46.8	2	0/20
Chloroquine	14.2	4	20/20
Phosphate	7.1	2	20/20

Blood schizontocidal efficacy of artesunate ig on *P knowlesi* The mean time for 50 % and 90 % reduction of parasitemia of oral artesunate administration was shown in Tab 4. Compared with equal dose groups, in case of 31.6 and 10 mg·kg^{-1}, the mean time for 90 % parasitemia reduction of oral artesunate administration was the same as that of chloroquine, but in 3.16 mg·kg^{-1} dose group artesunate was 13 and 18 h shorter than chloroquine in the time for 50 % and 90 % reduction. The rapidity of parasite clearance of oral artesunate administration on *P knowlesi* was obviously superior to that of chloroquine at 3.16 mg·kg^{-1} dosage (Tab 4).

Tab 4. Time of parasitemia clearance of artesunate and chloroquine ig on asexual form of erythrocytic stage of *P knowlesi*. $n = 3$ monkeys/group. $\bar{x} \pm s$.

Dose/mg·kg^{-1}		% Infected RBC before treatment	Time for 50 % reduction/h	Time for 90 % reduction/h	Time of parasitemia clearance/h
Artesunate	31.6	51.3	7.9 ± 0.5	13.9 ± 1.1	52 ± 3
	10.0	35.0	8.2 ± 0.8	13.7 ± 0.9	50 ± 3
	3.16	42.7	7.6 ± 0.4	13.3 ± 0.8	54 ± 10
Chloroquine	31.6	56.3	5.8 ± 0.9	13.3 ± 1.6	64 ± 4
Phosphate	10.0	39.3	5.9 ± 0.6	13.2 ± 0.7	64 ± 9
	3.16	49.0	21 ± 18	31 ± 20	76 ± 14

Curative efficacy of artesunate and chloroquine on *P knowlesi* was shown in Tab 5. Compared with equal dose groups, curative efficacy of artesunate against asexual form of *P knowlesi* was the same as that of chloroquine. In case of 31.6, 10.0, and 3.16 mg·kg^{-1}, all animals treated by artesunate and chloroquine got cured. Recrudescence did not occur in all monkeys of 3 dose groups on 105 d.

DISCUSSION

The suppressive therapeutic efficacy of oral artesunate on N strain and RC line of *P berghei* K_{173} was superior to chloroquine and no cross-resistance to chloroquine. The rapidity of parasite clearance of oral artesunate was higher than that of chloroquine. Moreover, the rapidity of parasite clearance of oral artesunate was as well as that of artesunate iv[1,2,3]. The curative efficacy of oral artesunate on asexual forming *P berghei* K_{173} N strain was inferior to

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chloroquine. When dose increased to 6 times of SD_{90}, all mice can be only protected, the dose was 140.4 $mg \cdot kg^{-1} \times 5$ d, while 2 times of equivalent dose (7.1 $mg \cdot kg^{-1} \times 5$ d) of chloroquine got 100 % cure rate[1,2].

Compared with equal dose groups, in 3.16 $mg \cdot kg^{-1}$ dose group, artesunate ig was 13 and 18 h shorter than chloroquine in the time for 50 % and 90 % reduction. Besides, as compared with artesunate iv, the blood schizontocidal efficacy of artesunate ig was equal to artesunate iv. Furthermore, the curative efficacy of artesunate ig on asexual form of *P knowlesi* was identical to that of chloroquine. In the case of 31.6, 10.0, and 3.16 $mg \cdot kg^{-1}$, all monkeys treated with artesunate and chloroquine got cured[3,11]. However, this was not the case observed in *P berghei*/mice experiments in which the curative effect of artesunate ig was poor. This may be due to species difference or due to the relative shorter of artesunate time course (5 d) in *P berghei*/mice experiments. In view of what above mentioned, therefore we suggest that artesunate regard as oral antimalarial drug is hopeful.

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口服青蒿琥酯对伯氏鼠疟和诺氏猴疟的杀灭效果

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关键词 青蒿琥酯；氯喹；伯氏疟原虫；诺氏疟原虫；抗疟药

目的：研究青蒿琥酯口服对伯氏鼠疟和诺氏猴疟的血液裂殖体杀灭效果。**方法**：分别在鼠疟和猴疟模型上采用"4-day 试验法"、"28-day 试验法"和"7-day 试验法"检测了青蒿琥酯和氯喹的药效。**结果**：口服青蒿琥酯对伯氏鼠疟 K_{173} 株的抑制效果低于氯喹，但其原虫血症下降 50 %、90 % 和转阴的时间比氯喹快 10-15 h，对抗株 RC/K_{173} 的疗效优于氯喹，无交叉抗性，I_{90} 仅为 1.4。青蒿琥酯和氯喹对诺氏猴疟在 31.6, 10.0 和 3.16 $mg \cdot kg^{-1}$ 剂量组的试验猴全部治愈。**结论**：口服青蒿琥酯在鼠、猴疟模型上的药效研究为临床研究提供有益参考。

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