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Early treatment of schistosomal infection with artemether and praziquantel in rabbits[1]

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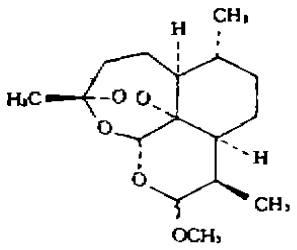
ABSTRACT Artemether (Art, β-methyl ether of artemisinin) first synthesized by Shanghai Institute of Materia Medica, Chinese Academy of Sciences, is effective against not only malaria but also schistosomiasis. When rabbits infected with *Schistosoma japonicum* cercariae for 7 d were treated ig with Art 10 $mg \cdot kg^{-1}$, the total worm reduction rates were 74.6—76.7 %. If Art (10 $mg \cdot kg^{-1}$) was given once weekly after the first treatment for 3—4 times, the total worm reduction rate was >98 %, and most of the rabbits were free from ♀ worms. When praziquantel (Pra) was given ig 40 $mg \cdot kg^{-1}$ to rabbits on d 21 after infection, and repeated once every week for 3 wk, most rabbits showed a total worm reduction rate >98 % with their livers showing normal or mild changes, and their parameters relevant to acute schistosomiasis were negative as compared to the controls. Hence Art and Pra are suggested to be used in field trial for control of acute schistosomiasis or reduction of the intensity of schistosomal infection.

KEY WORDS *Schistosoma japonicum*; artemether; praziquantel; combination drug therapy; drug administration schedule

When praziquantel (Pra) was given to mice infected with *Schistosoma japonicum* cercariae at an early stage after infection, most or even all of the ♀ worms before sexual maturity were killed[1]. When artemether (Art, first synthesized by Shanghai Institute of Materia Medica, Chinese Academy of Sciences[2] and found to be effective against schistosomes[3,4]) was given to mice at an early stage after infection, similar results were observed[5]. To evaluate appropriate regimens of Art and Pra used in early treatment for controlling schistosomiasis and reducing the intensity of schistosomal infection, this study was carried out in rabbits.



Artemether

MATERIALS AND METHODS

Parasites *Schistosoma japonicum* cercariae (Anhui isolate) was released from infected snail Oncomelania hupensis provided by the Department of Vector Biology of our Institute.

Rabbits New Zealand strain rabbits, ♀ and ♂, weighing 2.2±0.3 kg, were provided by the Animal Care Facilities of the Institute. Each rabbit was infected with 198—202 cercariae via shaved abdominal skin.

Drugs Pra was purchased from Shanghai 6th Pharmaceutical Factory and Art was the product of Kunming Pharmaceutical Factory. Pra and Art were

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suspended 5—30 mg·ml^{-1} in 1% tragacanth and the ig volume was 2.0 ml·kg^{-1}.

Regimens of Art and Pra Art was administered ig on d 7 after infection and repeated once in 1—2 wk for 3—4 times. The Pra treatment was started on d 21 after infection followed by repetition once in 1—2 wk for 2—3 times. To evaluate the influence of administration frequency on efficacy of Art, rabbits infected with cercariae were divided into 6 groups, in which one group was served as control and the other 5 groups were treated ig with Art 10 mg·kg^{-1} on d 7 and repeated the dosing once a wk for 0, 1, 2, 3 and 4 times.

Evaluation of efficacy The rabbits were killed 4—5 wk after treatment. The efficacy was evaluated by terms of the number of residual worms. The liver was evaluated as —: normal, fresh red color without egg tubercle; ±: the liver in fresh red color with few fine egg tubercles; +: the liver in light red color with some egg tubercles; ++: the liver in markedly red color with numerous small egg tubercles; +++: the liver in marked grey color with numerous larger egg tubercles.

Acute schistosomiasis Rabbits infected with 198—202 cercariae were divided into Group K, P and Q. The Group P (n=6) was treated ig with Art 10 mg·kg^{-1} on d 7 after infection and retreated weekly for 4 wk. In Group Q (n=5), Pra ig 40 mg·kg^{-1} was started on d 21 after infection, and repeated weekly for 3 wk. The Group K (n=5) was served as control. Four weeks after infection, the rectal temperature was measured and the blood was withdrawn from the ear vein for eosinophil count. The schistosomal eggs in feces were examined by hatching and light microscope. Serum was detected by circumoval precipitin test (COPT)[6] and dot-ELISA using monoclonal antibody[7] (n=4 in each group) for schistosomal antibody and circulating antigen, respectively, weekly during the 5th—9th wk after infection.

