RESEARCH NOTE

EFFECT OF PRAZIQUANTEL TREATMENT ON HEPATIC EGG GRANULOMAS IN MICE INFECTED WITH PRAZIQUANTEL-SUSCEPTIBLE AND -RESISTANT *SCHISTOSOMA JAPONICUM* ISOLATES

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Abstract. Currently, praziquantel is the drug of choice for treatment of human *Schistosoma japonicum* infections in China. Although no evidence of resistance to praziquantel has been detected following repeated and extensive chemotherapy with praziquantel for more than 30 years in endemic foci of the country, there were several patients with *S. japonicum* infection who failed in standard praziquantel treatment, and praziquantel-resistant isolates of *S. japonicum* have been successfully generated in a mouse model. This study assessed the effect of praziquantel treatment on pathological damages to mice infected with praziquantel-susceptible and -resistant *S. japonicum* isolates. Mice were each infected percutaneously with 20 cercariae of praziquantel-resistant and -susceptible *S. japonicum* isolates, and then randomly grouped. Mice in the treatment groups were administered a single dose (150 kg/mg) of praziquantel at day 35 post-infection, while infected but untreated mice served as controls. Mice were sacrificed on day 42 or 49 post-infection, the area and circumference of the hepatic egg granulomas in fresh liver specimens were measured. In infected but untreated mice, there were no significant differences in the area and circumference of the hepatic egg granulomas between praziquantel-resistant and -susceptible isolates, whereas in praziquantel-treated mice, the area or circumference of hepatic egg granulomas were significantly greater in mice infected with the praziquantel-resistant than in those harboring

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praziquantel-susceptible isolates ($p<0.01$). These results demonstrate praziquantel treatment causes more reductions in the size of hepatic egg granulomas in mice infected with praziquantel-susceptible *S. japonicum* isolates than in those infected with drug-resistant parasites, indicating eggs from praziquantel-susceptible isolates are more sensitive to praziquantel than those from the drug resistant isolates.

Keywords: *Schistosoma japonicum*, drug resistance, hepatic egg granuloma, praziquantel

INTRODUCTION

Schistosomiasis, caused by infection with blood flukes of the genus *Schistosoma*, remains a global public health concern (Colley *et al,* 2014). This neglected tropical disease is reported to be endemic in 78 countries and more than 200 million people are estimated to have the disease across the world, with a further 800 million at risk of infection (Qu *et al,* 2016). Currently, mass drug administration (MDA) with praziquantel is implemented as the major global strategy for schistosomiasis control (Ross *et al,* 2015). However, there is a growing concern on the emergence of praziquantel-resistant parasites (Wang *et al,* 2012b), following long-term and extensive use of the agent since its introduction in late 1970s (Gönnert and Andrews, 1977). In Africa, there are increasing evidences demonstrating praziquantel-resistant *S. mansoni* has already exists in endemic foci (Doenhoff *et al,* 2002; Melman *et al,* 2009; Bergquist *et al,* 2017; Vale *et al,* 2017).

Since the 1980s, praziquantel has replaced other schistosomicides to become the drug of choice for the treatment of human *S. japonicum* infections in China (Chen, 2005; Xiao *et al,* 2010). Following repeated and extensive chemotherapy with praziquantel for more than 30 years in ten endemic foci of the country, there is still no evidence of resistance to praziquantel; however, there are several patients with *S. japonicum* infection who fail in standard praziquantel treatment at a single oral dose of 40 mg/kg or at a single dose of 30 mg/kg administered for two successive days (Liang *et al,* 2001a; Wang *et al,* 2010, 2012a; Seto *et al,* 2011). In addition, praziquantel resistance was experimentally induced in *S. japonicum* in a mouse model, demonstrating that the parasites are capable of developing resistance to praziquantel when sufficiently given under drug pressure (Liang *et al,* 2011).

