Annals of Tropical Medicine & Parasitology, Vol. 105, No. 4, 329–333 (2011)

SHORT COMMUNICATION

Dihydroartemisinin-praziquantel combinations and multiple doses of dihydroartemisinin in the treatment of *Schistosoma japonicum* in experimentally infected mice

Currently, praziquantel is virtually the only drug used for the treatment of human schistosomiases (WHO, 2002). Although there is little direct evidence of the existence of praziquantel resistance in field isolates of schistosomes, decreased sensitivity to the drug has been reported in some *Schistosoma* species from several endemic areas (Tchuem-Tchuenté *et al.*, 2001; Alonso *et al.*, 2006; Melman *et al.*, 2009). The screening and development of novel antischistosomal drugs, as alternatives to praziquantel, therefore need to be given high priority. One derivative of artemisinin, dihydroartemisinin, shows activity against many parasites and, when administered orally to mammals, is rapidly absorbed, widely distributed and promptly excreted and metabolised (Tu, 2009). Single oral doses of dihydroartemisinin have recently been found effective against the 7-day-old schistosomula and 35-day-old adult worms of *Schistosoma japonicum* (Li *et al.*, 2011). The aim of the present study was to explore the effects of multiple doses of dihydroartemisinin and of combined treatments with dihydroartemisinin and praziquantel against *S. japonicum* in experimentally infected mice, mainly to see if such treatments led to increased efficacy.

ANIMALS AND METHODS

Dihydroartemisinin (kindly provided by Chongqing Holley Wuling Mountain Pharmaceutical Company, Zhongduo, China; batch no. 2006090131; purity=99.4%) was ground in a ball miller, with dimethyl sulphoxide, Tween-80 and distilled water to give aqueous solutions containing 8, 12, 16 or 24 g dihydroartemisinin/litre (Li *et al.*, 2011). Praziquantel (Sigma–Aldrich, Shanghai, China; batch no. CH09125) was dispersed in water containing 2.5% (w/v) cremophor EL (Sigma–Aldrich). Both drugs were administered by oral gavage.

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* snails, collected in Anhui province, China) and then randomly assigned to groups, of 10–12 mice each.

In the first experiment, designed to investigate the effect of multiple doses of dihydroartemisinin on the schistosomula and adult worms of *S. japonicum*, mice were given three daily doses, of 200, 300, 400 or 600 mg dihydroartemisinin/kg (in dose volumes of 25 ml/kg), on days 6–8 or 34–36 post-infection, respectively. An additional group of mice, infected but not given the drug, served as a control.

In the second experiment, designed to assess the effect of combination therapy with dihydroartemisinin and praziquantel against the schistosomula and adult worms of *S. japonicum*, infected mice were divided into six groups (Groups 1–6). Mice in two of the groups were each given a single treatment dose, either of dihydroartemisinin (at 300 mg/kg; Group 1) or praziquantel (at 300 mg/kg; Group 2), on day 7 or day 35 post-infection. Mice in Group 3 were each given both drugs (300 mg dihydroartemisinin/kg and 300 mg

© W. S. Maney & Son Ltd 2011
DOI: 10.1179/136485911X13018982159979

praziquantel/kg) on day 7 or day 35 post-infection. The animals in Group 4 were each treated with a single dose of praziquantel (at 300 mg/kg) on day 6 or day 34 post-infection, followed by a single dose of dihydroartemisinin (at 300 mg/kg) 1 day later. Mice in Group 5 were each given a single dose of dihydroartemisinin (300 mg/kg) on day 7 or day 35 post-infection, followed by a dose of praziquantel (300 mg/kg) 1 day later. Finally, mice in Group 6 were left untreated, and hence served as a control.

