

CONCENTRATION TIME COURSE OF PRAZIQUANTEL IN FILIPINOS WITH MILD *SCHISTOSOMA JAPONICUM* INFECTION

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Abstract. Despite extensive use of praziquantel, the current drug of choice for the treatment of schistosomiasis and other helminthic infections, little information is available about its pharmacokinetics in individuals living in geographic areas where such infections are endemic. We investigated the pharmacokinetics of praziquantel by determining its serum concentration-time course in four selected Filipino volunteers with mild *Schistosoma japonicum* infection who lived in an endemic area in the Southern Philippines. At specified intervals during a 24-hour time period after a single oral dose of praziquantel (25 mg/kg BW), intravenous samples of blood were drawn, processed and analyzed for praziquantel using reverse phase high pressure liquid chromatography. The same study was repeated one week later to assess pharmacokinetic reproducibility. A third study, simulating current field practice, consisted of dosing the patient four hours apart and analyzing for praziquantel in serial blood samples drawn at specified time intervals after the first and second dose. The following results were obtained:

- 1) Serum concentration-time course of praziquantel was reproducible for each patient but varied from patient to patient.
- 2) Praziquantel was rapidly absorbed in the gastrointestinal tract as measurable amounts appeared in the blood as early as 15 minutes after dosing. Time to peak serum concentration ranged from 1.50 to 6.00 hours with almost complete elimination from blood by 24 hours whether it was administered as a single dose (1×25 mg/kg BW) or as a twice a day dose (2×25 mg/kg BW) 4 hours apart. Half-life values ranged from 1.00 to 2.50 hours.
- 3) The highest praziquantel concentration attained was 1.20 ng/ml serum after a single dose and 1.80 ng/ml serum during the twice a day regimen. Using the twice a day dosing regimen, a significant drop in praziquantel level after the first dose was noted by the fourth hours, supporting the current therapeutic field practice of administering two doses of praziquantel to patients four hours apart.
- 4) *Schistosoma* ova were completely eliminated from stools of infected patients after treatment. No clinical adverse effects or changes in hematological, liver and renal function tests were observed.

INTRODUCTION

Praziquantel is a pyrazinoisoquinoline drug with efficacy against all species of schistosomes infecting man as well as activity against cestodes (Pearson and Guerrant, 1983; Gonnert and Andrews, 1977). It is the drug of choice for both individual and mass chemotherapy of schistosomiasis in the Philippines as well as in other countries endemic for *Schistosoma Japonicum*. Distinct advantages of praziquantel include high therapeutic efficacy after oral administration, low toxicity and a one-day treatment regimen. Previously

available antischistosomal drugs usually had to be given parenterally, were found to be too toxic, eg antimony potassium tartrate, or if given orally, like niridazole, had to be taken for a period of one week.

Previous clinical pharmacologic studies of praziquantel using doses that varied from 20 to 75 mg/kg body weight and from 14 to 44 mg/kg were limited to studies in normal German volunteers (Leopold *et al*, 1978; Buhring *et al*, 1978). This investigation is different from previous studies as it was done in four mildly infected Filipino

patients who were selected using stringent criteria. The patients had to have essentially normal physical and laboratory exams and be asymptomatic except for the presence of mild *Schistosoma japonicum* infection (1 - 100 eggs/g stool). They were examined and recruited from an endemic area in Leyte Province, transported to Manila and admitted to a government research hospital for the duration of the study.

MATERIALS AND METHODS

The objective of the study was to determine the concentration-time course of unchanged praziquantel and its reproducibility in Filipino patients after an oral dose of 25 mg/kg body weight. The dose selected was that being used in field trials of praziquantel in endemic areas in the Philippines. At specified time intervals after dosing, samples of blood were drawn and analyzed using the high pressure liquid chromatographic method (Xiao *et al*, 1983).

The study was composed of three stages:

1) The field stage consisted of recruitment and initial screening of patients with the cooperation of municipal health officers in 3 endemic rural communities of Leyte namely, Barugo, Jaro, and San Miguel.

2) The clinical stage consisted of selection of the final 4 patients after screening about 50 patients. The four patients selected were transported by boat to Manila where they were admitted to the Research Institute for Tropical Medicine (RITM).