Previous studies detected higher *in vitro* response to praziquantel at the egg, miracidial, cercarial and adult stages of praziquantel-susceptible *S. mansoni* isolates than drug-resistant isolates (Liang *et al*, 2001b). In addition, mice infected with praziquantel-resistant *S. mansoni* isolates shed more eggs in their feces than mice carrying drug-susceptible parasites, and the praziquantel-resistant isolates appear more pathogenic in mice than susceptible parasites (Liang *et al,* 2001c). Similarly, there is a clear cut reduction in the responses to praziquantel at the miracidial, cercarial and adult stages of praziquantel-resistant *S. japonicum* isolates than praziquantel-susceptible parasites (Li *et al,* 2011). However, the pathological damages of praziquantel-resistant *S. japonicum* to mammalian hosts remain unknown.

Hence, the present study was designed to assess the effect of praziquantel treatment on hepatic egg granulomas in mice infected with praziquantel-susceptible and -resistant *S. japonicum* isolates and, thereby, obtain information of the

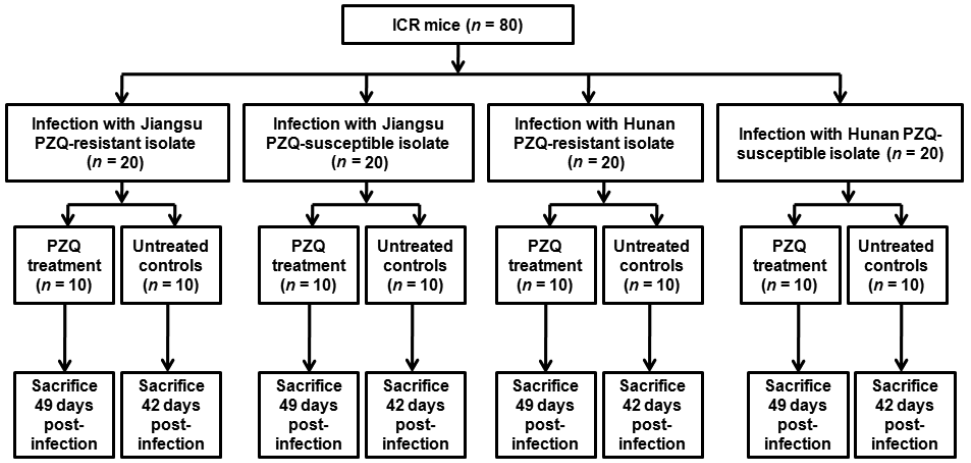

Fig 1–Diagram of study protocol. Praziquantel (PZQ) treatment was a single dose of 150 mg/kg by oral gavage at day 35 post-infection.

pathogenicity of praziquantel- resistant *S. japonicum* isolates in mice.

MATERIALS AND METHODS

Chemicals

Praziquantel (batch number: 0001449213) and 2.5% Cremophor number: BCBB1383) were from Sigma-Aldrich (Sigma-Aldrich Chemie, Munich, Germany). Praziquantel was ground in an ND-2L ball miller (Nanda Tianzu Electronics, Nanjing, China) with 2.5% Cremophor EL to produce a 12 g/l praziquantel suspension.

Parasites

Four *S. japonicum* isolates were employed, namely, Jiangsu isolate of the 12th praziquantel-selection passage (praziquantel ED_{50} = 565.5 mg/kg), the 11th passage of a Hunan isolate selected for praziquantel resistance (praziquantel ED_{50} = 467.2 mg/kg), non-drug treated Jiangsu isolate (praziquantel ED_{50} = 147.7 mg/kg), and non-drug treated Hunan isolate (praziquantel ED_{50} = 151.8 mg/kg) (Wang *et al*, 2014a,b). The four *S. japonicum* isolates were maintained at the Key Laboratory of the National Health and Family Planning Commission on Parasitic Disease Control and Prevention, Wuxi, China.

Animals

Eighty female six-week-old mice, ICR strain, each weighing 20 g, from the Center for Comparative Medicine, Yangzhou University, Yangzhou were housed at the Laboratory Animal Center at Key Laboratory of National Health and Family Planning Commission on Parasitic Disease Control and Prevention, Wuxi and given free access to food and water.

