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SHORT COMMUNICATION

Effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination in treatment of mice infected with *Schistosoma japonicum*

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Abstract Artemether and artesunate, derivatives of the antimalarial artemisinin, as well as their main metabolite, dihydroartemisinin, all exhibit antischistosomal activities. The purpose of the current study was to compare the effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination in treatment of mice infected with *Schistosoma japonicum*. We carried out experiments with mice, infected with 40 cercariae of *S. japonicu*m, and treated with artemether, aretesunate and dihydroartemisinin (all at a single dose of 300 mg/kg, and the dose of the mixed three drugs is also 300 mg/kg) at multiple doses or combination therapy on days 6–8 or 34–36 post-infection. Administration with artemether, artesunate or dihydroartemisinin for 3 successive days reduced total worm burdens by 79.5−86% (30.86±4.98 of mean total worm burden in control), female worm burdens by 79.4−86.7% (11.29±2.63 of mean female worm burden in control) (all P values <0.01 vs. control), depending on different treatment protocols given on days 6–8 post-infection. However, no differences were seen between each treatment group (all P>0.05). While the same treatment was given on days 34–36 post-infection, total worm burden reductions of 73.8−75.8% were achieved (29.44±3.36 of mean total worm burden in control), which were significant when compared with the untreated control group (all P values <0.01). In all different treatment groups, female worm reductions (ranging from 88.7% to 93.1%, while the mean female worm burden in control is 10.33±1.80) were consistently higher than the total worm reductions, resulting always in significantly lower female worm burdens when compared to the corresponding control (all P values<0.01). However, there were no significant differences found between each treatment group (all P values >0.05). It is concluded that artemether, artesunate and dihydroartemisinin can be used to control schistosomiasis japonica, as a strategy to prevent *S. japonicum* infection. Administration with artemether, artesunate and dihydroartemisinin at multiple doses or in combined treatment damages both juvenile and adult *S. japonicu*m, without statistically significant differences among the three drugs at the same dose.

Introduction

Praziquantel is the only current drug of choice for the treatment of human schistosomiases (WHO 2002). However, with the extensive, long-term repeated use of the drug for morbidity control, there is a growing concern that praziquantel resistance or reduced susceptibility may emerge (Cioli and Pica-Mattoccia 2003). In addition, the drug suffers from the problem of being less sensitive to young developmental stages (i.e., schistosomula); hence, retreatment is necessary to kill parasites that have since matured (Ribeiro-dos-Santos et al. 2006; Doenhoff et al. 2008) Screening and development of new antischistosomal agents,

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as an alternative to praziquantel, is therefore given a high priority (Cioli et al. 2008; Abdul-Ghani et al. 2009; Magalhães et al. 2009, 2010; Xiao et al. 2009).

Artemether and artesunate, derivatives of the antimalarial artemisinin, which is widely acknowledged for their antimalarial properties (McIntosh and Olliaro 2001), also exhibit antischistosomal activities (Xiao 2005, Xiao et al. 2010, 2011; Hua et al. 2010; Abdul-Ghani et al. 2011). It has been previously reported that dihydroartemisinin, the main metabolite of the mother compound artemisinins, as well as of the two derivatives, artemether and artesunate, is efficacious against 7-day-old schistosomula and 35-day-old adult worms of *Schistosoma japonicum* (Li et al. 2011). This study was designed to compare the effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination in treatment of mice infected with *S. japonicum*.

Materials and methods

Parasites, drugs and mice

Cercariae of *S. japonicum* (Jiangsu isolate) were obtained from infected *Oncomelania hupensis* snails (collected from the rural marshland of Dantu District, Zhenjiang City, Jiangsu Province, China), following routine procedures in our laboratory.

Artemether was obtained from Yunnan Kunming Pharmaceutical Corporation (Kunming, China); artesunate was presented by Guangxi Guilin Pharmaceutical Corporation (Guilin, China) and dihydroartemisinin was kindly provided by Chongqing Holley Wuling Mountain Pharmaceutical Company (Chongqing, China). All companies documented that their products showed high purity: 99.2% for artemether, 99.9% for artesunate and 99.4% for dihydroartemisinin. Each of the aforementioned drugs, together with DMSO, Tween 80, 1% carboxymethylcellulose sodium (Nanjing Trust Chemical Co., Ltd., Nanjing, China) and distilled water, was ground in a ball miller to give aqueous solutions at a final concentration of 12 g/l.