All mice were killed 50 days post-infection so that any adult *S. japonicum* worms in their hepatic and portomesenteric veins could be recovered, sexed and counted. The reductions in the numbers of worms and female worms recovered that were attributable to drug treatment were then calculated, as percentages, by comparison with the numbers of worms recovered from the untreated control mice. Using version 13.0 of the SPSS software package (SPSS Inc, Chicago, IL), the statistical significance of each reduction was estimated in Fisher's least-significant-difference tests, with a *P*-value <0.05 considered indicative of a statistically significant difference.

RESULTS

In the first experiment (see Table 1), single oral doses of dihydroartemisinin (at 200, 300, 400 or 600 mg/kg), given once on each of days 6–8 post-infection, reduced total-worm burdens by 69.2%–90.6% and female-worm burdens by 62.2%–92.2%, depending on dosage. Similar treatments given on days 34–36 post-infection reduced total-worm burdens by 73.9%–85.5% and female-worm burdens by 83.8%–95.3%.

Table 2 summarizes the antischistosomal efficacies of dihydroartemisinin combined with praziquantel against *S. japonicum* schistosomula and adult worms, as observed in the second experiment. Total-worm burdens were reduced by 19.7% and

TABLE 1. *Effects of three daily doses of dihydroartemisinin (at doses of 200, 300, 400 or 600 mg/kg.day), administered on days 6–8 or 34–36 post-infection, on the subsequent recovery, on day 50, of adult* Schistosoma japonicum

	Treatment of schistosomula (on days 6–8 post-infection)				Treatment of adult worms (on days 34–36 post-infection)			
Daily dose (mg/kg)	Mean (S.D.) no. of adult worms collected	Reduction compared with control (%)	Mean (S.D.) no. of adult female worms collected	Reduction compared with control (%)	Mean (S.D.) no. of adult worms collected	Reduction compared with control (%)	Mean (S.D.) no. of adult female worms collected	Reduction compared with control (%)
200	8.78 (2.86)	69.2	4.00 (1.41)	62.2	7.43 (3.10)	73.9	1.71 (1.11)	83.8
300	5.50 (2.47)	80.7	2.58 (1.16)	75.6	7.13 (4.58)	75.0	0.75 (0.88)	92.9
400	3.67 (1.51)	87.1*	1.73 (0.75)	87.1	4.50 (3.42)	84.2	0.62 (0.74)	94.1*
600	2.67 (1.97)	90.6*	0.83 (0.75)	90.2	4.12 (2.59)	85.5	0.50 (0.70)	95.3*
None (control)	28.47 (5.53)	–	10.58 (3.78)	–	28.47 (5.53)	–	10.58 (3.78)	–

*Significantly greater than value recorded with treatment at 200 mg/kg.day.

70.2% when both dihydroartemisinin and praziquantel were administered on day 7 or day 35 post-infection, respectively. The reduction in total-worm burden seen when the dihydroartemisinin–praziquantel combination was given on day 7 was significantly less than when only dihydroartemisinin was administered on day 7 ($P<0.01$). The administration of dihydroartemisinin on day 35 post-infection, combined with praziquantel treatment on day 34 or day 36, reduced total-worm burdens by 60.8% and 81.5%, respectively. The apparent efficacy of day-35 dihydroartemisinin plus day-36 praziquantel was significantly better than the efficacies recorded when day-35 dihydroartemisinin was given alone or with day-34 praziquantel ($P<0.05$ for each).

DISCUSSION

Li *et al.* (2011) already showed that a single oral dose of dihydroartemisinin (at 300 mg/kg) has activity against *S. japonicum*, being particularly effective against the 7-day-old schistosomula and 35-day-old adult worms (with total-worm burdens reduced by 64.8% and 60.5%, respectively, but no marked dose–response relationship seen when the dosage was varied). The present study — an extension of the investigation by Li *et al.* (2011) and using the same experimental model — was designed to investigate the effects, on *S. japonicum* in mice, of multiple doses of dihydroartemisinin or a combination of single-dose dihydroartemisinin with a single dose of the current treatment of choice (i.e. praziquantel), to