Infection and treatment protocol

Each mouse was infected percutaneously with 20 cercariae of praziquantel-resistant or -susceptible *S. japonicum* isolates via the shaved abdominal skin, and 20 mice with the same parasite infection were grouped together. Mice in the treatment groups were given a single dose of 150 mg/kg praziquantel suspension by oral gavage at day 35 post-infection, while infected but untreated mice served as controls. Animals in the treatment groups were sacrificed at day 49 post-infection, while untreated mice were sacrificed at day 42 post-infection (Fig 1).

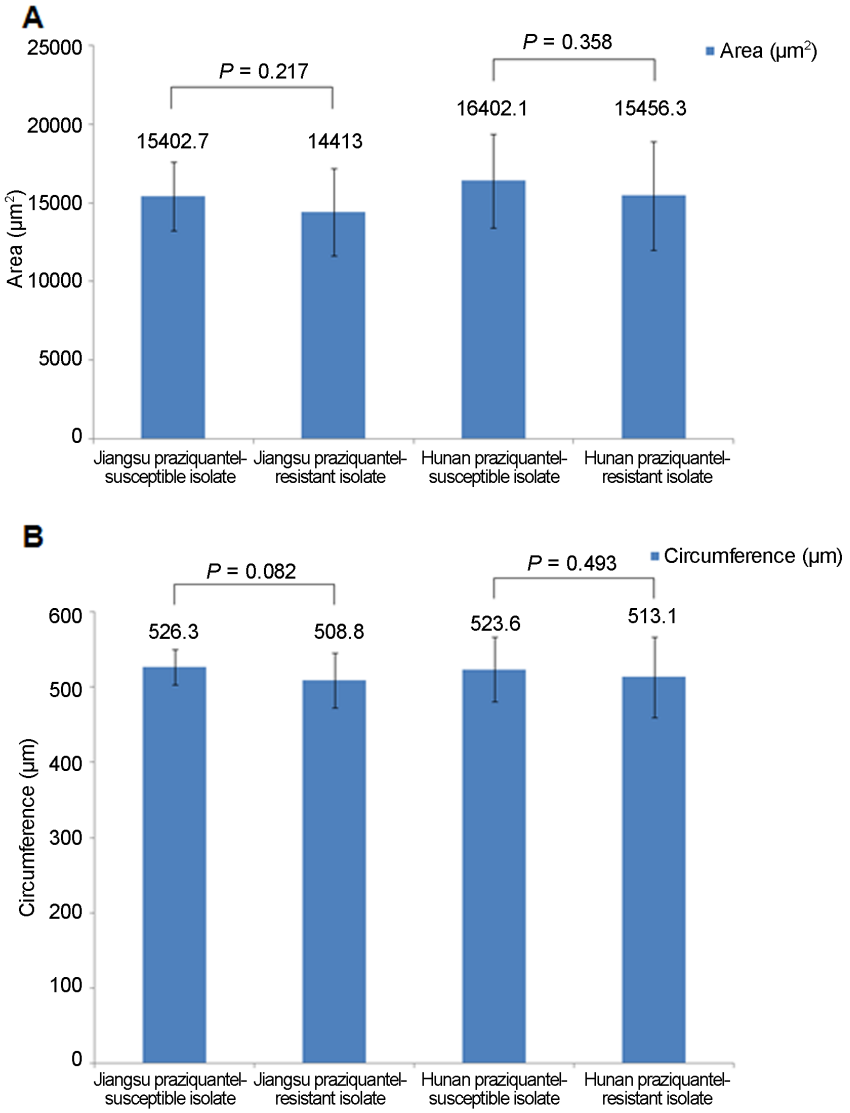

Fig 2–Area and circumference of hepatic egg granulomas of mice infected with praziquantel-susceptible and -resistant *Schistosoma japonicum* isolates. The experimental protocol is shown in Fig 1. A. Area of hepatic egg granulomas. B. Circumference of hepatic egg granulomas. Bar is mean ± SD. *P*, *p*-value.

