THERAPEUTIC EFFECT OF SUBCURATIVE DOSE PRAZIQUANTEL ON *SCHISTOSOMA MANSONI* INFECTED MICE AND RESISTANCE TO CHALLENGE INFECTION AFTER TREATMENT

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Abstract. The therapeutic effect of a subcurative dosage of praziquantel (PZQ) on *Schistosoma mansoni* infected mice and resistance to challenged worm infection after treatment were assessed and compared with conventional treatment using a curative dosage of PZQ. *S. mansoni* infected mice were treated with PZQ at a curative dosage (600 mg kg^{-1}) or a subcurative dosage (300 mg kg^{-1}) at 9 weeks after infection. Untreated mice and non-infected mice were added as controls. The therapeutic effect of the drug was evaluated in terms of the mortality of mice after treatment, and the parasitological and pathological findings in mice sacrificed at 1 week, 1 month, or 3 months after treatment. Another sample of mice was not killed but challenged with *S. mansoni* cercariae at 1 week, 1 month, or 3 months after treatment. Resistance to re-infection was evaluated by the extent of challenged worm reduction. In conclusion, there was no significant difference in mortality, or parasitological and pathological findings between mice treated with PZQ at the two dosages. However, resistance to challenged worm infection was more sustained in the group treated with subcurative dose PZQ, especially at 3 months after treatment.

INTRODUCTION

Recently, schistosomiasis has been successfully treated by using praziquantel (PZQ) (WHO, 1995; Stanley, 2004). However re-infection after curative treatment has occurred due to the continuous contact of host with the cercariae under natural conditions and the loss of resistance of the host after complete elimination of worms, eggs, and egg-associated pathology (Moloney *et al*, 1987; Wilkins, 1989; Giboda *et al,* 1994; Cioli, 1998).

Previous studies on the antischistosomal activities of PZQ were mostly based on a curative dosage of the drug. Nevertheless, the effects of a subcurative or low-dose PZQ were sporadically reported. A single dosage of 200 mg kg^{-1} PZQ caused both reversible and permanent damage on the reproductive organs of female worms (Shaw and Erasmus, 1988). Treatment of *Schistosoma mansoni* infected mice at 5 weeks of infection with a single subcurative dosage of 333 mg kg^{-1} PZQ reduced significantly the number of eggs in the viscera (Botros *et al*, 1989). A single or total dosage of 200 mg kg^{-1} PZQ caused extensive and long-term damage to the tegument of adult *S. mansoni* worms: the surface antigen was exposed and subsequently attached by the host antibody (Shaw, 1990; Fallon *et al*, 1996). Since adult worms were partially susceptible to subcurative dose PZQ, eggs, which are generally accepted as the major cause of pathogenesis, were subsequently reduced. Therefore, treatment of schistosomiasis with subcurative dosages of PZQ is possibly sufficient for reducing egg-associated pathology. However, resistance to re-infection is probably more slowly reduced, compared to treatment with a

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curative dose of PZQ, due to incomplete or partial destruction of worms.

This experiment was performed to investigate the possibility of using a subcurative dosage of PZQ for treatment of schistosome infection and partially sustaining resistance to re-infection after treatment in experimental animals.

MATERIALS AND METHODS

Parasite

The P.R. strain of *S. mansoni* was experimentally maintained in *Biomphalaria glabrata* snails and ICR mice at the Applied Malacology Centre, Faculty of Tropical Medicine, Mahidol University, Thailand.

Mouse and mouse infection

Female, 5-week old outbred ICR mice were obtained from the National Laboratory Animal Center, Thailand and used throughout the experiment. A total of 90 mice were percutaneously infected with 75 *S. mansoni* cercariae using the tail immersion method under anesthesia with pentobarbital sodium. A group of 30 normal mice was added as non-infected controls. All mice were kept under the same conditions and fed *ad libitum*.

Drugs and drug administration

A praziquantel 600 mg tablet (Praquantel™, Atlantic Pharmaceutical, Thailand) was suspended in distilled water at a concentration of 30 mg ml^{-1}. At 9 weeks after infection, all infected mice were randomly divided into 3 equal groups. PZQ was given orally to the first group of mice at a total dosage of 600 mg kg^{-1} (divided into 2 equal doses of 300 mg kg^{-1} given 8 hours apart) and to the second group at single dosage of 300 mg kg^{-1}. The third group of mice were not treated and kept as untreated infected controls.