TABLE 2. *Effects of combined treatment with dihydroartemisinin (at 300 mg/kg) and praziquantel (at 300 mg/kg) against the schistosomula and adult worms of* Schistosoma japonicum

Treatment	Mean (S.D.) no. of adult worms collected on day 50	Reduction compared with control (%)
TARGETED AT SCHISTOSOMULA		
Day-7 dihydroartemisinin only	11.56 (4.00)	60.3
Day-7 praziquantel only	29.00 (4.14)	0.0*
Day-7 dihydroartemisinin plus day-7 praziquantel	23.38 (3.62)	19.7*
Day-6 praziquantel plus day-7 dihydroartemisinin	16.60 (3.10)	43.0
Day-7 dihydroartemisinin plus day-8 praziquantel	12.38 (2.45)	57.5
None (control)	29.10 (4.70)	–
TARGETED AT ADULT WORMS		
Day-35 dihydroartemisinin only	10.44 (3.50)	64.1†
Day-35 praziquantel only	6.67 (3.20)	77.1
Day-35 dihydroartemisinin plus day-35 praziquantel	8.67 (4.12)	70.2
Day-34 praziquantel plus day-35 dihydroartemisinin	11.40 (4.06)	60.8†
Day-35 dihydroartemisinin plus day-36 praziquantel	5.38 (3.38)	81.5
None (control)	29.10 (4.70)	–

*Significantly lower than value recorded with day-7 dihydroartemisinin only ($P<0.01$).
†Significantly lower than value recorded with day-35 dihydroartemisinin plus day-36 praziquantel ($P<0.05$).

see if better schistosomicidal efficacy could be achieved. Three daily doses of dihydroartemisinin (each at 200, 300, 400 or 600 mg/kg) administered on days 6–8 post-infection reduced total-worm burdens by 69.2%–90.6%, whereas the same treatment regimen given on days 34–36 post-infection resulted in total-worm-burden reductions of 73.9%–85.5%. There was more evidence of a dose–response relationship in these multiple-dose treatments (see Table 2) than in the single-dose regimens explored by Li *et al.* (2011).

Dihydroartemisinin is the main metabolite of artemisinin and of two artemisinin derivatives, artemether and artesunate (Tu, 2009). The administration of artemether to mice, rabbits and dogs experimentally infected with *S. japonicum* resulted in different preventive effects, with no clear-cut dose–response relationships observed in the mice (Utzinger *et al.*, 2001; Xiao *et al.*, 2002). Whether dihydroartemisinin has similar efficacies against *S. japonicum* in hosts other than mice, and whether there might be any clear dose–response relationships with the drug at different dosages to those explored in the present study, are topics that merit further investigation.

The different developmental stages of *S. japonicum* are known to have different sensitivities to artesunate (Ru *et al.*, 2006). The addition of praziquantel did not appear to enhance the efficacies of artemether against the various developmental stages of *S. japonicum* (You *et al.*, 1994), and the co-administration of artesunate and praziquantel (or the administration of praziquantel, followed by artesunate) significantly reduced the activities of the artesunate against *S. japonicum* schistosomula (Wu *et al.*, 1998). In the latter investigation, however, the administration of artesunate first, followed by praziquantel, resulted in similar antischistosomal activities to those observed with artesunate alone. In the present study, similarly, treatment during the schistosomulum stage with co-administered dihydroartemisinin and praziquantel, or the administration of praziquantel, followed, a day later, by treatment with dihydroartemisinin, appeared less effective than day-7 treatment with dihydroartemisinin alone, whereas day-7 treatment with dihydroartemisinin followed by day-8 treatment with praziquantel gave a similar result to that seen with dihydroartemisinin alone. When the treatments were targeted at adult *S. japonicum* (and given on days 34, 35 and/or 36 post-infection), day-35 dihydroartemisinin followed by day-36 praziquantel appeared more effective than day-35 dihydroartemisinin given alone, or day-34 praziquantel followed by day-35 dihydroartemisinin. None of the combined treatments showed greater efficacy than that seen with praziquantel alone.

