

Microscopic observations on livers of rabbits and dogs infected with *Schistosoma japonicum* cercariae and early treatment with artemether or praziquantel¹

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AIM: To study the histopathological change of the liver of the hosts treated with artemether (Art) or praziquantel (Pra) in early stage after infection with *Schistosoma japonicum* cercariae. **METHODS:** Dogs infected once with schistosome cercariae were treated ig with Art 10 mg·kg⁻¹ or Art capsule (ArtC) 15 mg·kg⁻¹ on d 7, or praziquantel (Pra) 30-40 mg·kg⁻¹ on d 21 after infection, followed by the repeated dosing once every 1 or 2 wk for 2-4 times. In rabbits, infected with 48-52 schistosome cercariae once every other day for 5 times, were treated ig with Art 10 mg·kg⁻¹ or Pra 30 mg·kg⁻¹ was started on d 7 or on d 21 after the first infection, followed by the repeated dosing every 1 or 2 wk for 2-3 times. **RESULTS:** After above mentioned dogs or rabbits were treated ig with Art, ArtC or Pra, the female worm reduction rates were 92.1% - 100%. Histopathological examination showed that the reduction rates of total granuloma in the liver sections of the dogs and rabbits were 70.9% - 97.3% and 76.5% - 97.4%, respectively. Meantime, the structure of the hepatic lobules was normal with normal arrangement of the liver bundle. **CONCLUSIONS:** Early treatment with Art or Pra exhibited a promising effect of protection of the liver of the dogs and rabbits infected with schistosome cercariae.

Early treatment of infection with schistosome cercariae kills the ♀ worms before oviposition. Thus, the host will be protected from the damage

caused by schistosome eggs. Promising results were obtained when mice and rabbits received an early treatment with artemether (Art) or praziquantel (Pra)¹⁻³⁾. This work was to study the liver infected with cercariae and treated by intragastric gavage (ig) with Art, Art capsule (ArtC) or Pra in early stage after infection.

MATERIALS AND METHODS

Parasites Cercariae released from *Oncomelania hupensis* snails infected with *Schistosoma japonicum* miracidia (Anhui isolate) were provided by our Institute.

Drugs Art and ArtC (40 mg/capsule) were made by Kunming Pharmaceutical Factory (respective lot No 880701 and 930114); Pra was made by Shanghai 6th Pharmaceutical Factory (lot No 871212). Art and Pra were suspended in 1% tragacanth at concentration of Art 10-15 g·L⁻¹ and Pra 30-40 g·L⁻¹.

Animals New Zealand rabbits (♀ & ♂, n=44, weighing 2.2 ± s 0.2 kg) were provided by our Institute. Mongrel dogs (♀ & ♂, n=25, weighing 7.3 ± s 1.7 kg) were used. Dogs were infected each with 198-202 schistosome cercariae via the shaved abdomen skin. Rabbits were infected with 48-52 schistosome cercariae and the infection was repeated every other day for 5 times.

Early treatment Infected dogs were treated ig with Art at a single dose of 10 mg·kg⁻¹ or ArtC 15 mg·kg⁻¹ on d 7 after infection, and repeated the same dosing every 1 or 2 wk for 3-4 times. Other 2 groups of dogs were initially treated ig with Pra at a single dose of 30 or 40 mg·kg⁻¹ on d 21 after infection, followed by the repetition of the same dose once every 1 or 2 wk for 2-3 times. Similar dose schedules of Art and Pra were used in infected rabbits. Two groups of rabbits treated ig with a single dose of Art 15 mg·kg⁻¹ or Pra 30 mg·kg⁻¹ on d 49 after the first infection served as the treated control. After 4-5 wk of treatment, animals were killed for collection of schistosomes. The efficacy was evaluated by the number of residual worms. Besides, 3-4 livers of each group were fixed in 10% neutral formalin.