RESULTS

1 Early treatment with Art When Art 30 mg·kg^{-1} was given ig to rabbits on d 7 after infection, the total worm number was 9.0 ±4.9 (Group B), which was less than 127±7 of the control (Group A). The number of ♀ worms in Group B was also less than that in the control, but ♀ worms were still found in each rabbit of Group B. In groups treated ig with Art at the same dose once a wk (Group C) or 2 wk (Group D) followed the first dosing for 3—4 times, the total worm numbers of Group C and Group D were less than that of Group B. All 7 rabbits in Group C and 3/7 rabbits in Group D were cured (Tab 1), and only 1—2 ♀ worms remained in the other 4 rabbits of Group D. The rabbit livers in the two groups appeared to be normal.

When rabbits were treated ig with Art on d 7 after infection at 10 or 15 mg·kg^{-1}, the total worm reduction rates were 74.6 % (Group F) and 82.2 % (Group G). The liver changes in Group F and Group G were less apparent than that in control group (Tab 1). In another 3 groups, the rabbits were treated ig with Art 10 mg·kg^{-1} (Group H), 15 mg·kg^{-1} (Group I) or 30 (Group J) mg·kg^{-1}, and repeated the dosing once a week for 4 wk, the total worm reduction rates were >97 % (Tab 1). In these 3 groups, 3/8, 4/8 and 2/4 rabbits were free from ♀ worm, and only 1—3 ♀ worms remained in the other rabbits. Meantime, no apparent changes in the livers were detected in most of the rabbits, but in each group 2/8, 2/8 and 1/4 rabbits showed some fine egg tubercles in the liver with fresh red color and soft quality of the tissues.

2 Influence of administration schedule on efficacy of Art When Art was given ig to rabbits on d 7 (Group L) or d 7 and d 14 (Group M) after infection, the numbers of total and ♀ worms of these 2 groups were less than those of control, but 7—20 ♀ worms were found in 4/4 and 2/4 rabbits, respectively. The total worm reduction rates and ♀ worm reduction rates were similar in Group N, O and P treated ig with the same dose of Art once a wk for 3—5 times, being much

Tab 1. Effects of artemether (Art) and praziquantel (Pra) on rabbits infected with *Schistosoma japonicum* cercariae. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* corresponding Group C, J, K, P, T or Y.

Group	Medication d	Dose/ mg·kg^{-1} Art	Dose/ mg·kg^{-1} Pra	Rabbits without ♀ worms	Total worms	Worm reduction rate/%	♀ worms	♀ WRR/ %	Liver alteration
A		0	0	0/9	127±7	—	57±6	—	+−+
B	7	30	0	0/6	9.0±4.9^b	92.9	4.3±2.2^c	92.4	±−++
C	7,14,21,28,35	30	0	7/7	0	100.0	0	100.0	−
D	7,21,35,49	30	0	3/7	0.9±1.0^b	99.3	0.4±0.5^b	99.3	±
E		0	0	0/9	122±15	—	49±10	—	+−+
F	7	10	0	0/7	31±19^c	74.6	13±7^c	74.2	+
G	7	15	0	0/6	22±10^c	82.2	9.7±5.7^c	80.3	+
H	7,14,21,28,35	10	0	3/8	3.1±3.8^a	97.7	1.5±1.8^a	97.0	−−±
I	7,14,21,28,35	15	0	4/8	1.8±2.1^a	98.6	0.8±0.9^a	98.4	−−±
J	7,14,21,28,35	30	0	2/4	2.0±2.4	98.4	0.8±1.0	99.3	−−±
K		0	0	0/7	114±10	—	54±5	—	−+−
L	7	10	0	0/4	28±3^c	75.0	14±1	74.4	−
M	7,14	10	0	0/4	17±19^c	85.0	8.0±8.4^c	85.2	±−+−
N	7,14,21	10	0	2/4	1.0±1.2^a	99.1	0.5±0.6^a	99.1	−−±
O	7,14,21,28	10	0	2/4	2.0±2.8^a	98.2	1.0±1.4^a	98.1	−−±
P	7,14,21,28,35	10	0	5/8	1.6±2.4	98.6	0.8±1.2	98.5	−−±
Q	21,28,35,42	0	40	4/6	2.2±2.4^c	98.1	0.5±0.8^c	99.1	−−±
R		0	0	0/9	127±7	—	57±6	—	++−
S	21,28,35	0	40	0/8	15±17^b	87.7	7±9^b	88.2	±−++
T	21,35,49	0	40	3/3	0.7±0.6	99.5	0	100.0	−−±
U	7	30	0	0/6	9.0±4.9^c	92.9	4.3±2.2^b	92.4	±−++
V	7	30	0	3/6	1.0±1.1^a	99.2	0.5±0.5^a	99.1	−−±
	21,28,35	0	40						
W		0	0	0/9	122±15	—	49±10	—	+−+
X	7	15	0	0/6	22±7^c	82.2	10±6^c	80.3	+
Y	7	15	0	4/6	2.0±2.3	98.4	0.3±0.5	99.3	−−±
	21,28,35	0	40						
Z1	21,28,35	0	40	2/7	8.9±10^a	92.7	2.3±2.4^c	95.3	±−+
Z2	21,28,35,42	0	40	2/6	6.5±9.8^a	94.7	1.5±1.9^a	97.0	−±±