In conclusion, the in-vivo treatment of *S. japonicum* at the schistosomulum stage with a combination of praziquantel and either artesunate (Wu *et al.*, 1998) or dihydroartemisinin (present study) appears no more effective (and may be significantly less effective) than treatment with the artemisinin derivative alone. Further studies to investigate the detailed mechanisms of the antischistosomal action of all of these drugs seem justified.

ACKNOWLEDGEMENTS. This study was funded by the National Science and Technology Pillar Program of China (via grant 2009BAI78B06) and Jiangsu Department of Health (via grants X200707 and X200911). The authors are very grateful to two anonymous referees for their excellent comments.

H.-J. LI*
W. WANG*
Y.-H. TAO
G.-L. QU
Y.-T. XING
Y.-Z. LI
J.-Y. WEI
J.-R. DAI
Y.-S. LIANG
Jiangsu Institute of Parasitic Diseases,
117 Yangxiang, Meiyuan, Wuxi

214064, China, and Key Laboratory on Technology for Parasitic Disease Prevention and Control, Ministry of Health, 117 Yangxiang, Meiyuan, Wuxi 214064, China

Received 14 January 2011,
Revised 18 March 2011,
Accepted 23 March 2011

*These authors contributed equally to the work.Reprint requests to: Y.-S. Liang, Jiangsu Institute of Parasitic Diseases, 117 Yangxiang, Meiyuan, Wuxi 214064, China.
E-mail: wxliangyousheng@yahoo.cn.

REFERENCES

Alonso, D., Muñoz, J., Gascón, J., Valls, M. E. & Corachan, M. (2006). *American Journal of Tropical Medicine and Hygiene*, **74**, 342–344.

Li, H. J., Wang, W., Qu, G. L., Tao, Y. H., Xing, Y. T., Li, Y. Z., Wei, J. Y., Dai, J. R. & Liang, Y. S. (2011). *Annals of Tropical Medicine and Parasitology*, **105**, 181–185.

Melman, S. D., Steinauer, M. L., Cunningham, C., Kubatko, L. S., Mwangi, I. N., Wynn, N. B., Mutuku, M. W., Karanja, D. M., Colley, D. G., Black, C. L., Secor, W. E., Mkoji, G. M. & Loker, E. S. (2009). *PLoS Neglected Tropical Diseases*, **3**, e504.

Ru, W. W., Liang, Y. S., Dai, J. R., Xu, Y. L., Zhu, Y. C. & Xu, M. (2006). *Chinese Journal of Schistosomiasis Control*, **18**, 161–164.

Tchuem-Tchuenté, L. A., Southgate, V. R., Mbaye, A., Engles, D. & Gryseels, B. (2001). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **95**, 65–66.

Tu, Y. Y. (2009). Artemisia annua *and Artemisinin Drugs*. Beijing: Chemical Industry Press.

Utzinger, J., Xiao, S. H., N'Goran, E. K., Bergquist, R. & Tanner, M. (2001). *International Journal for Parasitology*, **31**, 1549–1562.

World Health Organization (2002). *Prevention and Control of Schistosomiasis and Soil-transmitted Helminthiasis: Report of a WHO Expert Committee*. Technical Report Series No. 912. Geneva: WHO.

Wu, L. J., Xu, P. S., Yang, M. J., Fan, J. T. & Li, S. W. (1998). *Chinese Journal of Schistosomiasis Control*, **10**, 65–68.

Xiao, S. H., Tanner, M., N'Goran, E. K., Utzinger, J., Chollet, J., Bergquist, R., Chen, M. & Zheng, J. (2002). *Acta Tropica*, **82**, 175–181.

You, J. Q., Mei, J. Y. & Xiao, S. H. (1994). *Chinese Journal of Parasitic Diseases*, **7**, 50–51.