

Effect of Praziquantel on Hatching of *Schistosoma haematobium* Eggs

Tatsuya KATSUMATA¹, D.K. MIGWI², N.D. MUHOHO²,
Eisaku KIMURA¹, Masaaki SHIMADA¹ and Yoshiki AOKI¹

¹Department of Parasitology, Institute of Tropical Medicine, Nagasaki University,
1-12-4 Sakamoto, Nagasaki 852, Japan

²Centre for Microbiology Research, Kenya Medical Research Institute, P.O. Box
54840, Nairobi, Kenya

Abstract: The hatchability of *Schistosoma haematobium* eggs excreted by patients treated with praziquantel (PZQ) was examined. The rate of hatching of the eggs excreted by the patients before treatment was 90.1%. The rates of hatching of the eggs excreted by the patients 3 hr, 24 hr and 48 hr after treatment were 25.5%, 19.2% and 29.9% respectively. In vivo PZQ inhibits the hatching of *S. haematobium* eggs. The result of in vivo experiment was strongly supported by the results of the in vitro experiments. The hatching of the eggs exposed to PZQ at a concentration of 0.1 µg/ml for one hour in vitro was inhibited.

Key words: *Schistosoma haematobium*, Egg, Hatching, Inhibition, Praziquantel

INTRODUCTION

Praziquantel (PZQ) is a potent antischistosomal drug. The widespread use of PZQ reduces contamination of the environment with schistosome eggs, thus reducing transmission; in addition it reduces and prevents morbidity (WHO, 1977). The egg production of adult female worms ceased immediately after treatment with PZQ (Andrew, 1981). However, the eggs were excreted for months after successful treatment (McMahon and Kolstrup, 1979; Doehring *et al.*, 1985). It is, therefore, obligatory subject to measure ovicidal effect of PZQ.

Since 1981, a research programme on urinary schistosomiasis has been carried out at Mwachinga village, Kwale, Kenya, under the joint sponsorship of the Kenya Government and the Japan International Cooperation Agency. The selective mass-chemotherapy with PZQ was attempted on July 1986. The present study was designed to examine the hatchability of *S. haematobium* eggs which were excreted by the patients treated with PZQ and those of eggs exposed to PZQ *in vitro*.

MATERIALS AND METHODS

Hatching of eggs excreted by patients treated with PZQ:

The subjects enrolled in this study were 20 schoolchildren of Mwachinga village between 10 and 13 years of age whose intensity of infection was moderate to severe. The schoolchildren received a single oral dose of 40mg/kg body weight of PZQ at noon. The urine was collected one hour before treatment, 3hr, 24 hr and 48hr after treatment. The hatchability of the eggs was examined immediately after collection of urine. Urine was filtered through a nuclepore membrane with 12 μ m porosity and 25 mm in diameter so as to leave 200 and more eggs on the membrane. Volume of urine filtered was then recorded. The membrane was then soaked in a small bottle filled with 4 ml of distilled water and left in day light for one hour. The hatching of the eggs was stopped by the addition of 1 ml of 10% formaline solution. The number of miracidium and unhatched eggs were then counted. The number of eggs excreted by a patient was expressed by egg counts/ 10 ml of urine.

Hatching of eggs exposed to PZQ *in vitro*:

S. haematobium eggs were obtained from the schoolchildren at Mtsangatam Primary School. Urine was filtered through a series of the meshes with different pore size (Nos. 5.5, 16 and 282). The eggs thus collected were washed repeatedly with Dulbecco's (-) phosphate-buffered saline (PBS), and incubated in PBS containing various concentrations of PZQ for different periods of time. The eggs then exposed to water after complete removal of PZQ solution. The hatching of the eggs was stopped by the addition of 10% formaline solution. The number of micracidia and unhatched eggs were then counted.

RESULTS

Hatching of eggs excreted by patients treated with PZQ:

The geometric mean of the number of eggs excreted by 20 schoolchildren before treatment was 537 ± 4 eggs/10 ml of urine. Those of the eggs excreted 3 hr, 24 hr, and 48 hr after treatment were 759 ± 7 , 246 ± 3 and 363 ± 3 eggs/10 ml of urine respectively. There was no significant difference in the egg output during the initial 48 hr after treatment (Scheffe's test).

When placed in water, the eggs of *S. haematobium* easily hatch. The rates of the hatching of eggs excreted by each of 20 schoolchildren before treatment varied from 76.3% to 97.6%, with an average of 90.1%. The hatching rates of eggs excreted 3 hr, 24 hr and 48 hr after treatment were 25.5%, 19.2% and 29.9% respectively. Fig 1. represents the change in the percentages of hatching of the eggs excreted by each of 20 schoolchildren during the initial 48 hr after treatment. Although individual variations exist, a definite pattern emerged from the results is that hatching tends to decrease during the initial 24 hr after treatment and increase slightly 48 hr after PZQ treatment.

Hatching of eggs exposed to PZQ *in vitro*:

Table 1 shows the hatchability of the eggs exposed to various concentrations of PZQ for different periods of time. The hatching rates of the eggs exposed to a high concentration of PZQ (10 $\mu\text{g}/\text{ml}$) for 10 minutes or more were lower than that of control eggs (chi square, $p < 0.01$). PZQ-inhibited hatching was depend on the concentration of PZQ and period of exposure to PZQ. PZQ, at a concentration of 0.01 $\mu\text{g}/\text{ml}$, did not affect the hatchability of the eggs.