higher than those of group L and M. No apparent changes in the livers were seen in 2/4, 3/4 and 6/8 rabbits of the 3 groups, but less and fine egg tubercles were detected in the remained rabbits of the 3 groups. In Group P more cured rabbits (5/8) were seen (Tab 1).

3 Early treatment with Pra Rabbits infected for 21 d were initially treated ig with Pra 40 mg·kg^{-1}, followed by repeated dosing once a week (Group S) or 2 wk (Group T) for 2 times. In Group T, all 3 rabbits were free from ♀ worms and only a few residual ♂ worms were found with a total worm reduction rate of 99.5 %. In Group S, the total and ♀ worm reduction rates were 87.7 % and 88.2%, respectively, being lower than those of Group T (Tab 1). The liver changes in Group S appeared to be darkish red color and a few fine egg tubercles. One rabbit in which 28 residual ♀ worms was found showed ap-

parent egg tubercles in its liver. In Group T the livers of 2 rabbits appeared to be normal, while the other one exhibited a few old and fine egg tubercles.

When Pra was given to the infected rabbits at the same regimen with a total administration time of 3 (Group Z1) or 4 (Group Z2), the efficacies were similar, but the residual ♀ worms in Group Z2 was less than that of Group Z1. In Group Q with the same regimen of Group Z2, the total and ♀ worm reduction rates were 98.1 % and 99.1 %, respectively, and 4/6 rabbits were free from ♀ worm. In the other 2 rabbits, only 1 or 2 ♀ worms were found and none or only mild changes in the livers were noted (Tab 1).

4 Pra + Art

When Art 30 mg·kg^{-1} was given ig to the rabbits on d 7, and Pra 40 mg·kg^{-1} was given on d 21, 28 and 35 (Group V), the numbers of total and ♀ worms were less than those treated with Pra (Group S) or Art (Group U) alone (Tab 1). In Group V, 3/6 rabbits were cured or free from ♀ worms, and most livers revealed no apparent change except for 1 rabbit which showed a few fine egg tubercles.

In above mentioned combined treatment the dose of Art decreased to 15 mg·kg^{-1} (Group Y) resulted in less numbers of total and ♀ worms as compared to Group X or Group Z1. In Group Y, 4/6 rabbits were cured, and in the other 2 rabbits only 1 residual ♀ worm remained. None or very mild changes in the liver were found (Tab 1).

5 Influence of Art and Pra on temperature, eosinophil count, and eggs in feces

5.1 Rectal temperature Four wk after infection, the temperature of rabbits in control group (Group K) was 39.5 ℃, and then gradually reached its peak value of 40.7 ℃ 6 wk later. Up to 9 wk after infection, the temperature of the control rabbits sustained around 40 ℃. The average temperature in Group P and Group Q, ie. treated ig with Art or Pra, fluctuated between 38.5—39.4 ℃ and 38.7—39.5 ℃, respectively 4—10 wk after infection (Fig 1A).