Mice of the Kunming strain, each weighing 20–24 g, were purchased from Model Animal Research Center of Nanjing University (Nanjing, China), and given free access to food and water.

Infection of mice and treatment protocol

Mice were each infected percutaneously with 40 *S. japonicum* cercariae via shaved abdominal skin, and then randomly assigned to groups, of 10–12 mice each.

In the first experiment, which was designed to investigate the effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination treatment against juvenile *S. japonicum*, mice were divided into eight groups, and treated as described in the following protocols. Mice in group 1 were administered with dihydroartemisinin (300 mg/kg), artesunate (300 mg/kg) and artemether (300 mg/kg) on days 6–8 post-infection, respectively. Mice in group 2 were given artesunate (day 6), artemether (day 7) and anddihydroartemisinin (day 8), respectively. Mice in group 3 were treated with artemether (day 6), dihydroartemisinin (day 7) and artesunate (day 8), respectively. Mice in group 4 were administered with the mixed drugs of artemether, artesunate and dihydroartemisinin at a dose of 300 mg/kg for consecutive 3 days on days 6–8 post-infection. Mice in groups 5–8 were administered three daily doses of 300 mg/kg of artemether, artesuante and dihydroartemisinin on days 6–8 post-infection for 3 successive days, respectively. An additional group of ten mice, infected but left untreated, served as control.

The second experiment was designed to assess the effects of the drugs on adult worms, and the administration doses and regimen were followed as mentioned above, but drugs were given to mice on days 34–36 post-infection.

Dissection of mice and statistical assessment

In both experiments, mice were sacrificed 50 days post-infection and any adult *S. japonicum* worms in the hepatic and portomesenteric veins were recovered, sexed and counted. SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was employed to calculate the total and female worm burdens for each treated and control group, and the reductions in the total and female worms recovered which were obviously caused by drug treatment, as percentages, by comparison with the corresponding control. Statistical significance of each reduction was estimated using Fisher's least significant difference (LSD) test, with a P value<0.05 considered as a statistically significant difference.

Results

In the first experiment, administration with artemether, artesunate or dihydroartemisinin for 3 successive days reduced total worm burdens by 79.5−86%, female worm burdens by 79.4−86.7%, which were significant when compared with the corresponding untreated control group (all P values<0.01), depending on different treatment protocols given on days 6–8 post-infection (Table 1). However, no differences were seen between each treatment group (all P values>0.05).

In the second experiment, while the same treatment was given on days 34–36 post-infection, total worm burden reductions of 73.8−75.8% were achieved, which were

Table 1 Effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination treatment against juvenile *S. japonicum* (*n*=number of mice)

Treatment regimen	Administration: days post-infection	Mice (*n*)	Mean total worm burden (SD)	Reductions (%)	Mean female worm burden (SD)	Reductions (%)
Dihydroartemisinin (6 days post-infection) plus artesunate (7 days post-infection) plus artemether (8 days post-infection)	6–8	10	5.17 (1.94)*	83.3	1.67 (1.03)*	85.2
Artesunate (6 days post-infection) plus artemether (7 days post-infection) dihydroartemisinin (8 days post-infection)	6–8	10	6.33 (3.14)*	79.5	2.33 (1.96)*	79.4
Artemether (6 days post-infection) plus dihydroartemisinin (7 days post-infection) plus artesunate (8 days post-infection)	6–8	10	4.50 (1.64)*	85.4	1.50 (1.05)*	86.7
Mixture of artemether, artesuante and dihydroartemisinin (300 mg/kg)	6–8	10	6.11 (1.90)*	80.2	2.11 (0.54)*	81.3
Administration of artemether daily for 3 consecutive days	6–8	10	4.33 (1.51)*	86	1.75 (1.33)*	84.5
Administration of artesunate daily for 3 consecutive days	6–8	10	5.33 (2.34)*	82.7	2.17 (1.60)*	80. 8
Administration of dihydroartemisinin daily for 3 consecutive days	6–8	10	5.83 (3.25)*	81.1	2.33 (1.63)*	79.4
None (control)	–	10	30.86 (4.98)	–	11.29 (2.63)	–

* $P<0.01$ vs. control

significant when compared with the untreated control group (all P values<0.01). In all different treatment groups, female worm reductions (ranging from 88.7% to 93.1%) were consistently higher than the total worm reductions, resulting always in significantly lower female worm burdens when compared to the corresponding control (all P values<0.01) (Table 2). However, there were no significant differences found between each treatment group (all P values>0.05).

