Is there reduced susceptibility to praziquantel in *Schistosoma japonicum*? Evidence from China

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SUMMARY

Praziquantel is widely used for the treatment of human schistosomiasis. However, in recent years, there has been increasing concern about the resistance of *Schistosoma* species to praziquantel. The study described here was designed to evaluate the current susceptibility to praziquantel in *S. japonicum* in China. During the non-transmission period of schistosomiasis, a random sample of 4760 subjects from the main endemic foci of China were examined using parasitological stool examination. In total, 584 subjects were identified as being infected with *S. japonicum*, with a prevalence rate of 12·27%. Among them, 565 stool-egg-positive subjects were treated with praziquantel in a single oral dose of 40 mg/kg. Six weeks post-treatment, among the 505 villagers re-examined, 480 (95·05%) had no detectable *S. japonicum* eggs. Twenty-one subjects still excreting eggs after the first treatment were treated with praziquantel for the second time. All stool samples, including those from those participants with second treatment were re-examined 6 weeks after the second treatment, and no stool-egg-positives were found. The results indicate that the current efficacy of praziquantel against *S. japonicum* is still high and has not changed after more than 2 decades of repeated, expanded chemotherapy in the main endemic areas of China. It is suggested that no evidence of tolerance or resistance to praziquantel in *S. japonicum* was detected in China.

Key words: *Schistosoma japonicum*, praziquantel, susceptibility, drug resistance, chemotherapy, China.

INTRODUCTION

Since praziquantel, a highly effective and safe anti-schistosomal drug, was developed (Gönnert and Andrews, 1977; Seubert *et al*. 1977) it has replaced all other schistosomacidal agents to become the only anti-schistosomal drug of choice for treatment against all the major species of schistosome (WHO, 1993). Praziquantel makes mass chemotherapy possible as the priority control strategy in almost all countries that are endemic for schistosomiasis worldwide (WHO, 1993). Under laboratory conditions it is possible to induce resistance of *Schistosoma mansoni* to praziquantel with multiple subcurative doses (Fallon and Doenhoff, 1994). Although there is little evidence of the existence of praziquantel-resistant field isolates, a decreased sensitivity of *S. mansoni* to praziquantel has been found in many endemic areas (Ismail *et al*. 1994*a*, 1996, 1999; Fallon *et al*. 1995; Stelma *et al*. 1995, 1997; Bennett *et al*. 1997; Tchuem-Tchuenté *et al*. 2001; Danso-Appiah and De Vlas, 2002; Melman *et al*. 2009). There are also several schistosomiasis cases caused by *S. haematobium* in which repeated standard treatment failed to clear the infection reported (Prociv, 1997; Silva *et al*. 2005; Alonso *et al*. 2006). Therefore, it is of great importance to monitor praziquantel efficacy in regions where the drug is widely used because firstly, there is concern that continued use of praziquantel may give rise to populations of resistant parasites, and secondly, there is currently no other drug being developed for treatment of this widespread disease.

Schistosomiasis japonica, which is still a major public health problem in China, remains endemic in the marshland and lake regions of 5 provinces (Hunan, Hubei, Anhui, Jiangsu and Jiangxi) along the middle and lower reaches of the Yangtze River, and in some mountainous areas in the provinces of Sichuan and Yunnan (Zhou *et al*. 2004; Hao *et al*. 2008; Wang *et al*. 2008; Li *et al*. 2009). Currently, about 726 000 people living in China are thought to have this disease (Zhou *et al*. 2007). Since 1992, the World Bank Loan Project for Schistosomiasis Control initiated in China, praziquantel-based chemotherapy has been conducted to control the morbidity and reduce the prevalence and intensity of *S. japonicum* infection (Chen *et al*. 2005), and the strategy, which was an important part of Chinese National Schistosomiasis Control Program, has been proved to be generally effective (Chen, 2005; Xiao, 2005; Zhou *et al*. 2007; McManus *et al*. 2009). After extensive, long-term repeated praziquantel chemotherapy, the possibility of reduced susceptibility of praziquantel against *S. japonicum* has been widely

