Effect of praziquantel treatment on experimental porcine *Schistosoma japonicum* infection

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SUMMARY

The aims of the study were to assess the effect of praziquantel on *Schistosoma japonicum* infection in pigs, and to elucidate the level of resistance to reinfection after treatment. Pigs were given a single infection with *S. japonicum* followed by a praziquantel treatment (40 mg/kg) week 8, reinfection week 12 and perfusion week 20 post-primary infection. Relevant control groups were included. Worm burdens, faecal and tissue egg counts, gross- and histopathology of the liver and specific liver enzymes were assessed. The results showed a 100% cure rate of praziquantel against *S. japonicum* in pigs. Worm nodules were present in equal numbers in the intestinal wall 4 and 14 weeks post-treatment. Treatment did not significantly reduce the number of tissue eggs neither 4 nor 14 weeks post-treatment. No worm nodules or worm-induced lesions were found in the livers of the treated pigs and the levels of liver enzymes were comparable in treated and non-treated infected pigs. Periportal and septal fibrosis regressed following treatment. Faecal egg counts were significantly reduced 2 (56%) and 4 (82%) weeks after treatment. Challenge infection 4 weeks post-treatment did not result in establishment of new worm pairs. Praziquantel proved to be highly effective against *S. japonicum* in pigs without causing pathological side-effects in the liver.

Key words: *Schistosoma japonicum*, pigs, praziquantel.

INTRODUCTION

Porcine schistosomiasis japonica is endemic in several provinces along the Yangtze river in China and domestic pigs are believed to play a major role in the transmission of *Schistosoma japonicum* to both man and other animals (Wu *et al*. 1992; Su *et al*. 1994; Brindley *et al*. 1995). Much effort has been put into control of schistosomiasis but although several vaccine candidates have been tested in the past decade, no schistosomiasis vaccine is commercially available today (Waine & McManus, 1997). Hence, treatment remains one of the main approaches to control the disease (Wu *et al*. 1993). Today, praziquantel is the drug of choice for treatment of schistosomiasis, clonorchiasis, opisthorchiasis, paragonimiasis, cycticercosis and many intestinal tapeworms, and it is considered a safe drug with only mild side-effects (King & Mahmoud, 1989). However, Johansen *et al*. (1996*b*) found severe liver lesions attributable to dead worms after praziquantel treatment of goats infected with *S. bovis*.

Porcine schistosomiasis japonica is, apart from being a serious problem in China, believed to be a good model system for human schistosomiasis since *S. japonicum* is a zoonosis and because pigs and man share many features with regard to anatomy and physiology (Miller & Ullrey, 1987; Willingham & Hurst, 1996).

Thus, the present study sought to gain basic information about the efficacy and potential side-effects of the drug and effect of treatment on resistance to infection.

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MATERIALS AND METHODS

Experimental animals

Thirty-five, helminth naive, specific pathogen-free Danish Landrace × Yorkshire × Duroc and/or Hampshire cross-breed pigs (20 females and 15 castrated males) initially 7–9 weeks old and with a weight range of 13–20 kg, were used. The pigs were housed together randomly under helminth-free conditions and fed a standard ratio of a ground barley and protein supplement with water provided *ad libitum*. They were allocated according to sex and weight into 7 groups of 5 pigs. *S. japonicum* cercariae used in the study was a Chinese mainland strain, maintained in *Oncomelania hupensis hupensis* snails and NMRI mice at the Danish Bilharziasis Laboratory.

Study design

The study design is presented in Table 1. The pigs to be infected were each given 2000 cercariae

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Table 1. Schematic illustration of the study design

Group	No. of pigs	Infection (week 0)	Treatment (week 8)	Challenge (week 12)	Perfusion (week 22)
PT1	5	+	+		+*
PT2	5	+	+		+
PTC	5	+	+	+	+
PC	5	+		+	+
P	5	+			+
C	5			+	+
T	5		+		
Total	35	25	20	20	30

* Pigs perfused week 12 p.i.

suspended in Iscoves medium at both the primary (P) and challenge (C) infections by intramuscular injection according to the method described by Willingham *et al*. (1998). Treatment (T) with praziquantel (40 mg/kg) was given via a stomach tube in a 5 % glyceryl solution. Prior to perfusion the pigs were sedated by an intramuscular injection of 0·06 mg/kg of Zoletil (zolazepam/tiletamin) and 0·1 mg/kg of Narcoxyl (Xylazinum NFN) and killed by an overdose of pentobarbital given intravenously.