Histopathological observation In each group, 25 sections with 50 mm² area were selected randomly from all liver sections. The number of schistosome egg granulomas in the

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50 mm² was counted and the eggs inside the granuloma was morphologically compared with that of the control group. The protective effect was based on the pathological changes in the liver.

Data analysis The *t* test was used

RESULTS

1 Efficacy of early treatment

When Art 10 mg·kg⁻¹ or ArtC 15 mg·kg⁻¹ was initially given ig to the dogs on d 7 after infection, or Art 15 mg·kg⁻¹ was initially given ig to rabbits repeatedly infected with cercariae on d 7 after the first infection according to the above-mentioned regimens, the numbers of total and ♀ worms of Group B, C, F, and G, or Group J and K were less than those of the control (Tab 1). Similar results were seen in dogs of Group D and H, or in rabbits of Group M and N initially treated ig with Pra 30 or 40 mg·kg⁻¹ regimens and started on d 21 after infection (Tab 1). In rabbits repeatedly infected with cercariae were treated ig with Art 15mg·kg⁻¹ (Group L) or Pra 30 mg·kg⁻¹ (Group O) on d 49 after the first infection, less worm reduction rate was seen in Group L, while in Group O, it was higher, but the livers of the rabbits showed apparent trace of damage induced by schistosome eggs.

2 Effect on liver

2.1 Histological observation After dogs were infected with schistosome cercariae for 10 wk (Group A), their livers showed softness and dark red color with many yellow miliary egg tubercles distributed unevenly on the surface of the liver. In control rabbits (Group I), the livers appeared in dark red grey color with numerous white-yellow miliary egg tubercles or white fibrous tissue distributed intensively over the whole surface, which resulted in hardness quality of the liver. Histological examination showed that in control dogs, some changes included swelling and cloudy of liver cells, narrow sinusoid, deposition of more or less eggs in portal vein area, formation of inflammatory and fibrous granuloma, and swelling of Kupffer's cells containing many dark grey particles (Fig 1A, Plate 1) were seen. Similar results were seen in rabbits, but a lot of eggs at different developmental stages deposited in the portal vein area accompanied by formation of extensive granuloma involved scarred shistosoma egg granuloma, which resulted in severe damage of the liver lobule. When the dogs and rabbits were treated ig with Art, ArtC or Pra (Group B, C, F, G, J, K, M, and N) in early stage after infection, their livers showed red color or

Tab 1. Effects of artemether (Art), artemether capsule (Art C) and praziquantel (Pra) given ig at a single dose to dogs (Group A-H) and rabbits (Group I-O) on d₇ or d₂₁ after infection with *Schistosoma japonicum* cercariae and followed by a dose once every week for 2-4 times. $\bar{x} \pm s$. **P*>0.05, ^c*P*<0.01 vs control.