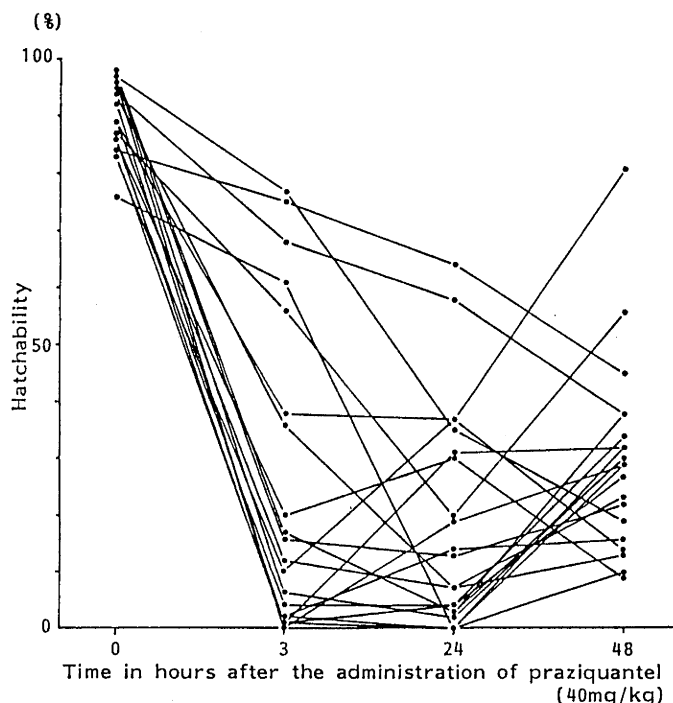


Fig. 1. Hatching of eggs excreted by patients during initial 48 hr after administration of praziquantel.

Table 1. Hatching of eggs exposed to various concentrations of praziquantel for different periods of time *in vitro*

Incubation Period in PBS containing Praziquantel (min)	Concentration of Praziquantel ($\mu\text{g}/\text{ml}$)				
	0	0.01	0.1	1	10
	M/M+UE	M/M+UE	M/M+UE	M/M+UE	M/M+UE
10	96/238 (40.3)	130/304 (42.8)	129/289 (44.6)	124/354 (35.0)	90/359 (25.1)*
30	225/473 (47.6)	127/294 (43.2)	108/261 (41.4)	151/449 (33.6)*	41/145 (28.3)*
60	80/214 (37.4)	76/218 (34.9)	34/188 (18.1)*	62/260 (23.8)*	36/226 (15.9)*
120	116/300 (38.7)	130/331 (39.3)	70/388 (18.0)*	48/306 (15.7)*	19/253 (7.5)*
360	93/206 (45.1)	136/272 (50.0)	56/335 (16.7)*	57/307 (18.6)*	25/274 (9.1)*

Eggs were pre-incubated in PZQ solution. After complete removal of PZQ by repeated washing, eggs were allowed to hatch in water.

M: miracidium, UE: unhatched eggs, (): % hatched

* Differences were statistically significant (chi square, $p < 0.01$).

DISCUSSION

Since ova of *S. haematobium* take several days to pass through the bladder wall into urine (Belding, 1965), the eggs examined in this study were evidently those which had already been deposited in the venules of the bladder at the time of treatment. The present study, therefore, was successfully designed to examine the effect of PZQ on the eggs of *S. haematobium in vivo*. The present study revealed that the hatching of eggs excreted after treatment was inhibited. It is a growing belief that active miracidia is responsible for breakage of the eggshell (Samuelson *et al.*, 1984; Katsumata *et al.*, 1988; Katsumata *et al.*, 1989). It would appear that PZQ has injurious effects upon the miracidia enclosed within the eggshell *in vivo*.

When a single oral dose of 40 mg/kg body weight of PZQ was given the healthy volunteers, the maximum plasma concentration of PZQ reached 0.7 µg/ml 3 hr later, and the high plasma concentration of PZQ (over 0.1 µg/ml) was observed for 24 hr after administration of PZQ (Mandour *et al.*, 1990). The eggs examined in our study, therefore, were probably exposed to the high concentration of PZQ (over 0.1 µg/ml) for several hours. The result of *in vivo* study was strongly supported by the results of *in vitro* experiments. When the eggs were incubated in PBS containing PZQ at a concentration of 0.1 µg/ml for one hour, the hatching of the eggs was inhibited.

Andrew (1978) reported the fact that *in vivo* PZQ inhibited hatching of *S. mansoni* miracidia for 24 hr after administration of 500 mg/kg to infected mice. Interestingly enough, the eggs passed after 48 hr hatched normally and were infective to *Biomphalaria glabrata* snails. In our study, the hatchability of the eggs excreted by patients 48 hr after treatment with PZQ was likely to recover partially. PZQ-inhibited hatching of the eggs of *S. haematobium* may be temporary in man as in a case of *S. mansoni*-mouse model, the inhibitory action of PZQ may be longer in man than in mice though.

It has been well known that the patients with urinary schistosomiasis excretes eggs for several months after treatment (McMahon and Kolstrup, 1979). Doehring *et al.* (1985) reported that many viable eggs were excreted by the patients during the initial 5 days after treatment with PZQ, the hatchability of the eggs was not examined though. The present study revealed that the hatchability of the eggs excreted by the patients treated with PZQ was inhibited, even if the inhibitory action of PZQ would be temporary. The majority of the patients treated with PZQ can be expected to contribute little to transmission of disease immediately after treatment with PZQ.

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