Parasitology and haematology

All pigs were weighed at the beginning of the experiment and at the time of perfusion, and faecal and blood samples were collected every second week throughout the study. Faecal egg counts were determined as described by Willingham *et al*. (1998).

Additional faecal samples were collected from the PTC-group and the PC-group 2 days before and 1, 5 and 13 days after treatment to determine egg hatchability. Perfusion was performed according to the method described by Willingham *et al*. (1998). Worms were counted according to sex and maturity. Tissue samples were collected at necropsy from caecum, 3 equally divided parts of colon and from rectum and the mucosa of each section was digested in 3 % KOH for 18 h at 37 °C as described by Johansen *et al*. (1996*a*). the liver egg counts were assessed as described by Bøgh *et al*. (1996). Serum concentrations of the specific liver enzymes, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were determined kinetically (Cobas-fara/Roche).

Pathology

Following perfusion, intestinal organs were removed and examined for residual worms, worm nodules and pathological lesions. Each worm nodule was regarded as a dead and decaying worm pair (Willingham *et al*. 1998). The liver was inspected and each lobe was cut in 1 cm thick slices to look for worm nodules. The degree of pathological changes of the liver was graded as none, mild, moderate or severe. For histopathology, a sample was taken from the central part of the left hepatic lobe and fixed in 10 % neutral-buffered formalin. After conventional processing and paraffin wax embedding, 2 sections (*ca*. 1 cm² in surface area) from each liver sample was prepared and stained with haematoxylin and eosin (H & E). Each section was examined for the presence of worm-induced lesions and a semi-quantitative assessment was made of periportal and septal fibrosis.

Statistical analyses

One-way analysis of variance was used to compare group means of body weight gain, ALAT and ASAT, worm numbers and tissue and faecal egg counts. Tissue and faecal egg counts were log 10 $(x+1)$ transformed before the analyses.

Faecal egg count reduction was calculated according to the formula: % reduction $= 100(1-(T2/T1)\times(C1/C2))$ where $T =$ the infected and treated groups ($PT+PTC$-groups); $C =$ the infected non-treated group ($P+PC$-groups); $1 = 1$ day before treatment and $2 = 2$ or 4 weeks post-treatment (Prichard *et al*. 1980).

RESULTS

Infections with *S. japonicum* were established in all exposed pigs. Clinical signs of the infections were restricted to light diarrhoea 6–8 weeks post-infection. No statistically significant differences were observed in mean weight gains nor in the 2 examined liver enzymes, ALAT and ASAT, between the groups (data not shown).

Worm recovery

The mean number of *S. japonicum* worms and worm nodules are presented in Fig. 1. Treatment resulted in a 100 % cure rate as no immature or adult live worms were found in the PT1-group or in the PT2-group. The number of worm nodules in the PT1-