| Group | Drug | Day of medication/d | Dose/mg·kg ⁻¹ | Dogs without ♀ worm | Total worms | WRR/% | ♀ worms | FWRR/% |
|-------|---------|--|--------------------------|---------------------|------------------------|-------|------------------------|--------|
| A | Control | - | - | 0/2 | 105 ± 3 | - | 40 ± 5 | - |
| B | Art | d ₇ d ₁₄ d ₂₁ | 10 | 1/3 | 3.3 ± 3.1 ^c | 96.9 | 1.0 ± 1.0 ^c | 97.5 |
| C | Art | d ₇ d ₁₄ d ₂₁ d ₂₈ d ₃₅ | 10 | 2/3 | 1.7 ± 1.5 ^c | 98.4 | 0.3 ± 0.6 ^c | 99.3 |
| D | Pra | d ₂₁ d ₂₈ d ₃₅ d ₄₂ | 40 | 3/3 | 0 ^c | 100 | 0 | 100 |
| E | Control | - | - | 0/4 | 105 ± 4 | - | 47 ± 2 | - |
| F | ArtC | d ₇ d ₁₄ d ₂₁ d ₂₈ d ₃₅ | 15 | 1/4 | 3.5 ± 4.4 ^c | 96.5 | 1.5 ± 1.3 ^c | 96.8 |
| G | ArtC | d ₇ d ₂₁ d ₃₅ d ₄₉ | 15 | 0/3 | 3.3 ± 2.5 ^c | 96.7 | 1.5 ± 1.3 ^c | 96.8 |
| H | Pra | d ₂₁ d ₃₅ d ₄₂ | 30 | 3/3 | 0.3 ± 0.6 ^c | 99.9 | 0 ^c | 100 |
| I | Control | - | - | 0/7 | 113 ± 12 | - | 51 ± 5 | - |
| J | Art | d ₇ d ₁₄ d ₂₁ d ₂₈ d ₃₅ | 15 | 4/6 | 2 ± 3 | 98.2 | 0.8 ± 1.6 | 98.4 |
| K | Art | d ₇ d ₂₁ d ₃₅ d ₄₉ | 15 | 2/5 | 2.6 ± 2.4 ^a | 97.7 | 1.2 ± 1.1 ^a | 97.6 |
| L | Art | d ₄₉ | 15 | 0/7 | 59 ± 14 ^c | 47.8 | 26 ± 6 ^c | 49.0 |
| M | Pra | d ₂₁ d ₂₈ d ₃₅ d ₄₂ | 30 | 3/7 | 18 ± 15 | 84.1 | 4 ± 6 | 92.2 |
| N | Pra | d ₂₁ d ₃₅ d ₄₉ | 30 | 1/6 | 27 ± 15 ^a | 76.1 | 3.5 ± 6 ^a | 93.1 |
| O | Pra | d ₄₉ | 30 | 1/6 | 33 ± 16 ^a | 70.8 | 10 ± 9 ^a | 80.4 |

WRR = worm reduction rate; FWRR = ♀ worm reduction rate.

light red color with no or few miliary egg tubercles scattered on the surface with more soft quality. In the section of the liver tissue, few eggs were found deposited in the portal vein area, no or much less imflammatory and fibrous egg granuloma was detected, whereas the structure of the hepatic lobule and the arrangement of the liver bundle remained normal (Fig 1B, C, D; Fig 2B, C, D, Plate 2). In the rabbits treated with Art or Pra once every week (Group J, M), the damage of the liver induced by schistosome eggs was similar to that treated with the same drug once every 2 wk (Group K, N). When the rabbits were treated ig with a single dose of Art (Group L), or Pra (Group O) on d 49 after the first infection, their livers appeared dark red with more miliary egg tubercles on the liver surface. Meantime, inflammatory, fibrous or scarred schistosome egg granuloma were found in the liver tissues, but less than the control and part of the hepatic lobules remained normal (Fig 2F, G).

3 Schistosome egg granuloma

3.1 Number of schistosome egg granuloma

When infected dogs and rabbits were treated ig with Art, ArtC or Pra in the early stage after infection, the average numbers of the egg granuloma in the liver were much less than that of the control group (Tab 2). In rabbits (Group L, O), the numbers

of the total egg granuloma were also less than that in the control group but significantly higher than that in the groups treated ig with either drug in the early stage after infection (Tab 2).

3.2 Types of schistosome egg granuloma In the control dogs, 2 types of granuloma, ie inflammatory and fibrous schistosome egg granuloma were found in the liver section (Tab 2). In the liver section of the control rabbits, besides above-mentioned 2 types of granuloma, another type of granuloma (ie scarred schistosome egg granuloma) was also detected (Tab 2). after early treatment with Art, ArtC or Pra, the numbers of each type of granuloma in the liver of dogs or rabbits were reduced in different degrees or even significantly lower than those of the control (Tab 2). When the rabbits were treated ig with Art, or Pra at a single dose on d 49 (Group L, O) after infection, apart from less inflammatory egg granuloma, no apparent difference was seen in the number of fibrous and scarred egg granuloma as compared with the control (Tab 2).