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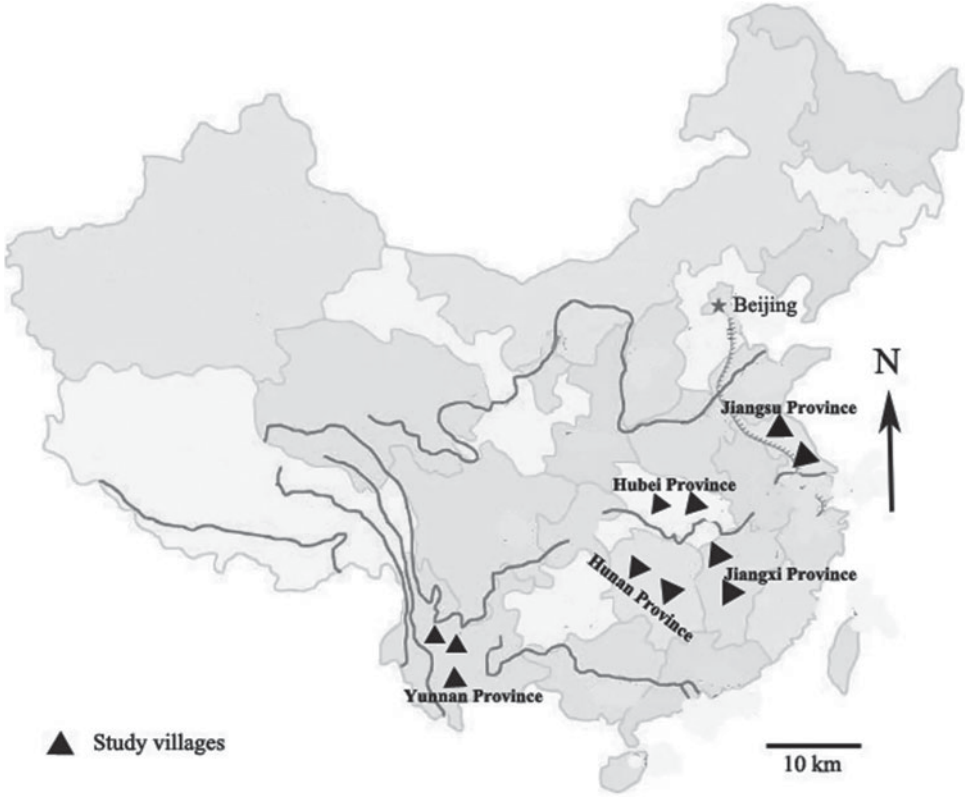

Fig. 1. Locations of the study villages in China.

investigated (Mitchell *et al*. 1990; Yue *et al*. 1990; He *et al*. 1992; Liang *et al*. 2001; Yu *et al*. 2001). Here, a field study was carried out in the main schistosomiasis-endemic foci of China to survey and evaluate the current sensitivity to praziquantel in *S. japonicum*.

MATERIALS AND METHODS

Study area and subjects

Eleven villages in 5 provinces that are endemic for *S. japonicm* along the Yangtze River and in mountainous areas were selected (Fig. 1), namely Biaoen and Changshan villages in Poyang County of Jiangxi Province, with populations of 1035 and 1682, and 39% and 40% infection rates of *S. japonicum*, respectively (local epidemiological data in 2003), Nandi and Chehun villages in Hanshou County of Hunan Province, with populations of 1742 and 1246, and 16% and 19% of the people infected, respectively (local epidemiological data in 2003), Gudi and Fanhu villages in Jiangling County of Hubei Province, with populations of 2753 and 1017, and 17% and 16% of the people infected, respectively (local epidemiological data in 2003), Tanzhu and Sanguan villages in Dantu District of Jiangsu Province, with populations of 1078 and 1156, and 2·8% and 1·8% of the people infected (local epidemiological data in 2003), and Shujie, Gumudi and Duma villages in Weishan County of Yunnan Province, with total population of 4863 and 21·3% total infection rates (local epidemiological data in 2003). Mass synchronous chemotherapy for both humans and domestic animals (mainly bovine) with praziquantel has been successively carried out in these villages for more than 10 years with the World Bank Loan Project for Schistosomiasis Control.

Survey of general information and symptoms and history related to schistosomiasis

An individual questionnaire was designed and then used for the study. Staff from local schistosomiasis control institutions were employed to obtain information including name, age, sex, symptoms related to the disease and history of treatment for schistosomiasis, etc.