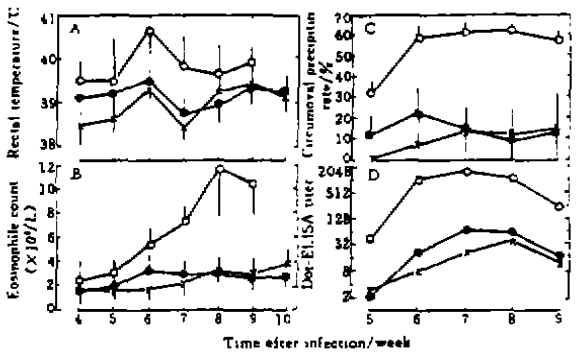

Fig 1. Rectal temperature (A, $n=5-6$, $\bar{x}\pm s$), eosinophil count (B, $n=5-6$, $\bar{x}\pm s$), circumoval precipitin rate (C, $n=5-6$, $\bar{x}\pm s$) and antigen level (D, $n=4$, $\bar{x}\pm s$) in infected rabbits treated ig with artementer 10 mg·kg^{-1} (×) or praziquantel 40 mg·kg^{-1} (●). The untreated infected rabbits were the control (○).

5.2 *Eosinophil count* The number of eosinophils increased gradually since 5 wk after infection and reached its peak value of (11.7±4.1)×10^9/L 8 wk later. Afterwards, the number of eosinophils decreased, but still much higher than that before 4 wk. No apparent alteration was detected in groups treated with Art or Pra during 4—10 wk after infection (Fig 1B).

5.3 Eggs in feces At 6—9 wk, schistosomal eggs in the feces were found in control group, but not in the groups treated with Art or Pra.

6 Influence of Art and Pra on antibody and antigen level in serum

6.1 Antibody COPT was used for determination of antibody level. Five wk after infection, all rabbits showed positive reaction with a circumoval precipitin rate of 33±5 %, and then reached and sustained around 57—63% 6—9 wk later (Fig 1C). In all rabbits

treated ig with Art, positive reaction revealed in 6 wk after infection with a circumoval precipitin rate of 8 %, afterwards, the circumoval precipitin rates sustained around 15 — 21 % (Fig 1C). Five to 9 wk after infection, the circumoval precipitin rates in rabbits treated ig with Pra were 9—23 % (Fig 1C).

6.2 Antigen Dot-ELISA method using monoclonal antibody was used for determination of antigen. Five to 9 wk after infection, the average serum titre in groups treated with Art or Pra were 3.4 — 38 and 2 — 76, respectively, while those in control group were 45 — 1720 (Fig 1D).

DISCUSSION

The results showed that when appropriate regimen of Art or Pra[2,3] was given to rabbits at an early stage of infection with schistosome cercariae, a significant effect protecting the hosts from damage induced by schistosome eggs, and apparent reduction in numbers of total and ♀ worms were observed. It is interesting to note that the dose of Pra used in early treatment of rabbits was equal to the curative dose used in clinical treatment. Further study showed that in experimentally infected dogs the minimal effective dose of Pra used at an early stage of infection was as low as 30 mg $\cdot kg^{-1}$ (Xiao SH, *et al.* Unpublished data), indicating that the dose recommended for field trial may be below 30 mg $\cdot kg^{-1}$. Since the dose of Art used in the early treatment of mice was 300 mg $\cdot kg^{-1}$ [5], higher doses of Art were tested initially in rabbits. Unexpectedly, the effect of Art 30 mg $\cdot kg^{-1}$ on 7-day-old schistosomulae in rabbits was rather high even at a single dose, indicating that the effect of Art on 7-day-old schistosomulae in rabbits was more promising. Further experiments demonstrated that the minimal effective dose of Art used in early treatment of rabbits was as low as 10 mg $\cdot kg^{-1}$, and the efficacy of Art medication commencing on d 7 was dependent on the following repeated dosing times.

The results also demonstrated that when rabbits were treated with Art or Pra at the early stage after infection, the parameters relevant to acute schistosomiasis[8] were negative, indicating that most or all of the female worms were killed. On the other hand, the levels of both specific antibody and antigen in the rabbits of the two treated groups were apparently lower than those of the controls. Therefore, the regimens of Art and Pra might be used in controlling acute schistosomiasis and reducing the rate and intensity of infection.

REFERENCES

1 Xiao SH, You JQ, Mei JY, Jiao PY. Early treatment of schistosomal infection with praziquantel in mice. Acta Pharmacol Sin 1993; **14**: 533—8.