Mortality

The numbers of mice that died with lesions of schistosomiasis in each group were counted and calculated for percentage of mortality. A final number of 30 mice in each group was achieved by the substitution of dead mice with equal numbers of infected or treated mice. This total number of 30 mice in each group was then equally divided into 2 parts. The first half was used for study on the effects of the drug in terms of parasitological and pathological findings. The other half was used for the study of resistance to re-infection after treatment.

Effects of drug in terms of parasitological and pathological findings

At 1 week, 1 month, and 3 months after treatment, five mice from each group were killed by cervical dislocation. Gross lesions in visceral organs were observed. The livers were divided into 2 portions, weighed, and separately cut into small pieces and placed in heparinized normal saline for 30 minutes. Worms emigrating out of tissue were counted. The numbers of worms in mesentery and lung were similarly examined. The first portion of liver was then fixed in 10% neutral buffered formalin for histopathological examination, while the other portion was frozen at -20°C for tissue egg count. Lesions of hepatomegaly and splenomegaly were determined by comparing the weights of liver and spleen as percentage of body weight. Intestines were weighed and kept at -20°C. Pieces of tissues that consisted of lesions were collected and fixed in 10% neutral buffered formalin.

Frozen samples of liver and intestines were later digested in 4% potassium hydroxide at 37°C for 18 hours. The number of eggs in the digest were counted and then calculated to determine the number of egg per gram of tissue. Formalin-fixed tissues were embedded in paraffin, sectioned at 4 µm thickness, and stained routinely with hematoxylin and eosin. Some slides were studied for more detail by staining using Masson's trichrome method. The diameter of hepatic periovular granuloma was measured.

Resistance to challenged worm infection after treatment

At 1 week, 1 month, and 3 months after treatment, 5 mice from each group were challenged with 150 *S. mansoni* cercariae using the same method as described above. Six days later, they were sacrificed by ether fumigation. The lung recovery assays were done and the numbers of recovered schistosomula were counted. The resistance to re-infection was considered as a percentage of challenged worm reduction that was calculated from the equation:

$$\% R = \frac{C - E}{C} \times 100$$

where % R = Percentage of challenged worm reduction, C = Number of recovered schistosomula in non-immune control group, and E = Number of recovered schistosomula in experimental group.

Statistical analysis

The number of worms, the number of eggs per gram of tissues, weight of liver and spleen as percentage of body weight, and the diameter of the hepatic periovular granuloma were analyzed using Mann-Whitney, Kruskal-Wallis tests and ANOVA. p-values >0.05 were considered to be statistically significant. SPSS 12.0 for Windows, was used in the data analysis.

RESULTS

Mortality

The number of mice that died after treatment and the percentage of mortality in each group are shown in Table 1. Statistical analysis showed no significant difference in the percentages of mortality between groups treated with PZQ at curative and at subcurative dosages (p=0.074).

Worm burden

The mean number of recovered worms is shown in Table 2. No worms were found in any mice after treatment with the curative dose of PZQ at 1-week post-treatment. A few worms were found in mice treated with the subcurative dosage of PZQ at 1 week and 1 month after treatment. However, almost all mice treated with the subcurative dosage of PZQ were free from worms at 3 months after treatment, and the percentage of worm reduction was increased (by 97.78%) to nearly 100%.

Tissue egg count

The mean numbers of eggs per gram of liver and intestines in the groups treated with PZQ at both dosages were significantly and similarly reduced according to time after treatment (Table 3). Tissue-deposited eggs were sporadically observed in liver and intestines at 3 months after treatment with PZQ at both dosages. There was no significant differences in the numbers of eggs per gram of liver between groups treated

Table 1
Mortality of mice after treatment.

Group (n=30)	Number of died mice	Mortality (%)[a]
PZQ (curative dose)	0	0
PZQ (subcurative dose)	1	3.3
Untreated, infected control	8	26.7
Non-infected control	0	0

[a]Mortality (%) = $\frac{\text{Number of mice died after treatment}}{\text{Number of treated mice}} \times 100$

Table 2
Number of worms and their distribution.