3.3 The quality of schistosome egg inside the granuloma In control dogs, less egg was seen in the liver, and none of calcified egg was found (Tab 2). In control rabbits, more eggs including fresh, degenerated and calcified egg were detected in the liver (Tab 2). After early treatment with Art,

Tab 2. Schistosome egg granuloma in liver tissues (section with 50 mm²) of dogs (Group A - G) and rabbits (Group I - O) treated ig with Art or Pra. n = 25, $\bar{x} \pm s$. ^aP > 0.05, ^bP < 0.05, ^cP < 0.01 vs control.

| Group | Drug | Day of medication /d | Dose/ mg·kg ⁻¹ | Dogs or rabbits without granuloma | Total number of granuloma | Granuloma quality | | | Egg quality | | |
|-------|---------|--|------------------------------|-----------------------------------|---------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | | | | | | Inflam-matory | Fibrous | Scarred | Fresh | Degenerated | Calcified |
| A | Control | - | - | 0/4 | 11 ± 5 | 9.9 ± 2.2 | 1.1 ± 1.5 | - | 2.2 ± 1.5 | 1.2 ± 1.3 | - |
| B | Art | d ₇ d ₁₄ d ₂₁ | 10 | 0/3 | 2.6 ± 2.0 ^c | 2.4 ± 2.1 ^c | 0.2 ± 0.5 ^b | - | 0.2 ± 0.4 ^c | 0.1 ± 0.2 ^c | - |
| C | Art | d ₇ d ₁₄ d ₂₁ d ₂₈ d ₃₅ | 10 | 1/3 | 0.3 ± 0.6 ^c | 0.3 ± 0.6 ^c | 0 ^c | - | 0.2 ± 0.9 ^c | 0.1 ± 0.2 ^c | - |
| D | ArtC | d ₇ d ₁₄ d ₂₁ d ₂₈ d ₃₅ | 15 | 0/4 | 3.2 ± 1.8 ^a | 2.5 ± 1.4 ^a | 0.5 ± 0.9 ^a | - | 0.7 ± 1.0 ^a | 0.6 ± 1.1 ^a | - |
| E | ArtC | d ₇ d ₂₁ d ₄₉ | 15 | 0/4 | 2.6 ± 2.2 ^c | 1.2 ± 1.0 ^c | 1.4 ± 1.6 ^a | - | 0.5 ± 0.9 ^a | 1.1 ± 1.7 ^a | - |
| F | Pra | d ₂₁ d ₂₈ d ₃₅ d ₄₂ | 40 | 1/3 | 0.3 ± 0.6 ^c | 0.3 ± 0.6 ^c | 0 ^c | - | 0 ^c | 0 ^c | - |
| G | Pra | d ₂₁ d ₃₅ d ₄₂ | 30 | 0/4 | 1.6 ± 1.8 ^a | 1.1 ± 1.3 ^c | 0.3 ± 1.7 ^a | - | 0.4 ± 0.7 ^a | 0.6 ± 1.2 ^a | - |
| I | Control | - | - | 0/3 | 34 ± 4 | 15 ± 5 | 13 = 2 | 6 ± 4 | 41 ± 11 | 18 ± 14 | 0.6 ± 0.9 |
| J | Art | d ₇ d ₁₄ d ₂₁ d ₂₈ d ₃₅ | 15 | 2/3 | 0.9 ± 1.5 ^c | 0.4 ± 0.7 ^c | 0.4 ± 0.7 ^a | 0 ^c | 0 ^c | 0 ^c | 0 ^c |
| K | Art | d ₇ d ₂₁ d ₃₅ d ₄₉ | 15 | 0/3 | 8 ± 7 ^c | 3 = 3 ^c | 4 ± 3 ^c | 1 = 2 ^c | 5 ± 5 ^c | 4 = 4 ^c | 0 ^c |
| L | Art | d ₄₉ | 15 | 0/3 | 17 ± 4 ^c | 5 = 3 ^c | 8 ± 5 ^c | 5 = 4 ^a | 12 ± 10 ^c | 29 ± 21 ^c | 0.6 ± 0.7 ^c |
| M | Pra | d ₂₁ d ₂₈ d ₃₅ d ₄₂ | 30 | 0/3 | 9 ± 6 ^c | 6 ± 4 ^c | 3 ± 2 ^c | 0.3 ± 0.5 ^c | 4 ± 5 ^c | 3 ± 3 ^c | 0 ^c |
| N | Pra | d ₂₁ d ₃₅ d ₄₂ | 30 | 0/3 | 8 = 6 ^c | 4 ± 3 ^c | 2 ± 4 ^c | 2 ± 2 ^c | 4 = 4 ^c | 3 ± 2 ^c | 0 ^c |
| O | Pra | d ₄₉ | 30 | 0/3 | 19 = 9 ^c | 5 ± 3 ^c | 9 = 5 ^c | 5 ± 4 ^c | 6 ± 5 ^a | 54 = 27 ^c | 0.8 ± 1.1 ^a |