2 Li Y, Yu PL, Chen YX, Li LQ, Gai YZ, Wang DS, *et al.* Synthesis of some derivatives of artemisinine. Kexue Tongbao 1979; **24**: 667—9.

3 Le WJ, You JQ, Yang YQ, Mei JY, Guo HF, Yang HZ, *et al.* Studies on the efficacy of artemether in experimental schistosomiasis. Acta Pharm Sin 1982; **17**: 187—93.

4 You JQ, Mei JY, Xiao SH. Effect of artemether against *Schistosoma japonicum*. Acta Pharmacol Sin 1992; **13**: 280—4.

5 Xiao SH, You JQ, Mei JY, Jiao PY. Effects of early treatment of artemether against schistosomiasis in mice. Chin J Parasitol Parasitic Dis 1994; **12**: 7—12.

6 Dignostic Laboratory of Shanghai Institute of Parasitology. Dignosis of schistoaomissia japonica by dry ova precipitation reaction. Chin J Intern Med 1979, **18**: 197—200.

7 Yan ZZ, Lu ZY, Wang W, Wu Y, Xu FN, Qing XY, *et al.* Detection of schistosome ova antigen by Dot-ELISA with monoclonal antibody. Shanghai J Immunol 1990; **10**: 113—6.

8 Yue WJ, Yang YQ, Yu SH, Yu QF. Experimental study on rabbits after infection with schistosomiasis. Natl Med J China 1980; **60**: 467—70.

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用蒿甲醚和吡喹酮早期治疗兔的血吸虫感染

R978.6

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摘要 兔于感染血吸虫尾蚴后 d 7或 d 21分别开始 ig 1次蒿甲醚(Art) 10 mg·kg^{-1}和吡喹酮(Pra) 40 mg·kg^{-1}，然后每隔1 wk ig 1次相同剂量的 Art 或 Pra 连给3－4次，减虫率达98%以上，且部分兔无虫，上述兔经 Art 或 Pra 早期治疗后，其肝脏与正常兔相仿，或仅有轻度变化，一些与急性血吸虫病有关的指标测定亦为阴性.

关键词 日本血吸虫；蒿甲醚；吡喹酮；联合药物疗法；用药计划表

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维拉帕米对大鼠脑突触体内 Ca^{2+} 和 $Ca^{(2+)}Mg^{(2+)}$-ATP 酶的影响[1]

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R965.2

Effect of verapamil on Ca^{2+} and $Ca^{(2+)}Mg^{(2+)}$-ATPase activity in rat brain synaptosomes

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ABSTRACT To elicit the correlation between the adrenergic transmitter release and calmodulin (CaM), the effect of verapamil on the free Ca^{2+} concentration was measured with fluorescence analysis and $Ca^{(2+)}Mg^{(2+)}$-ATPase activity in rat synaptosomes were studied.

When stimulated with high-K^+ or norepinephrine, the concentration of free Ca^{2+} in rat synaptosome was increased by verapamil 10, 50, and 100 μmol·L^{-1}. But the free Ca^{2+} concentration in the resting synaptosome was reduced by verapamil. The activity of $Ca^{(2+)}Mg^{(2+)}$-ATPase in synaptosome was remarkably inhibited by verapamil in a dose-dependent manner. These results support our hypothesis that CaM not only acts directly on the vesicles to enhance the transmitter release, but also acts on the activity of $Ca^{(2+)}Mg^{(2+)}$-ATPase to reduce the free Ca^{2+} in the cytosol, and indirectly inhibited the transmitter release.

KEY WORDS calcium; $Ca^{(2+)}Mg^{(2+)}$-ATPase; synaptosomes; verapamil; calmodulin; potassium; brain; norepinephrine

摘要 维拉帕米(Ver) 10，50和100 μmol·L^{-1}能增加高 K^+ 和去甲肾上腺素(NE)所致大鼠脑突触体内游离 Ca^{2+} 的浓度，但使静息状态突触体内游离 Ca^{2+} 浓度下降. Ver 还抑制突触体 $Ca^{(2+)}Mg^{(2+)}$-ATP 酶活性. 结果提示：与静息状态不同，在神经末梢受到刺激时，Ver 可能是通过抑制 CaM，进而抑制 $Ca^{(2+)}Mg^{(2+)}$-ATP 酶活性，使胞浆内游离 $Ca^{(2+)}$ 升高，引起递质释放量增加.

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