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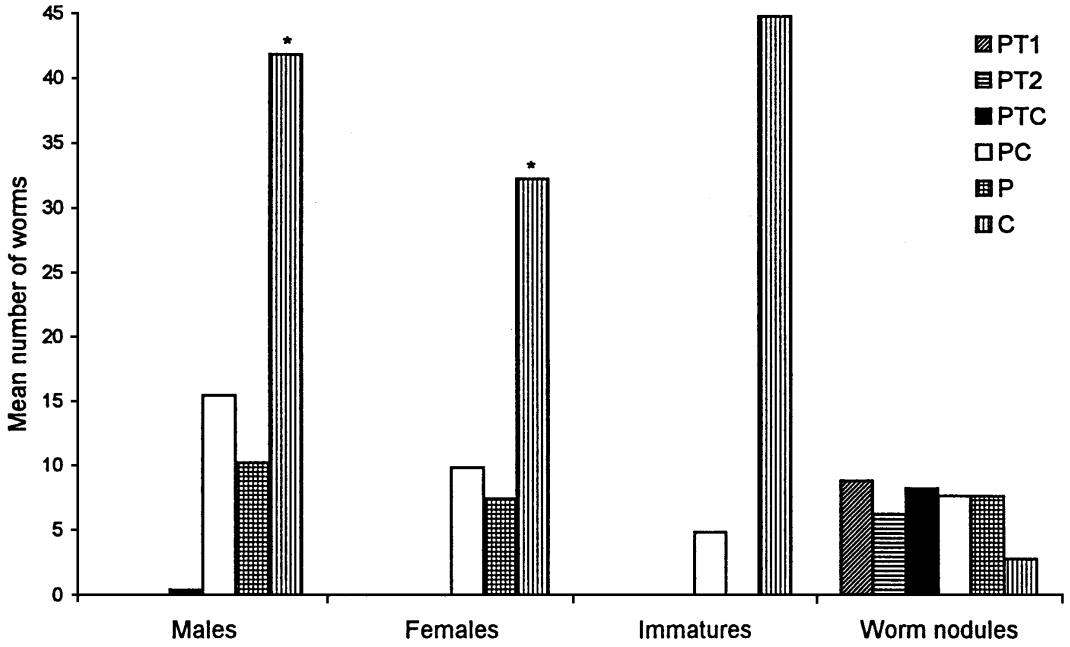

Fig. 1. Mean number of *Schistosoma japonicum* worms and worm nodules in pigs. P, primary infection with 2000 cercariae; T, treatment with praziquantel (40 mg/kg) 8 weeks post-primary infection; 1, pigs killed 4 weeks post-treatment; 2, pigs killed 14 weeks post-treatment; C, challenge infection with 2000 cercariae 12 weeks post-primary infection. * Significantly different from treated groups ($P < 0{\cdot}05$).