3) The laboratory stage consisted of processing of the blood samples collected in the hospital and frozen at -20°C for subsequent praziquantel analysis.

Field stage

The criteria for selection of the final four patients were as follows:

- a. Must be between 16 and 50 years of age;
- b. A stool examination positive for *S. japonicum* (1-100 ova/g of stool) by the modified thick smear technique with or without concomitant ascariis, hookworm, and *Trichuris* infections;
- c. normal physical examination and normal

laboratory tests assessing hematologic status, liver function, and renal function as well as a normal chest x-ray;

d. no history of treatment with any anti-schistosomal drug such as stibophen, niridazole and 2/ praziquantel;

e. no history of excessive smoking, excessive alcohol intake, or drug intake for three months prior to the study.

All patients under consideration were placed under the care of their respective municipal health officers or village physicians until the final selection was made. The patients and their parents were informed about the objective of the study. Informed consent forms were signed by both the patients and their parents if appropriate, before the patients were transported by boat from their home province of Leyte to Manila. The rest of the patients who participated in the screening process but were not selected were treated with two oral doses of praziquantel (25 mg/kg BW) by the municipal health officers.

Clinical stage

Four patients, all single (2 females NC and MP, ages 18 and 19 respectively, and 2 males FS and NB, ages 22 and 25 respectively) were entered into the study. Their weights ranged from 45 kg (FS) to 71 kg (NB) and heights from 147 cm (NC and FS) to 170 cm (NB). Upon arrival in Manila, they were taken to the RITM hospital in Alabang, Metro Manila (50 km southeast of Manila) and confined for a period of one month. The first week was a period of acclimatization to the hospital and its surroundings including hospital diet. The first part of the pharmacokinetic experiment was done during the second week of hospitalization. A single oral dose of 25 mg/kg BW of praziquantel was administered to each of the patients with orange juice after an overnight fast. A pre-treatment blood sample and serial blood samples at different time intervals after dosing were collected through an intravenous line. The same study was repeated during the third week of hospitalization. During the fourth and last week, the patients were given two oral doses of praziquantel (25 mg/kg BW) four hours apart in order to simulate current chemotherapeutic practice in the endemic areas of Leyte. Stool examinations taken at weekly intervals were consistently negative for *S. japonicum* eggs after the first dose of praziquantel given

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during the second week of hospitalization.

The blood samples were allowed to clot at 0°C and centrifuged to separate the serum from cells. The sera were stored at -20°C until analysis.

All four patients had mild *S. japonicum* infection with egg counts ranging from 1 - 100 eggs/g stool. NB and MP had 46 eggs/g stool whereas NC and FS had 92 eggs/g stool. In addition, all had mild *Trichuris trichiura* infection while MP and FS had mild hookworm and moderate ascariis infections all less than 100 eggs/g stool.

Prior to receiving praziquantel the patients were fasted overnight before each experiment. They were then each given a glass of orange juice along with the calculated dose of praziquantel. Each clinical study took 24 hours. Patients were served the regular hospital lunch (approximately 6 hours after dosing) and supper (approximately 12 hours after dosing) which consisted of a cup of rice, vegetable soup, a slice of meat, a piece of fish and fresh fruit). The patients consumed the meals over an average of 30 minutes.

In the first experiment (A), each patient was orally dosed once with 25 mg/kg BW praziquantel (Fig 1). Blood samples each measuring 8 ml were collected at zero time (just before dosing) and at 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, and 24.00 hours. The second experiment (B), which was a repeat of the first experiment was done during the third week (Fig 2). The third experiment (C) was conducted during the fourth and last week of hospital confinement. During this period, each of the patients were given 2 doses of praziquantel orally (25 mg/kg BW) at four hours apart. Blood samples were collected at zero time, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 4.25, 4.50, 4.75, 5.00, 5.50, 7.00, 8.00, 10.00, 12.00, 14.00, 16.00, and 24 hours. Data on time to peak values in terms of hours from the three experiments are shown in Table 1.

No adverse effects were experienced by any of the patients after each dose of praziquantel. Stool examinations were done at the end of the second, third and fourth weeks. While eggs of intestinal nematodes were still present, *S. japonicum* eggs were eradicated from stools of each patient. Before departure of the patients for their home province of Leyte, they received treatment with 100 mg mebendazole (Antiox) twice a day for three days.

