SHORT COMMUNICATION

In-vivo activity of dihydroartemisinin against *Schistosoma japonicum*

Schistosomiasis japonica, caused by *Schistosoma japonicum*, is still a major public-health concern in China, afflicting thousands of people (Zhou *et al.*, 2007; Li *et al.*, 2009). Since 1992, when the World Bank Loan Project for Schistosomiasis Control was initiated in China, praziquantel-based chemotherapy has been used to control the disease (Chen, 2005). Unfortunately, in the long term, repeated praziquantel treatment may lead to resistance to the drug in some *Schistosoma* species (Fallon *et al.*, 1995; Ismail *et al.*, 1996; Wang *et al.*, 2010), and the development of new schistosomicidal drugs, as alternatives to praziquantel, is therefore a priority.

Artemisinin is a sesquiterpene lactone endoperoxide isolated from a Chinese medicinal plant [*Artemisia annua* L. (qinghao)] that has been used as a herbal remedy for fever and malaria for more than 1000 years (Klayman, 1985; Trigg, 1989). Dihydroartemisinin is a derivative of artemisinin (in which the C-10 lactone group has been replaced by hemiacetal) and is the active metabolite of several other artemisinin derivatives such as artemether and artesunate (Janse *et al.*, 1994). Although, in both laboratory and field experiments, artemether and artesunate have each been found active against *S. japonicum* (Utzinger *et al.*, 2001b; Li *et al.*, 2005; Xiao, 2005; Keiser and Utzinger, 2007; Hua *et al.*, 2010; Xiao *et al.*, 2011), the effect of dihydroartemisinin on schistosomes remains unclear. The main aim of the present study was to explore the effects of dihydroartemisinin treatment on *S. japonicum* in experimentally infected mice, as a further step towards the development of novel schistosomicidal drugs.

MATERIALS AND METHODS

The dihydroartemisinin used was kindly provided by the Chongqing Holley Wuling Mountain Pharmaceutical company (batch 2006090131; 99.4% purity). The drug was ground in a ball miller with dimethyl sulphoxide (DMSO), Tween-80 and distilled water to give aqueous solutions containing 8, 12, 16 or 24 g dihydroartemisinin/litre, 4%–8% (v/v) DMSO and 0.5% (v/v) Tween-80.

Mice of the Kunming strain, each weighing 20–24 g, were purchased from Yangzhou University (Yangzhou, China), and given free access to food and water. They were each infected percutaneously with 39–41 *S. japonicum* cercariae (from naturally infected *Oncomelania hupensis* collected in Anhui province, China) and then randomly assigned to groups, of 10 mice each.

In the first experiment, designed to assess the efficacy of dihydroartemisinin against the different developmental stages of *S. japonicum*, infected mice were left untreated (10 mice) or each treated with a single oral dose of the drug (at 300 mg/kg bodyweight, in a dose volume of about 0.5 ml) 2 h or 3, 5, 7, 10, 14, 18, 21, 28 or 35 days post-infection (with one group of mice used for each treatment time).

In a second experiment, designed to investigate the dose–response relationships of dihydroartemisinin against the schistosomula and adult worms of *S. japonicum*, single oral doses (of 200, 300, 400 or 600 mg/kg bodyweight, in a dose volume of about 0.5 ml) were given to mice that had been infected either 7 or 35 days previously.

Again, similarly infected but untreated mice served as controls.

In both experiments, the mice were killed 50 days post-infection and any adult *S. japonicum* worms in the hepatic and portomesenteric veins were recovered by perfusion, sexed and counted. The reductions in the total number of worms recovered and in the number of female worms recovered that were apparently caused by drug treatment were then estimated, as percentages, by comparison with the numbers of worms recovered from the untreated control mice. The statistical significance of each reduction was then estimated using Fisher's least-significant-difference (LSD) tests and version 11.0 of the SPSS software package (SPSS Inc, Chicago, IL), with a *P*-value of <0.05 considered indicative of a statistically significant difference.