The study was approved by the Ethical Review Committee of Jiangsu Institute of Parasitic Diseases (permission number: IRB00003927). All animal experimentations were performed following the Guidelines for the Care and Use of Laboratory Animals (Jones-Bolin, 2012).

Measurement of hepatic egg granulomas

After mice were sacrificed, fresh liver specimens were immediately fixed in absolute ethyl alcohol for 24 hours. Then, the liver specimens were fixed in a series of methanol (90%, 80% and 70%) for 24 hours, embedded in paraffin, sliced into 7 μm sections using an AO 860 sliding microtome (American Optics, Ontario, Canada), and stained with hematoxylin-eosin (HE). The area and circumference of the granuloma surrounding a single, mature egg were measured in the mouse liver using a BA310 biological light microscope (Motic Electric Group, Xiamen, China), and 20 hepatic egg granulomas were measured for each parasite isolate.

Data analysis

Data were analyzed using a Microsoft Excel version 2010 (Microsoft, Redmond, WA) and a statistical software SPSS version 17.0 (IBM, Armonk, NY). Data are expressed as mean ± SD and difference of means between groups was tested for statistical significance with Student's *t*-test. A p-value <0.05 is considered statistically significant.

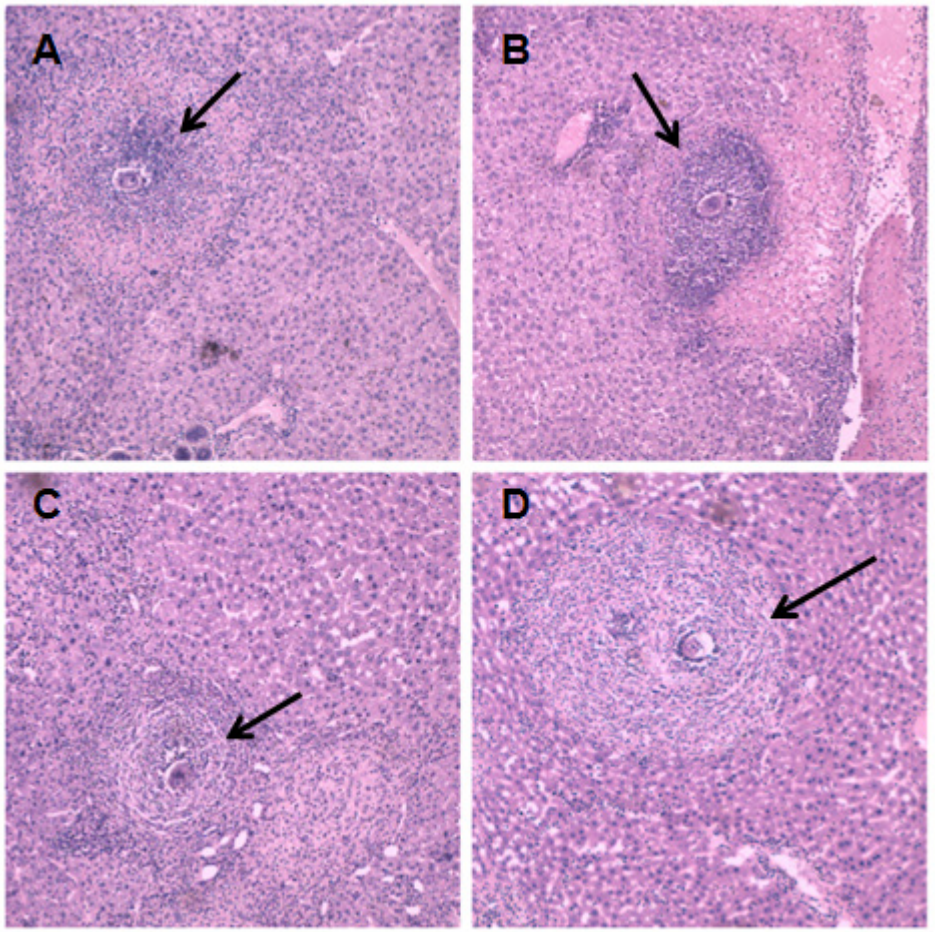

Fig 3–Hematoxylin-eosin stained livers of mice infected with praziquantel-susceptible and -resistant isolates of *Schistosoma japonicum*. The experimental protocol is shown in Fig 1. A. Female ICR mouse infected with praziquantel-susceptible *S. japonicum* isolate. B. Female ICR mouse infected with praziquantel-resistant *S. japonicum* isolate. C. Female ICR mouse infected with praziquantel-susceptible *S. japonicum* isolate following praziquantel treatment. D. Female ICR mouse infected with praziquantel-resistant *S. japonicum* isolate following praziquantel treatment. Arrow indicates hepatic egg granuloma. Light microscope magnification, 100x.

RESULTS

In infected but untreated mice, there are no significant differences between praziquantel-resistant and -susceptible isolates in the area and circumference of hepatic egg granulomas (Figs 2 and 3). In mice administered a single dose of 150 mg/kg praziquantel at day 35 post-infection, the area and circumference of the hepatic egg granulomas are statistically larger in mice infected with praziquantel-resistant *S. japonicum* isolates than in those harboring drug-susceptible parasites (p <0.01) (Figs 3 and 4).

DISCUSSION

Elucidation of the biological characteristics of praziquantel-resistant *Schisto-*