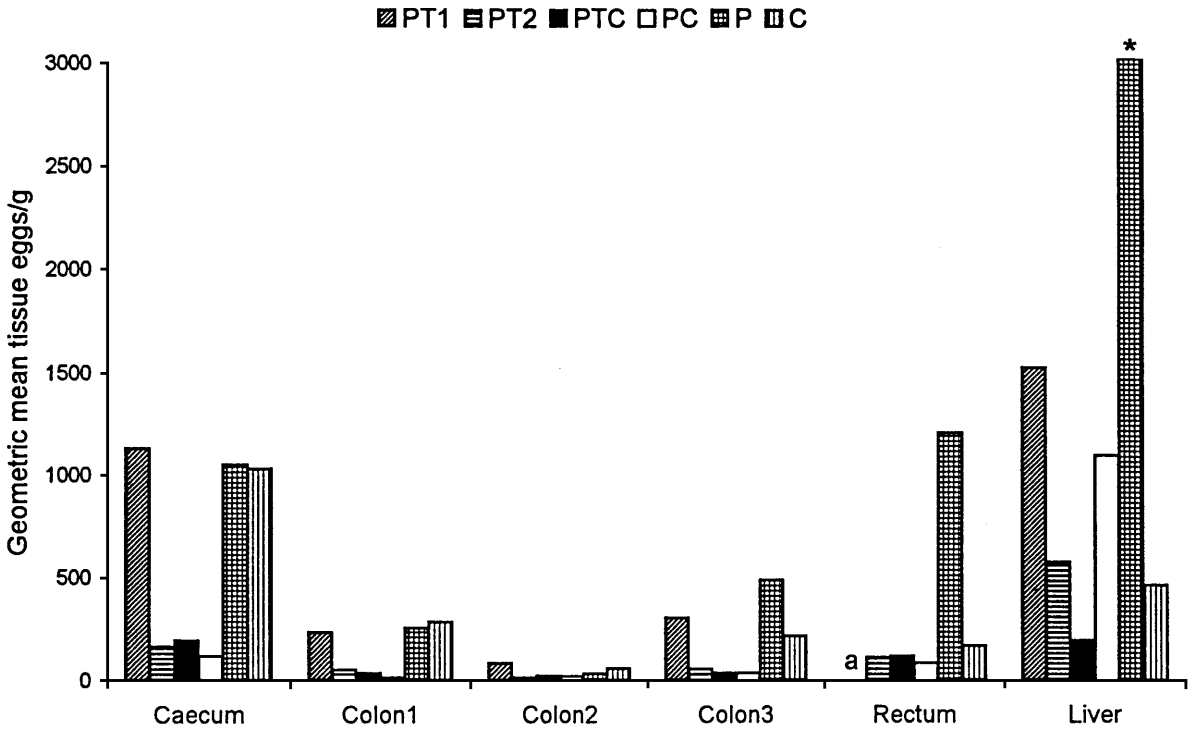

Fig. 2. Geometric mean tissue egg counts from pigs experimentally infected with *Schistosoma japonicum* and treated with praziquantel. P, primary infection; T, treatment; 1, pigs killed 4 weeks post+treatment; 2, pigs killed 14 weeks post-treatment; C, challenge infection 12 weeks post-primary infection. * Significantly higher than PTC-group in the liver. a, No samples collected.

group and the PT2-group were comparable to the number of nodules found in the other groups. In the PTC-group no live worms were recovered except in 1 pig where 2 adult males were found. Equal numbers of worms and worm nodules were recovered in the PC-group and the P-group but immature worms were found only in the PC-group. Significantly more adult males and females were found in the C-group as compared to the treated groups ($P < 0{\cdot}05$). Although the mean number of

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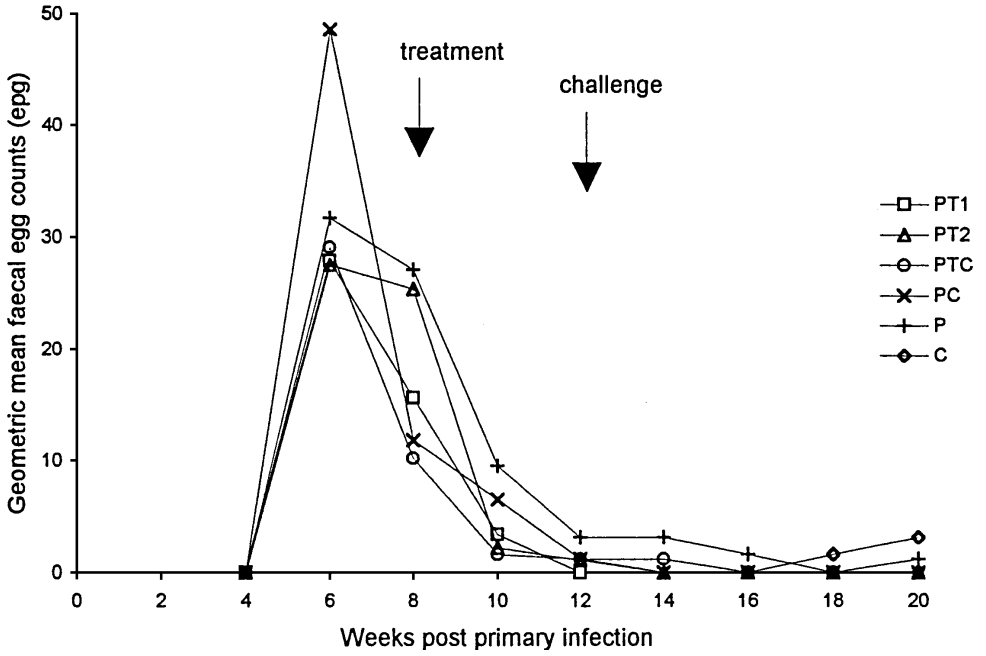

Fig. 3. Geometric mean faecal counts from pigs experimentally infected with *Schistosoma japonicum* and treated with praziquantel. P, primary infection; T, treatment; 1, pigs killed 4 weeks post-treatment (no faecal samples from week 14 onwards); 2, pigs killed 14 weeks post-treatment; C, challenge infection 12 weeks post-primary infection.

immature worms was much higher in the C-group as compared to the other groups this was not significant due to large individual variations ($P = 0{\cdot}1$).