RESULTS

In the first experiment, single oral doses of dihydroartemisinin (at 300 mg/kg) reduced total worm burdens by 1.07%–64.81% and

female-worm burdens by 11.90%–90.48%, depending on when, relative to infection, treatment was given (Table 1). The greatest reductions were seen when treatment was given either 7 or 35 days post-infection (Table 1).

In the second experiment, a single treatment on day 7 post-infection reduced total and female-worm burdens significantly — by 46.8% and 59.7%, respectively — when the lowest dose (200 mg/kg) was tested (Table 2). Although use of the drug at 300 mg/kg on day 7 led to slightly greater reductions in the total number of worms (60.6%) and number of female worms (72.3%), further increases in the dose (to 400 or 600 mg/kg) caused no further increase in the reductions seen.

When given on day 35 post-infection, a single oral dose of either 200 or 300 mg/kg resulted in reductions in total and female-worm burdens ranging from 47.2% to 89.4% (*P*<0.01 for each; Table 2). Although the use of higher doses on day 35 led to even greater reductions, there was no clearcut dose–response relationship (Table 2).

TABLE 1. *Effects of dihydroartemisinin treatment (a single dose of 300 mg/kg) on the recovery of adult Schistosoma japonicum from mice that had been experimentally infected 50 days earlier*

Mice	Mean (S.D.) no. of worms collected	Reduction* (%)	Mean (S.D.) no. of female worms collected	Reduction* (%)
ANIMALS TREATED:				
2 h post-infection	27.8 (3.73)	1.1	8.9 (2.42)	15.3
3 days post-infection	23.8 (8.27) [†]	15.2	8.1 (3.84) [†]	22.9
5 days post-infection	19.3 (8.08) [‡]	31.3	5.8 (3.58) [‡]	44.8
7 days post-infection	9.9 (2.42) [‡]	64.8	2.8 (1.75) [‡]	73.8
10 days post-infection	21.9 (5.57) [†]	22.0	8.3 (2.87)	21.0
14 days post-infection	18.9 (4.34) [‡]	32.7	7.9 (2.57) [†]	24.9
18 days post-infection	23.0 (3.46) [†]	18.1	8.0 (2.98) [†]	23.8
21 days post-infection	24.0 (3.21)	14.5	9.3 (2.66)	11.9
28 days post-infection	14.5 (4.08) [‡]	48.5	4.3 (2.33) [‡]	59.3
35 days post-infection	11.1 (2.47) [‡]	60.5	1.0 (1.15) [‡]	90.5
Untreated controls	28.1 (4.27)		10.5 (2.68)	

*Compared with the corresponding values for the untreated (control) mice.

[†]Significant reduction compared with control (*P*<0.05).

[‡]Very significant reduction compared with control (*P*<0.01).

TABLE 2. Effects of four different single doses of dihydroartemisinin, given 7 or 35 days post-infection, on the recovery of adult *Schistosoma japonicum* from mice that had been experimentally infected 50 days earlier

Drug dosage (mg/kg)	Treatment 7 days post-infection (of schistosomula)				Treatment 35 days post-infection (of adult worms)			
	Mean (S.D.) no. of worms collected	Reduction* (%)	Mean (S.D.) no. of female worms collected	Reduction* (%)	Mean (S.D.) no. of worms collected	Reduction* (%)	Mean (S.D.) no. of female worms collected	Reduction* (%)
200	13.8 (6.81) [†]	46.8	4.2 (3.01) [†]	59.7	13.7 (3.43) [†]	47.2	4.2 (1.81) [†]	59.7
300	10.2 (2.49) [†]	60.6	2.9 (1.69) [†]	72.3	9.8 (3.76) [†]	62.3	1.1 (1.64) [†]	89.4
400	10.5 (4.52) [†]	59.6	2.9 (2.11) [†]	72.6	6.2 (2.91) [†]	76.3	0.9 (1.08) [†]	89.7
600	10.3 (2.00) [†]	60.2	2.4 (1.24) [†]	76.6	4.3 (1.91) [†]	83.6	0.6 (0.74) [†]	94.0
None (control)	26.0 (5.36)		10.4 (3.78)		26.0 (5.36)		10.4 (3.78)	

*Compared with the corresponding values for the untreated (control) mice.

[†]Very significant reduction compared with control ($P < 0.01$).