Discussion

It has been shown that artemisinin derivatives such as artemether and artesunate exhibit effectively antischistosomal activities (Hua et al. 2010; Abdul-Ghani et al. 2011; Xiao et al. 2011), and the two drugs present similar activity against *S. japonicum* (Xiao 2005). However, artemether displays consistently higher activities in treatment of *S. mansoni*-infected mice (Shaohong et al. 2006; Utzinger et al. 2002).

Table 2 Effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination treatment against adult *S. japonicum* (*n*=number of mice)

Treatment regimen	Administration: days post-infection	Mice (*n*)	Mean total worm burden (SD)	Reductions (%)	Mean female worm burden (SD)	Reductions (%)
Dihydroartemisinin (6 days post-infection) plus artesunate (7 days post-infection) plus artemether (8 days post-infection)	34–36	10	7.50 (4.23)*	74.5	0.75 (0.75)*	92.7
Artesunate (6 days post-infection) plus artemether (7 days post-infection) dihydroartemisinin (8 days post-infection)	34–36	10	7.67 (3.61)*	74	0.83 (0.75)*	92
Artemether (6 days post-infection) plus dihydroartemisinin (7 days post-infection) plus artesunate (8 days post-infection)	34–36	10	7.14 (1.95)*	75.8	0.71 (0.75)*	93.1
Mixture of artemether, artesuante and dihydroartemisinin (300 mg/kg)	34–36	10	7.71 (2.21)*	73.8	0.79 (0.69)*	92.4
Administration of artemether daily for 3 consecutive days	34–36	10	7.38 (2.45)*	74.9	0.81 (0.65)*	92.2
Administration of artesunate daily for 3 consecutive days	34–36	10	7.17 (1.94)*	75.7	1.17 (0.75)*	88.7
Administration of dihydroartemisinin daily for 3 consecutive days	34–36	10	7.25 (3.11)*	75.4	0.75 (0.71)*	92.7
None (control)	–	10	29.44 (3.36)	–	10.33 (1.80)	–

*$P<0.01$ vs. control

Our previous studies also showed that dihydroartemisinin, the main metabolite of the mother compound artemisinins, as well as the two derivatives artemether and artesunate, is efficacious against 7-day-old schistosomula and 35-day-old adult worms of *S. japonicum* (Li et al. 2011). Considering that these three drugs all appear similarly effective against *S. japonicum*, the current study, therefore, was conducted to assess to compare the effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination against *S. japonicum* infection.

Our findings showed that oral administration with artemether, artesunate and dihydroartemisinin at multiple doses or combination therapy is toxic to juvenile or adult *S. japonicum*. It is indicated that all three drugs can be used in the control of schistosomiasis japonica as a strategy to prevent *S. japonicum* infection. However, there is no significant difference between each treatment group (all $P>0.05$). It is speculated that similar drug formulation and administration route leads to failure in prolonged or superpositioned peak plasma drug concentrations of the drugs, and the resultant difficulty in enhanced efficacy of combined treatment.

We also observed that treatment with artemether, artesunate and dihydroartemisinin administered orally at multiple doses or in combination achieved 79.5−86% reductions of total worm burdens against juvenile *S. japonicum*, which were higher than those in adult worms (73.8−75.8%). However, the same administration protocol reduced 79.4−86.7% of female worm burdens against juvenile *S. japonicum*, which were lower than those of 88.7−93.1% in adult *S. japonicum*. It is estimated that the phenomenon is associated with the effects of artemisinin derivatives on different developmental stages of *S. japonicum* and their mechanism of actions. The mature adult worms have higher anti-oxygen free-radical effect than the immature schistosomula (Meshnick et al. 1989; Xiao et al. 2002; Haynes et al. 2010), resulting in more toxicity to juvenile *S. japonicum* with the same treatment protocols. The female worms of adult *S. japonicum* need to uptake lots of red cells for absorption of nutrition to lay eggs, with much more hemin and iron-porphyrin than male worms (Zhai et al. 2002). The artemisinin derivatives react with the hemin and iron ions to yield free radical and chelates (Xiao et al. 2002), which damage the adults, and the toxicity to adult *S. japonicum* is increased with the amount of the hemin. Therefore, the administration of the three drugs at multiple doses or combination therapy killed more female worms of adults than juvenile *S. japonicum*, achieving more reductions of female worm burdens of adult worms. Further studies should be carried out to investigate the mechanism of actions of the variations in the effects of artemisinin derivatives against *S. japonicum*, and the effects of the drug combination against other *Schistosoma* species.

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