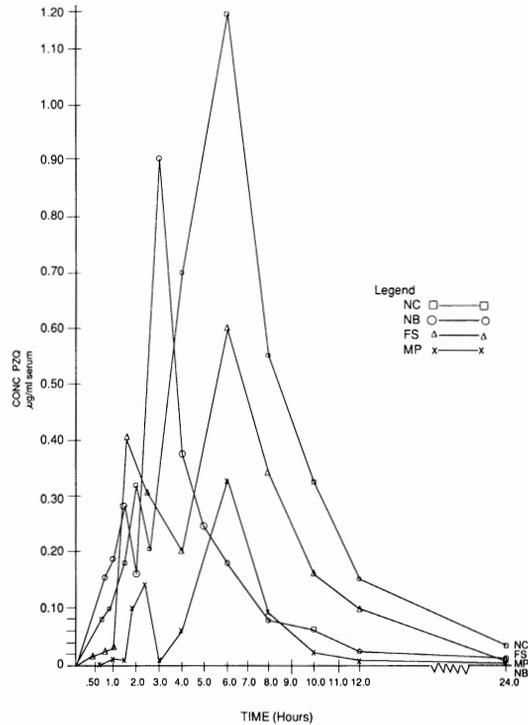


Fig 1—Concentration-time curve of PZQ with once-a-day dosing (A Experiment)

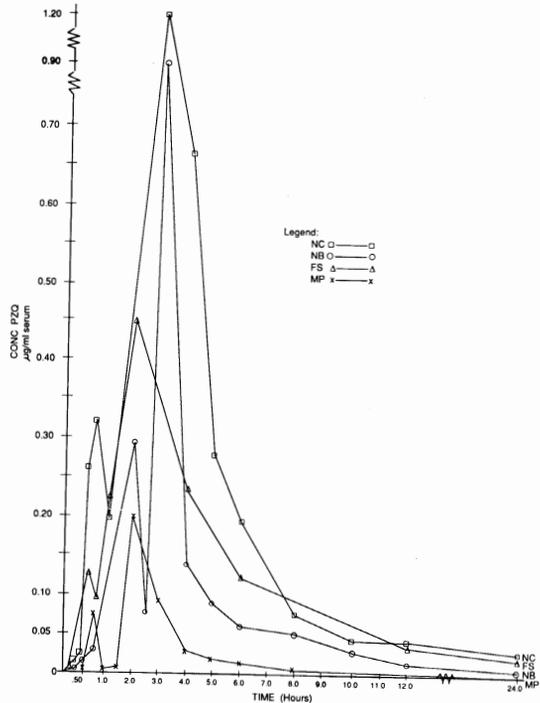


Fig 2—Concentration-time curve of PZQ with once-a-day dosing (B Experiment)

Table 1
Time to peak values in hours.

Sequence of experiments	NC	MP	FS	NB
First experiment (A)	6.0	6.0	6.0	6.0
Second experiment (B)	3.0	2.0	2.0	3.0
Third experiment (C)				
after first dosing	3.0	1.5	1.5	2.5
after second dosing	6.0	5.5	5.5	6.0

Follow-up stool examinations one month after their return to Leyte showed that their stools were free of all intestinal helminths and schistosome eggs.

Laboratory stage

Serum Assay: Standards and Solvents:

1. 40% aqueous acetonitrile
2. water-saturated ethyl acetate
3. standard praziquantel solution, 1.00 mg dissolved in 10 ml of 38% aqueous acetonitrile
4. internal standard : cycloheptyl analog of praziquantel, 0.80 mg, dissolved in 10 ml of 38% aqueous acetonitrile

Both acetonitrile (Burdick and Jackson Labs, Muskegon, MI, USA) and ethyl acetate (Fisher Scientific, Pittsburg, PA, USA) were HPLC grade. The ethyl acetate was washed three times with equal volumes of MilliQ-grade distilled water before use.