DISCUSSION

Under laboratory conditions, it is possible to induce praziquantel resistance in *S. mansoni* by exposing the parasites to multiple sub-curative doses of the drug (Fallon and Doenhoff, 1994). Fortunately, there is little direct evidence of the existence of such resistance in field isolates of schistosomes, although decreased sensitivity to praziquantel has already been reported in *S. mansoni* in many endemic areas (Fallon *et al.*, 1995; Ismail *et al.*, 1996; Tchuem-Tchuente *et al.*, 2001; Melman *et al.*, 2009) and repeated standard treatment with the drug has failed to clear *S. haematobium* from several patients (Silva *et al.*, 2005; Alonso *et al.*, 2006).

Since praziquantel, a highly effective and safe antischistosomal drug, was developed, it has been used (virtually alone) for the treatment and control of schistosomiasis japonica in China (Chen, 2005). Praziquantel-based chemotherapy remains an important part of the Chinese National Schistosomiasis Control Programme and has generally been found effective (Chen, 2005; Xiao, 2005). The possibility that, in the long term, repeated praziquantel treatments will, however, reduce the susceptibility of schistosomes, such as Chinese *S. japonicum*, to the drug is a major concern (Liang *et al.*, 2001; Yu *et al.*, 2001; Wang *et al.*, 2010) and one that drives the current search for new antischistosomal drugs.

Some artemisinin derivatives, notably artemether and artesunate, have already been used to prevent and control mammalian infection with some *Schistosoma* species (Utzing *et al.*, 2000, 2001b; Li *et al.*, 2005; Keiser and Utzing *et al.*, 2007; Hua *et al.*, 2010; Xiao *et al.*, 2011).

The administration of artemether (in four, weekly doses, each of 300 mg/kg, from day 7 post-infection) to mice infected with *S. japonicum*, for example, resulted in female-worm reductions that exceeded 90% (Utzing *et al.*, 2001b). Whether administered, from 1–3 weeks

post-infection, in weekly doses of 300 mg/kg (to mice infected with *S. japonicum*), 15 mg/kg (to rabbits infected with the same parasite) or 10 mg/kg (to dogs infected with the same parasite), artemether exhibited a broad spectrum of activity (Utzinger *et al.*, 2001b). As artemether and praziquantel have their greatest efficacies against juvenile and adult schistosomes, respectively (Xiao, 2005; Keiser and Utzinger, 2007; Xiao *et al.*, 2011), it has been suggested that a combination of these two drugs might be more effective than either drug alone. Utzinger *et al.* (2001a) confirmed that combined treatment with praziquantel and artemether is safe and more effective against *S. japonicum* and *S. mansoni* than treatment with praziquantel alone.

Compared with artemether, artesunate appears similarly effective against *S. japonicum*, leading to worm reductions of 90%–99.5% when given — in four to six, weekly doses from day 7 post-infection — to infected mice (at 300 mg/kg in each dose), rabbits (at 20 mg/kg) or dogs (at 30 mg/kg), schistosomula aged 6–9 days showing the greatest sensitivity to the drug (Xiao, 2005). When artesunate was given as a single dose (of 300 mg/kg) to mice infected with *S. mansoni*, treatment on days 14 or 21 days post-infection (when the parasites are still schistosomula) led to the greatest worm-burden reductions, of 84% and 93%, respectively (Keiser and Utzinger, 2007; Xiao *et al.*, 2011).

In the treatment of mice infected with *S. japonicum*, the present results indicate that dihydroartemisinin is particularly effective against 7-day-old schistosomula and 35-day-old adult worms. No marked dose–response relationship was observed, probably because dihydroartemisinin administered orally to mammals is rapidly absorbed, its blood concentration peaking just 1 h (mice) or 2 h (rabbits and dogs) post-administration and then falling rapidly (Zhao and Song, 1990, 1993). The activity of dihydroartemisinin against *S. japonicum* would therefore probably be enhanced

by the use of multiple doses. Further studies should be carried out to investigate the antischistosomal effects of combined treatments with dihydroartemisinin and praziquantel, and the efficacy of dihydroartemisinin against other schistosome species.

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