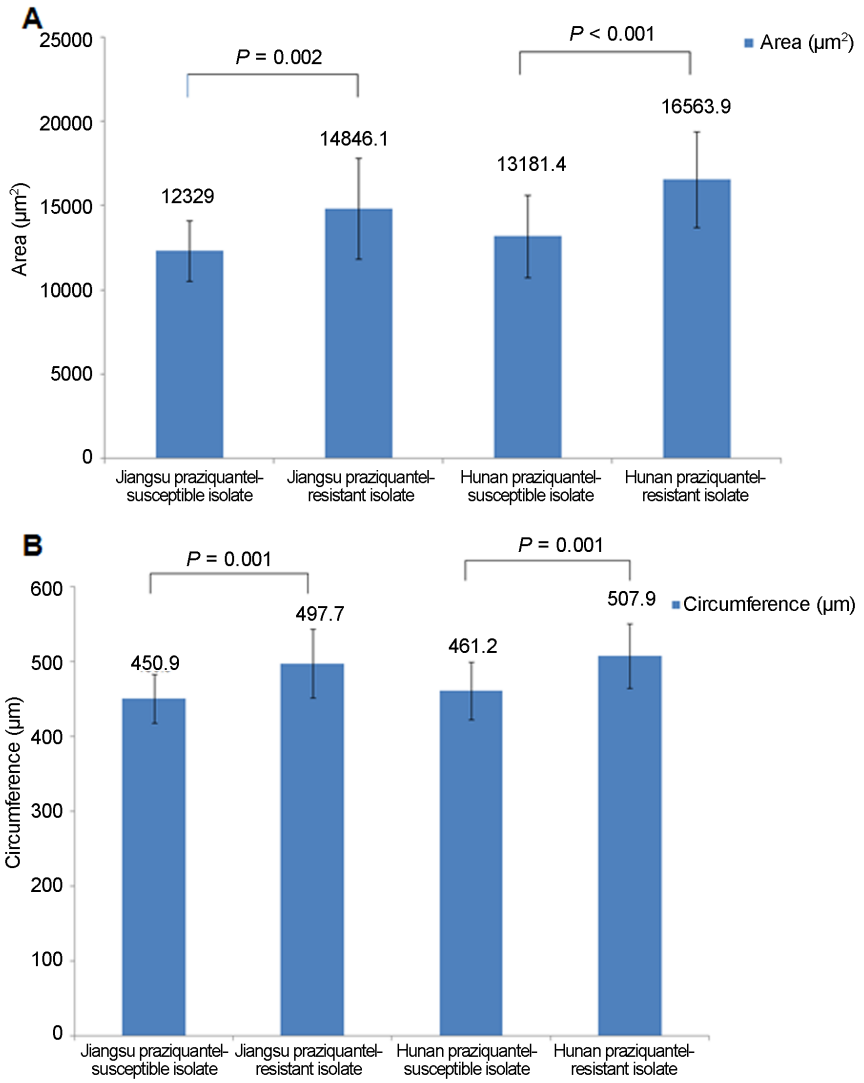

Fig 4–Area and circumference of hepatic egg granulomas of mice infected with praziquantel-susceptible and -resistant *Schistosoma japonicum* isolates following praziquantel treatment. The experimental protocol is shown in Fig 1. A. Area of hepatic egg granulomas. B. Circumference of hepatic egg granulomas. Bar is mean ± SD. *P*, *p*-value.

soma species, such as parasite infectivity to definitive hosts, cercarial production and pre-patent period of the parasite may facilitate understanding of the transmission dynamics of drug-resistant parasites, while investigating the egg production and pathological damages caused by praziquantel-resistant isolates to definitive hosts is of great significance to assess the virulence and pathogenicity of such parasites (Hsu and Hsu, 1958; Liang *et al*, 2001c). Currently, a variety of parameters have been proposed to assess the virulence of *S. japonicum* in definitive hosts, including number of adult worms recovered from hosts, host mortality, size of hepatic egg granulomas, severity of hepatic fibrosis, and proportion of hepatomegaly and splenomegaly (Hsu and Hsu, 1960). Because the primary pathological signs and changes of *S. japonicum* infections stem from egg granuloma formation following parasite eggs deposition in liver (Warren *et al*, 1975), the size of egg granulomas and the severity of granulomatous inflammation have been widely used to evaluate the virulence and pathogenicity of *S. japonicum* (Warren *et al*, 1978; Cheever, 1985; Doenhoff, 1997; Huang *et al*, 2011).

In this study, a mouse model designed to have the same intensity and duration