Time after treatment	Group (n=5)	No. of mice without living worm	No. of worms					% R[b]
			MSV+HPV[a]		Liver		Total	
			(mean)	(%)	(mean)	(%)	(mean ± SD)	
1 week	PZQ (curative)	5	0	0	0	0	0	100
	PZQ (subcurative)	0	0.2	11.1%	1.6	88.9%	1.8±0.84	90.2
	Untreated, infected control	0	15.4	83.7%	2	16.3%	18.4±2.3	0
1 month	PZQ (curative)	5	0	0	0	0	0	100
	PZQ (subcurative)	1	0.2	11.1%	1.6	88.9%	1.8±1.3	90.8
	Untreated, infected control	0	17	86.7%	2.6	13.3%	19.6±1.67	0
3 months	PZQ (curative)	5	0	0	0	0	0	100
	PZQ (subcurative)	4	0	0%	0.2	100%	0.2±0.45	98.8
	Untreated, infected control	0	14.2	86.6%	2.2	13.4%	16.4±2.3	0

[a]MSV+HPV = Mesenteric vein and hepatic portal vein

[b]%R (Percentage of worm reduction) = $\frac{\text{mean no. of worm in untreated group – mean no. of worm in treated group}}{\text{mean no. of worm in untreated group}} \times 100$

Table 3
Number of eggs per gram of liver and intestines.

Time after treatment	Group (n=5)	No. of eggs in liver (epg)[a]		No. of eggs in intestines (epg)	
		mean ± SD	%R[b]	mean ± SD	%R
1 week	PZQ (curative)	3,684 ± 638.46	44.5	5,546 ± 980.43	21.8
	PZQ (subcurative)	3,530 ± 630.30	46.8	5,420 ± 973.94	23.5
	Untreated, infected control	6,635 ± 908.60	0	7,088 ± 811.77	0
1 month	PZQ (curative)	3,483 ± 691.16	48.5	5,404 ± 217.71	43.1
	PZQ (subcurative)	3,257 ± 666.15	51.8	5,179 ± 829.44	45.5
	Untreated, infected control	6,967 ± 1,280.75	0	9,498 ± 1,493.46	0
3 months	PZQ (curative)	23 ± 31.51	99.6	134 ± 84.217	98.4
	PZQ (subcurative)	164 ± 147.26	97.4	329 ± 347.76	96
	Untreated, infected control	6,216 ± 1,268.54	0	8,208 ± 1,417.92	0

[a]epg = eggs per gram
[b]%R (Percentage of egg reduction) = $\frac{\text{mean no. of egg in untreated group – mean no. of egg in treated group}}{\text{mean no. of egg in untreated group}} \times 100$

Table 4
Weight of liver and spleen as % body weight, and diameter of hepatic periovular granuloma (μm).

Time after treatment	Group (n=5)	Weight of liver as % BWT[a,b] (mean±SD)	Weight of spleen as % BWT (mean±SD)	Diameter of granuloma (mean ± SD, μm)
	PZQ (curative dose)	7.67±0.7	1.26±0.37	315.07±67.46
1 week	PZQ (subcurative dose)	6.59±0.55	0.88±0.19	302.58±59.32
	Untreated, infected control	7.44±0.47	1.02±0.04	314.32±70.21
	Non-infected control	4.84±0.29	0.27±0.05	-
	PZQ (curative dose)	6.51±0.43	0.72±0.1	238.76±65.61
1 month	PZQ (subcurative dose)	6±0.52	0.86±0.2	225.14±49.65
	Untreated, infected control	7.98±0.53	1.13±0.08	254.56±48.25
	Non-infected control	4.55±0.21	0.32±0.01	-
	PZQ (curative dose)	4.76±0.13	0.45±0.11	165.82±40.43
3 months	PZQ (subcurative dose)	4.68±0.72	0.52±0.22	190.41±41.66
	Untreated, infected control	5.90±1.08	0.79±0.12	217.43±45.77
	Non-infected control	4.83±0.38	0.28±0.06	-

[a]Weight of liver or spleen as % body weight = $\frac{\text{Weight of liver or spleen (g)}}{\text{Body weight (g)}} \times 100$

[b]BWT = body weight

with PZQ at curative and subcurative dose at 1 week, 1 month, and 3 months after treatment ($p=0.465$, $p=0.251$, and $p=0.054$, respectively). Statistical analysis also showed no significant differences in numbers of eggs per gram of intestines at the three different times of necropsy ($p=0.347$, $p=0.602$, and $p=0.113$, respectively).