Parasitological examination for Schistosoma japonicum *infection*

During the period of schistosomiasis non-transmission (from November, 2004 to March, 2005), a random sample of 4760 volunteers aged from 6 to 70 years from the study villages was involved in the present study, but pregnant women were excluded. The villagers were detected for *S. japonicum* infection with parasitological stool examinations using the Kato-Katz technique (examination of 1 stool sample with 3 thick smears) (Katz *et al*. 1972). Briefly, each faecal sample was pressed through a sieve and an

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amount of 41·7 mg sieved stool measured by a standard template was transferred to a microscope slide where a piece of cellophane soaked in glycerine was pressed onto the sample. Three Kato-Katz thick smears were made from each stool specimen and the total number of eggs detected in each Kato-Katz thick smear was recorded.

Praziquantel treatment

Praziquantel tablets (Nanjing Pharmaceutical Factory Co. Ltd, Nanjing, China; Batch No. 20040202) were administered to those villagers whose stool examinations were positive with a single oral dose of 40 mg/kg. Fecal samples were collected for parasitological stool examinations 6 weeks post-treatment. Those villagers still excreting eggs were treated a second time with the same dose of praziquantel. The stool samples of the villagers, including those re-treated with praziquantel, were collected and re-examined 6 weeks after the second treatment. Those villagers remaining positive were treated for a third time with a dose of 60 mg/kg of praziquantel.

Ethical consideration

This study was approved by the Ethics Review Committee of Jiangsu Institute of Parasitic Diseases. Informed consent was obtained from all participants following a detailed description of the purpose and potential benefits of the study. Praziquantel (a single oral dose of 40 or 60 mg/kg) was offered to those cases with stool-egg without charge, and all subjects accepted the treatment strategy.

Statistical analysis

All data were entered in Excel (Microsoft Corporation; Redmond, WA, USA) and all statistical analyses were performed using the statistical software Statistical Package for the Social Sciences v. 11.0 (SPSS 11.0, SPSS Inc., Chicago, IL, USA). Differences of proportions were tested for statistical significance with the chi-square test; *t*-test and 95% confidence intervals of means were used to compare groups. A P value $< 0{\cdot}05$ was considered significant.

RESULTS

The infection rate, age, sex ratio, intensity of infection, symptom related to schistosomiasis and history of treatment for schistosomiasis with praziquantel of all the subjects from 11 villages of 5 provinces pre-treatment are shown in Table 1. Of the 4760 villagers examined, 584 had *S. japonicum* eggs in the first faecal sample, with a prevalence rate of 12·27%, among whom 565 infected subjects received praziquantel treatment in a dose of 40 mg/kg. Six weeks after treatment, 505 of the treated villagers were re-examined and 480 (95·05%) had no detectable *S. japonicum* eggs. Twenty-one subjects still excreting eggs after the first treatment were administered with praziquantel for the second time. Six weeks after the second treatment, the stool samples from all treated villagers, including those with the second treatment, were collected and re-examined, no stool-egg-positives were detected. There were no significant differences in cure rates among the 11 villages (P value $>0{\cdot}05$) and no differences between either age groups or male or female villagers, among different intensity of infection groups (all P values $>0{\cdot}05$) (Table 2). The 21 cases with high intensity of infection (EPG $\geqslant 400$) were successfully treated with a single dose.

DISCUSSION

Since the 1980s praziquantel has been the drug of choice for the treatment of *S. japonicum* infection and it is used widely in endemic areas of China. Over this period, it is estimated that more than 10 million infected people in China have been treated with the drug (Chen, 2005; Anon, 1986). Treatment of a population for schistosomiasis with praziquantel at a dose of 40 or 50 mg/kg usually results in short-term parasitological cure rates of 97·6%–100%. However, increased dosage produced no significant improved cure rates (Fu *et al*. 1988). As a result of experimental and field studies, since 1984 either a single dose of 40 mg/kg or a total daily dose of 40 mg/kg divided into 2 doses given 6 h apart has been recommended (MOH, 1993). Our findings showed that the cure rate reached 95·1% with a single oral dose of 40 mg/kg of praziquantel and 100% with 2 treatments with praziquantel. The results from this study demonstrate that the efficacy of praziquantel against *S. japonicum* is still high in main endemic areas of China. The present study shows that the current susceptibility of praziquantel against *S. japonicum* has not changed after more than 2 decades of repeated, expanded chemotherapy in China. This is important information for both the public health workers and health policy makers in the field of schistosomiasis control, considering that praziquantel plays an essential role in the current Chinese National Schistosomiasis Control Program. Earlier studies (Liang *et al*. 2001; Yu *et al*. 2001) also reached the same conclusion. However, only 1 endemic province was selected for each study. In this study, we chose 11 villages from 5 out of 7 main schistosome-endemic provinces both in marshland and lake regions and in mountainous areas. The conclusion we draw, therefore, is more persuasive.