of *S. japonicum* infection as in the natural state was employed. Our findings are in agreement with a previous report performed with *S. mansoni* (Liang *et al,* 2002). The present study detected a lower *in vivo* response to praziquantel in eggs of praziquantel-resistant *S. japonicum* isolates relative to drug-susceptible parasites is consistent with a previous *in vitro* assay showing lower responses to praziquantel at the miracidial, cercarial and adult stages of praziquantel-resistant compared to -susceptible *S. japonicum* isolates (Li *et al,* 2011).

Praziquantel treatment is effective in causing a clear-cut and rapid reduction in the size of hepatic egg granulomas of mice harboring *Schistosoma* species (Mehlhorn *et al,* 1981, 1982; Huang *et al,* 2011). Praziquantel ability to ameliorate the pathological damages is considered to be closely associated with its activity against the parasite eggs deposited in the host liver (Huang *et al,* 2011). As eggs have been identified to play a critical role during the pathogenic process of *Schistosoma*-induced diseases (Wilson *et al,* 2007), a reduction in parasite egg production and alleviation of egg-induced pathological damage are therefore of great importance in improving the prognosis of schistosomiasis even if infection cannot be completely suppressed (Gray *et al,* 2011). Because eggs of praziquantel-resistant *S. japonicum* isolates exhibited a lower response to praziquantel than the susceptible isolates, praziquantel treatment may fail to decrease egg-induced pathological damage. The underlying mechanisms responsible for the parasite-host interactions, host immune responses, and immunomodulation during egg granuloma need further investigations, which should facilitate the elucidation of our understanding of the immune mechanisms in schistosomiasis, and thereby allow exploration of the mechanism(s) of praziquantel resistance in schistosomiasis.

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