Pathological findings

The number of whitish spots distributed on liver and intestinal walls were significantly reduced after treatment with PZQ at both dosages and rarely found at 3 months after treatment. Enlargements of liver and spleen were gradually reduced over time after treatment (Table 4). In general, there were no significant differences in the sizes of liver and spleen between groups treated with PZQ at curative and subcurative dose at any time of necropsy after treatment ($p>0.076$). Surprisingly, the sizes of both liver and spleen in group treated with a subcurative dose of PZQ were significantly smaller than the

Table 5
Number of schistosomulae in mice after challenge.

Time of challenge	Group (n=5)	No. of schistosomulae (mean ± SD)	%R[a]
1 week after treatment	PZQ (curative)	4.8 ± 2.17	86.9
	PZQ (subcurative)	8.8 ± 1.30	76
	Untreated, infected control	5.6 ± 1.82	82.5
	Non-immunized control	36.6 ± 4.16	0
1 month after treatment	PZQ (curative)	9.8 ± 1.48	72.8
	PZQ (subcurative)	9.6 ± 1.52	73.3
	Untreated, infected control	10.8 ± 0.84	70
	Non-immunized control	36.0 ± 5.1	0
3 months after treatment	PZQ (curative)	29.0 ± 2.92	18.1
	PZQ (subcurative)	21.2 ± 4.55	40.1
	Untreated, infected control	10.6 ± 1.67	70.1
	Non-immunized control	35.4 ± 3.36	0

[a]%R = Percentage of challenged worm reduction $= \frac{C - E}{C} \times 100$

where C = mean no. of schistosomulae in non-immunized control group
E = mean no. of schistosomulae in experimental group

sizes of liver and spleen in the group treated with a curative dose of PZQ at 1 week after treatment (p=0.047 and p=0.047, respectively). At 3 months after treatment, there were no significant differences in the sizes of the liver among the group treated with curative dose of PZQ, the group treated with subcurative dose of PZQ, and the non-infected group (p=0.595). However, statistical analysis showed a significant difference in the size of the spleen at 3 months post-treatment (p=0.016).

Microscopic lesions were similarly observed in the groups treated with PZQ at both dosages. Non-viable eggs in periovular granuloma were commonly found in both groups at 1 week after treatment (Figs 1 and 2). Granuloma surrounding dead worms was commonly observed in both treatment groups. At 1 month after treatment, the hepatic periovular granuloma was much reduced in the number of inflammatory cells, and the major components were dense concentric collagen fibers surrounding non-viable schistosome eggs. Inflammatory cell infiltration in liver and intestinal wall, hepatic congestion, and coagulative necrosis of hepatocytes were not commonly found at 1 month after treatment. At 3 months after treatment, microscopic lesions were rarely observed in the groups treated with PZQ at both dosages. A few small periovular granulomas with egg debris and fibrous scars were sporadically found in liver and intestines. In contrast, the periovular granuloma, with viable eggs and numerous eosinophils, was still commonly found in the untreated group at the same time period.

Diameter of hepatic periovular granuloma

At 1 week after treatment, there were no significant differences in the diameters of the granuloma (p=0.557). The diameters of the hepatic periovular granuloma in all groups were similarly decreased according to time of infection, and there were significant differences among groups at 1 month and 3 months after treatment (p=0.022 and p=0.005, respectively). However, there were no significant differences in the diameters of the granuloma between groups treated with PZQ, at a curative dose and a subcurative dose, at any time of necropsy after treatment (p=0.340, p=0.196 and p=0.157, respectively).

Challenged worm reduction

The number of recovered schistosomulae was consistently highest in the non-immune control group (35.4-36.6 schistosomulae) at any time of challenge (Table 5). In contrast, the number

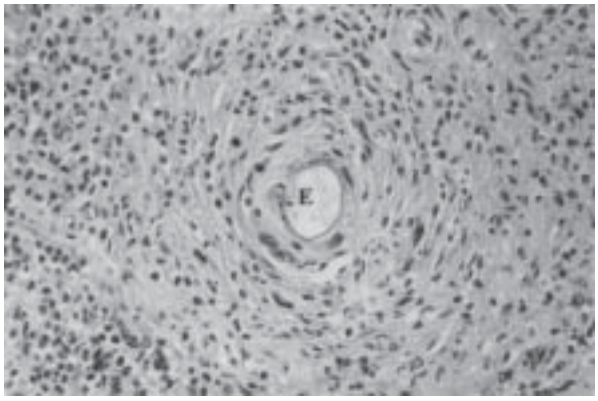

Fig 1–Hepatic periovular granuloma in a mouse at 1 week after treatment with curative dose of PZQ, shows empty non-viable schistosome egg (E) surrounded by inflammatory cells and fibrous tissues (H&E, 200 x).