Tissue egg counts

Fig. 2 shows mean tissue egg counts per gram (epg) of tissue from the intestines and the liver. Eggs were predominantly located in the liver and caecum in all groups. Although reductions were observed in the tissue egg counts in the examined sections following treatment, comparing the PT1-group to the C-group and the PT2-group to the P-group respectively, they were not statistically significantly different ($P > 0{\cdot}05$). The liver egg counts of P-group was very high as compared to the other groups, but it was only significantly higher than the PTC-group ($P < 0{\cdot}01$).

Faecal egg counts and hatchability

Faecal egg excretion of the groups of infected pigs are presented in Fig. 3. In all primary infected groups faecal egg excretion started between week 4 and week 6 post-infection (p.i.), peaked at week 6 and declined markedly to week 12 p.i. The faecal egg excretion declined more steeply in the treated groups than in the non-treated groups. Faecal egg count reduction following treatment was 56 % 2 weeks and 82 % 4 weeks post-treatment, the difference between the groups being highly significant ($P < 0{\cdot}01$) at both times. Only 1 of the 15 treated pigs excreted eggs at 4 and 6 weeks post-treatment, but at a very low level (1 epg). Challenge infection of the treated group (PTC-group) did not result in excretion of any eggs neither 6 nor 8 weeks post-challenge. The faecal egg excretion of the C-group remained at a very low level 6 and 8 weeks post-challenge and was not significantly different from the other groups ($P > 0{\cdot}05$).

Miracidial hatching of faecal eggs was observed at 1 and 5 days but not 13 days after treatment in the PTC-group as compared to the PC-group where the hatchability did not change during the test period (data not shown).

Pathology

Gross pathological lesions of the liver were found in all infected pigs. They were characterized by disseminated small firm grey–white nodules accompanied by multifocal or generalized interlobular connective tissue to various degrees. No worm nodules were found in any of the pig livers. Severe liver fibrosis was found in all challenge-infected non-treated pigs, but was additionally found in 2 out of 5 pigs in the PT1-, the PTC- and the P-group. All other infected pigs had mild to moderate fibrosis with least affections found in PT2-group. The livers from the pigs in the PT1- and PT2-groups showed no evidence of worm-induced lesions. Periportal and septal fibrosis were found in all infected pigs independent of the praziquantel treatment. Most severe fibrosis was found in the C-group whereas PT2- and PTC-groups had less pronounced fibrosis.

DISCUSSION

The present study showed that praziquantel was 100 % effective against *Schistosoma japonicum* in pigs at 40 mg/kg when treated 8 weeks after a primary

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infection. As no worms were found either at 4 or at 14 weeks after treatment it appears that praziquantel was additionally effective against immature worms which, for comparison, were found in high numbers in the C-group after 10 weeks of infection. This is somewhat different from the observations made for *S. bovis* in goats (Johansen *et al*. 1996*a*) and *S. mansoni* in mice (Andrews, 1981; Shaw, 1990). However, worm nodules were still observed in the intestinal wall of the pigs 14 weeks after treatment in numbers comparable to nodules in the infected non-treated pigs.

Treatment did not significantly reduce the number of *S. japonicum* eggs in the intestinal mucosa or in the liver up to 14 weeks after treatment. This stands in contrast to the observations seen in *S. bovis* infected and treated goats, where the tissue egg count reduction was highly significant 4 weeks post-treatment as compared to infected non-treated goats (Johansen *et al*. 1996*a*). The reason for the low elimination rate in the pigs in the present study remains an open question.