Unlike *S. mansoni* and *S. haematobium, S. japonicum* is truly a zoonosis and besides humans, livestock, especially cattle, which are considered as the main sources of infection in China, are incriminated as

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Table 1. Demographical features of schistosomiasis patients from 11 villages in 5 *Schistosoma japonicum*-endemic provinces of China

	Yunnan Province			Jiangsu Province		Hunan Province		Jiangxi Province		Hubei Province	
Characteristic	Shujie Village	Gumudi Village	Duma Village	Tanzhu Village	Sanguan Village	Nandi Village	Chehun Village	Changshan Village	Biaoen Village	Gudi Village	Fanhu Village
Infection rate (%)	26·3	28·8	33·2	3·1	2·3	15·8	10·9	16·9	4·1	23·0	13·6
No. of villagers examined	410	52	196	574	668	575	303	611	822	291	258
No. of cases infected with *S. japonicum*	108	15	65	18	15	91	33	103	34	67	35
Age range (years)	9–60	8–49	8–62	26–64	15–63	7–64	11–64	6–65	7–62	8–64	8–68
Age group (years):											
5–20	3	3	10	0	1	22	7	41	13	9	6
⩾20	105	12	55	18	14	69	26	62	21	58	29
Sex:											
Male	55	8	23	8	9	71	26	71	20	38	15
Female	53	7	42	10	6	20	7	32	14	29	20
Intensity of infection:											
EPG < 10	53	5	35	9	–	13	5	1	14	–	–
EPG 10–100	45	7	25	6	–	59	24	86	17	–	–
EPG 100–400	8	2	2	3	–	14	1	10	2	–	–
EPG ⩾400	2	1	3	0	–	5	3	6	1	–	–
Geometric mean EPG	41·9	129·6	64·1	10·2	–	39·5	37·9	31·3	22·6	–	–
No. of cases with history of treatment with praziquantel	54	10	16	15	13	61	28	86	28	58	30
Symptom:											
Diarrhoea	31	8	21	5	4	27	7	30	11	25	9
Blood or mucus stool	17	4	9	2	1	9	5	15	8	11	10
Weakness	40	10	25	6	10	35	24	41	19	30	12

Table 2. Efficacy of praziquantel (40 mg/kg) against *Schistosoma japonicum* in villagers in 5 provinces of China

		First examination			Second examination		
Province	Village	No. cases treated	No. cases examined	Cure rate (%)	No. cases re-treated	No. cases re-examined	Cure rate (%)
Yunnan	Shujie	97	79	98·7	1	79	100
	Gumudi	16	15	93·3	1	15	100
	Duma	56	44	90·9	4	44	100
Jiangsu	Tanzhu	18	18	100	0	18	100
	Sanguan	15	15	100	0	15	100
Hunan	Nandi	91	87	90·8	8	87	100
	Chehun	33	30	90	3	30	100
Jiangxi	Changshan	103	89	98·9	1	88	100
	Biaoen	34	32	100	0	32	100
Hubei	Gudi	67	62	98·4	1	61	100
	Fanhu	35	34	94·1	2	32	100
95% confidence intervals	–	–	–	91·8–100	–	–	100–100