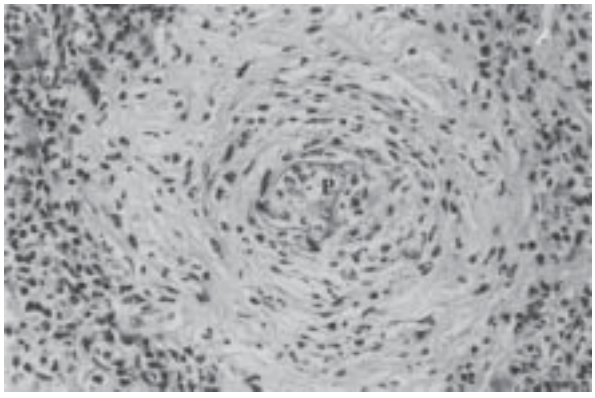

Fig 2–Hepatic periovular granuloma in a mouse at 1 week after treatment with subcurative dose of PZQ, shows phagocytosed non-viable schistosome egg (P) surrounded by inflammatory cells and fibrous tissues (H&E, 200 x).

of schistosomulae was lowest in the untreated and infected, or immunized, control group (5.6-10.8 schistosomulae), and the percentage of challenged worm reduction was constantly high. In the other treated groups, the percentages of challenged worm reduction were decreased according to the drug of treatment and the time after treatment. For the group treated with PZQ at a curative dose, the percentage of challenged worm reduction immediately dropped at 3 months after treatment. It more gradually reduced in the group treated with PZQ at a subcurative dose, and there was a significant difference in number of schistosomulae ($p = 0.009$).

DISCUSSION

Treatment of *S. mansoni* infected mice with subcurative dosage of 300 mg kg^{-1} PZQ effectively reduced parasitological and pathological findings as treatment with curative dose, although all worms were not rapidly killed within 1 week after treatment, and a very low mortality was observed. Previous studies demonstrated that PZQ caused rapid damage to adult worms by causing paralysis and by shifting adult worms from the mesenteric vein to the liver where they were finally destroyed by the phagocytic system. PZQ also caused damage to the worm tegument. A consequence of this damage is the exposure of tegumental antigens, which in turn become accessible to the parasite-specific antibody within 1 hour (Fallon *et al*, 1995). The drug-immune synergistic effect similarly occurred in mice treated with a single dosage of 200-300 mg kg^{-1} PZQ (Shaw and Erasmus, 1988; Shaw, 1990; Fallon *et al*, 1996). Therefore, a subcurative dose of PZQ might be sufficient for the slow killing of adult worms by drug-immunity synergy.

The mature egg is also destroyed by PZQ. The action of the drug on eggs *in vivo* is to stimulate hatching, which is followed by the death of the miracidium. However, an immature egg is not damaged, but develops into a miracidium that later degenerates into a granulated and calcified egg (Matsuda *et al*, 1983; Giboda *et al*, 1994). The direct effect of a subcurative dose of PZQ on tissue-deposited eggs has not been previously reported. However, the observation of non-viable eggs in the livers in the present study, at 1 week post-treatment, with a subcurative dose of PZQ, which is similar to a curative dose of PZQ, might indicate that 300 mg kg^{-1} of PZQ was sufficient to damage the mature egg. This finding might be useful for the treatment of schistosomiasis because the egg is generally accepted as the major cause of the pathogenesis of schistosomiasis.

The results of this present study demonstrated that gross and microscopic lesions, the numbers of tissue-deposited eggs, and the diameters of hepatic periovular granuloma were significantly reduced after treatment with PZQ at both dosages. However, resistance to chal-

lenged worm infection was more maintained at 3 months post-treatment with subcurative dose, and that might have resulted from the slower disappearance of adult worms and their associated antigen. The schistosomula stage is also vulnerable to host immunity (Terry, 1994; Ridi *et al*, 2001). In our study, we did not compare the immune response to *S. mansoni* antigen in infected mice treated with both dosages to demonstrate the resistance of mice in subcurative dose. However, it is possible that the treatment of schistosomiasis mansoni with a subcurative dose, not only causes reduction in life-threatening pathology, but also partially maintains resistance to re-infection. However, the disadvantage of using a subcurative dose of an anthelmintic drug should be carefully considered, especially regarding drug resistance (Fallon and Doenhoeff, 1994).

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