Neither worm nodules nor worm-induced lesions were found in the liver of any of the pigs, liver enzymes remained at control levels after treatment, and nor furthermore did the treatment reduce the amount of periportal and septal fibrosis in the organ within 14 weeks. Since no dead worms were found in the livers of the treated animals either at 4 or at 14 weeks after treatment, it might be suggested that either the elimination of the worms occurs very fast in the liver or the treatment does not dislocate the worms to the liver, but rather gives rise to the killing of the worms at their location in the mesenteric veins. However, if the dead worms remain in the mesenteric veins, the number of worm nodules in the treated groups should have been comparable to the total number of worms in the primary group, which was not found. Whether praziquantel treatment accelerates the phagocytosis of the dead worms in the intestinal wall remains unanswered. As for comparison, treatment of goats with *S. bovis* caused a massive inflammatory response to dead worms in the liver, which was still pronounced 4 weeks after treatment (Johansen *et al*. 1996*b*). Mehlhorn *et al*. (1981, 1982) found dislocated *S. mansoni* worms in the liver of praziquantel treated mice causing severe lesions as late as 12 weeks after treatment. Morcos *et al*. (1985) found that treatment with praziquantel of murine *S. mansoni* infection resulted in arrest but only partial reversal of liver fibrosis. This was confirmed by El-Badrawy *et al*. (1991) and Kresina *et al*. (1993) who additionally showed that specific timing of treatment is essential for the reversibility of the liver fibrosis in murine schistosomiasis.

Faecal egg counts were significantly reduced 2 weeks after treatment and at that time hatching of the excreted eggs was no longer possible. For comparison, Huang *et al*. (1992) have reported a 100 % egg-negative rate in 4 *S. japonicum*-infected pigs after treatment with praziquantel at either 30 or 40 mg/kg, but did not indicate how long after treatment the pigs were examined. Bushara *et al*. (1982) reported a near 100 % reduction in faecal egg counts in cattle infected with *S. bovis* and treated with praziquantel, at 20 mg/kg, after 3 weeks, and Markovics *et al*. (1985) observed a 97·6 % reduction after 1 week with a similar set up. In sheep infected with *S. bovis* the reduction was only 75·2 % after 1 week but 96·8 % after the second week (Markovics *et al*. 1985).

In the present study, pigs were almost 100 % resistant to reinfection 4 weeks after treatment as almost no worms established from the challenge infection. It cannot be excluded that the 2 male worms found in 1 pig from the PTC-group originated from the primary infection, but since no worms were found in the PT-groups, it is most likely that the 2 male worms established from the challenge infection. Hence, resistance to reinfection was more likely linked to the large number of tissue eggs present at the time of challenge. For comparison, Moloney, Hinchliffe & Webbe (1987) showed that mice infected with *S. japonicum* had lost their acquired resistance 5 weeks after treatment with praziquantel. In cattle naturally infected with *S. bovis*, treated with praziquantel (20 mg/kg) and challenged with *S. bovis* 7 weeks post-treatment, Bushara *et al*. (1983) found an 85 % reduction in worm counts as compared to challenge controls 16 weeks after challenge. Tawfik & Colley (1986) demonstrated in the *S. mansoni*/mouse model that resistance to reinfection after treatment depends on the duration of the primary infection prior to treatment and length of time between treatment and challenge. Wilkins (1989) reviewed reinfection after treatment of human schistosome infections and concluded that the great variation seen in reinfection rates, ranging from weeks to years, was due to variation between time, places and persons. Similar conclusions were drawn from a Chinese study (Wu *et al*. 1993). However, comparisons to human studies are difficult, since very different approaches have been used in those studies.

In conclusion, this study showed that praziquantel was highly effective against *S. japonicum* in pigs, caused reduction in periportal and septal fibrosis within 14 weeks, reduced faecal egg counts to nearly zero within 2 weeks and prevented establishment of a challenge infection given 4 weeks after treatment.

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