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ArtC or Pra, the numbers of fresh and degeneration egg detected in the liver of the dogs were significantly lower than those of the control except for the numbers of degenerated egg in 2 groups treated with ArtC (Tab 2). In the treated rabbits (Group J, K, M, N), the numbers of fresh, degenerated and calcified egg were also reduced significantly as compared with those of the control (Tab 2). When the rabbits were treated ig with Art or Pra on d 49 after infection, the numbers of degenerated and calcified eggs were higher than or similar to those of the control (Tab 2).

DISCUSSION

For evaluation of efficacy of early treatment, the pathological change in the liver was one of the important parameters except for the total number of the worms, especially the ♀ worm reduction rate. Our study indicated that when the infected dogs and rabbits were treated with Art, ArtC or Pra in early stage after infection, the protection of the host liver from egg-induced damage was seen, providing the evidence concerning the rationality of the early treatment with Art or Pra. Since the early treatment is started before the oviposition of the female worm, which is quite different from the treatment after the oviposition of the female worm, and resulted in avoidance or diminution of egg damage.

The results of our study also indicated that after the early treatment, the protective effect of Art, or Pra given every week on the liver was similar to that of the 2 drugs given every 2 wk. Therefore, it is reasonable to suggest that in field trial, the recommended intervals for Art or Pra medication is every 2 wk, which would also be convenient for application of the regimen of the 2 drugs in the field.

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蒿甲醚或吡喹酮早期治疗感染血吸虫尾蚴兔和犬的肝脏显微镜检查

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血吸虫病 尾蚴 感染

关键词 日本血吸虫; 显微镜检查; 肉芽肿; 蒿甲醚; 吡喹酮; 用药计划表 肝脏

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目的: 观察感染血吸虫尾蚴后早期用蒿甲醚(Art)或吡喹酮(Pra)治疗, 对宿主肝组织的影响. 方法: 犬感染 198-202 条尾蚴后 d 7 ig Art 10 mg·kg⁻¹, Art 胶囊(ArtC) 15 mg·kg⁻¹或感染后 d 21 ig Pra 30-40 mg·kg⁻¹, 1-2 wk 重复给药 1 次, 共 2-4 次; 兔每隔日感染 48-52 条尾蚴, 共 5 次, 并于第 1 次感染后 d 7 或 d 21 ig 上述剂量的 Art 和 Pra, 停药后 4-5 wk 剖杀取肝作切片观察. 结果: 犬与兔经 Art 或 Pra 早期治疗后, 减♀虫率达 92.1%-100%, 肝切片中的总虫卵肉芽肿数分别减少 70.9%-97.3% 和 76.5%-97.4%, 肝叶结构和肝索排列正常. 结论: 2 种药物早期治疗对宿主肝脏有保护作用.

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