reservoir hosts (Wang, 2005, 2009; Wang *et al*. 2005; Yang *et al*. 2009; Yu *et al*. 2009). In all villages in this study, mass synchronous chemotherapy for both humans and livestock has been systematically implemented for more than 10 years. There was, therefore, the possibility that *S. japonicum* might begin to develop resistance to praziquantel. One of the purposes of the present study was to determine whether tolerance or resistance to praziquantel exists in *S. japonicum* populations, as there have been many reports of diminished susceptibility or tolerance to praziquantel against *S. mansoni* in Africa (Ismail *et al*. 1994*a*, 1996, 1999; Fallon *et al*. 1995; Stelma *et al*. 1995, 1997; Bennett *et al*. 1997; Tchuem-Tchuenté *et al*. 2001; Danso-Appiah and De Vlas, 2002; Melman *et al*. 2009; Gryseels *et al*. 1994; Guisse *et al*. 1997). There were also some case reports of failure of repeated standard praziquantel treatment to clear *S. haematobium* infections (Prociv, 1997; Silva *et al*. 2005; Alonso *et al*. 2006). Tolerance of *S. mansoni* to praziquantel has already been induced in the laboratory (Fallon and Doenhoff, 1994; Ismail *et al*. 1994*b*). The normal cure rates of a single dose of 40 mg/kg of praziquantel, as recommended by the World Health Organization for the treatment of *S. mansoni* in both adults and children, are usually 60%–90% (WHO, 1993; Jordon *et al*. 1993; Kumar and Gryseels, 1994), while the cure rates of *S. japonicum* exceed 90%. It was concluded that the adults of *S. japonicum* are more sensitive to praziquantel than *S. mansoni* by comparing the *in vitro* responses of adult *S. japonicum* and *S. mansoni* (Sobhon and Upatham, 1990).

The Kato-Katz technique was used to detect *S. japonicum* infection in this study. Currently, the Kato-Katz technique (3 thick smear slides for 1 stool specimen) is still the golden standard used for the diagnosis of schistosomiasis (Zhou *et al*. 2007). It has been shown that the routine Kato-Katz technique underestimates the real prevalence of *S. japonicum* in endemic areas with low-intensity infections (Lin *et al*. 2008; Zhang *et al*. 2009). Considering that several EPG scores in infected individuals were lower than 10, the missing situation of *S. japonicum*-infected villagers cannot be excluded. The search for a better diagnostic test that can be applied in the endemic field situation in China is therefore essential and should be given high priority.

The present study described here indicates that the efficacy of praziquantel remains high despite its extensive use, and no evidence of reduced susceptibility of praziquantel in *S. japonicum* populations was detected. The fact that the 21 infected cases who needed to be re-treated is thought probably to be due to the presence of the immature worms, which are known to be less sensitive to praziquantel (Xiao *et al*. 1985, 1987; Sabah *et al*. 1986; You *et al*. 1986). For example, the 3-h schistosomula and the adult worms aged more than 28 days of *S. japonicum* are susceptible to the drug, but those schistosomula aged 3–21 days are not sensitive to praziquantel. It is also thought that the 3-h schistosomula are more susceptible to praziquantel than those aged 12–48 h. Those patients with high intensity of infection are usually re-infected cases (Wu *et al*. 1993), among whom both adult worms and schistosomula are found in body. The adults that are susceptible to praziquantel were killed during the initial treatment. And after 6 weeks, the immature schistosomula developed futher to mature adult worms, which are sensitive to praziquantel, and then were given the second treatment, all patients turned negative. It is indicated that the positive cases after the first treatment are due to the remaining schistosomula. It is also proved that

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the adults developing from the schistosomula remain susceptible to praziquantel.

The current efficacy of praziquantel against *S. japonicum* appears satisfactory in China, however, it does not mean that resistance can not occur nor that in different geographical regions the response of *S. japonicum* will be the same. We, therefore, should not reduce our vigilance to the possible development of drug resistance by the parasite. Fortunately, the drug resistance can be easily detected by testing miracidia hatched from eggs passed by patients (Liang *et al*. 2000). Further periodical studies monitoring both the efficacy of schistosomes to the drug and the development and epidemiology of praziquantel resistance of different geographical isolates of *S. japonicum* in China are still required, and these would be further used to develop field surveillance of drug susceptibility in *S. japonicum* in China.

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CONFLICTS OF INTEREST

The authors have declared that no competing interests exist.

AUTHOR CONTRIBUTIONS

W.W., D.J.R. and Y.S.L conceived and designed the study. W.W., H.J.L. and X.H.S. collected baseline data. X.H.S. provided logistical support for part of the fieldwork. W.W., H.J.L., J.R.D. and Y.S.L. took part in all of the fieldwork. W.W. carried out the statistical analysis and interpretation of the data and prepared the manuscript. Y.S.L revised the manuscript. All authors read and approved the final manuscript. Y.S.L. is guarantor of the paper.

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