Sample treatment

Blood samples were centrifuged at 1,600 g for 10 minutes to separate the supernatant serum from the clotted red blood cells. Two ml of serum was drawn out and transferred to a cryotube (4.5 ml capacity Nunc, Denmark) spiked earlier with internal standard (0.08 µg/ml). The cryotubes were stored at -20°C. Exactly 1.00 ml of serum was drawn out from the cryotube by means of a serological pipette and transferred to a glass tube. The serum was mixed with 2 ml of water-saturated ethyl acetate and shaken. The clear upper organic layer was drawn out and transferred to a 25 ml pear-shaped evaporator flask. This procedure was repeated 3 times and the organic layer drawn out each time was pooled in the same flask. The ethyl acetate extract was dried under vacuum using a

rotary evaporator (Haake Buchler Instruments Inc, Saddle Brook, NJ, USA) and the dried residue was redissolved in 40% aqueous acetonitrile, transferred to a 1.0 ml capped polypropylene vials (Eppendorf, Germany) for storage at -20°C until the time for HPLC analysis.

HPLC determination

Aliquots of 25 µl from these final sample solutions were injected into a High Pressure Liquid Chromatography (HPLC) apparatus (Waters Associates Model 510, Millipore Company, MA, USA) equipped with an ultraviolet detector at 210 nm. Sample runs were compared with standard HPLC runs using pure praziquantel and internal standard (provided by Bayer Philippines from its head office in Leverkusen, Germany) to quantify sample peaks in the chromatogram. The following formula was utilized: $C_2 = (A_2 \times C_1) / (A_1 \times 1.13)$ where C_1 = concentration of internal standard added to serum, C_2 = concentration of PZQ in serum, A_1 = area under the internal standard peak in the HPLC chromatogram, and A_2 = area under the PZQ peak in the HPLC chromatogram.

HPLC operating conditions were as follows:

1. column or stationary phase - radial pak C18 10 cm × 5.0 mm analytical cartridge with precolumn or radial pak insert
2. mobile phase - 40% HPLC grade aqueous acetonitrile
3. spectral region - 210 nm, ultraviolet
4. flow rate - 1.5 ml/minute
5. operating pressure - 700 to 1,700 psi
6. AUFS (absorbance unit full scale) - 0.005 to 0.01 for sample; 0.05 for standard.

RESULTS AND DISCUSSION

A series of representative reverse-phase chromatograms of a standard and a sample run demonstrated a consistent elution time of unchanged praziquantel (PZQ) at 7 minutes and its cycloheptyl analog which served as the internal standard (IS) at 11 minutes. The limit of sensitivity of the HPLC for PZQ detection was 2.5 ng/ml.

It is well known that praziquantel is rapidly absorbed from the gastrointestinal tract into the systemic circulation with an extensive first pass effect after oral administration (Buhning *et al*, 1978). The present study showed that as early as

15 minutes (0.25 hour), PZQ was already measurable in the blood (Fig 1).

The concentration-time curves of the parent drug PZQ obtained from three experiments A, B, and C are shown in Fig 1 - 3. For experiments A and B which involved a once-a-day dosing with PZQ, there seems to be a large interindividual variability in the concentration-time pattern among the four patients within each experiment. This finding agrees with that observed in healthy volunteers given single and multiple doses of oral PZQ using the gas chromatographic analysis (Leopold *et al*, 1978). This was attributed to distinct interindividual variation in "first-pass-effect". The patterns however were fairly consistent for each patient from one experiment to another. The peak values from experiment A were constant at 6.00 hours whereas those from experiment B ranged from 2.00 to 3.00 hours. The reason behind the decrease in time to peak values in experiment B is not clearly understood. However, in experiment C which was a twice-a-day dosing with PZQ there was a common bimodal-looking pattern for each of the patients consisting of two con-

secutive peaks which corresponded to the first and second dosing. Peak values after the first of two doses ranged from 1.50 to 3.00 hours whereas peak values after the second dosing ranged from 5.50 to 6.00 hours (Table 1).

Notably, NC consistently showed the greatest area under the curve (AUC) throughout all 3 experiments followed by NB, FS and MP in consistently decreasing order. Maximum serum concentrations demonstrated by the four patients ranged from 0.075 to 1.20 µg/ml for both experiments A and B and from 0.50 to 1.80 µg/ml for experiment C. A previous study estimated that the oral administration of 46 mg/kg BW yielded a peak level of 1 µg/ml in the serum within 70 - 120 minutes (Patzschke *et al*, 1979).

The steep decline of the curves from their maximum peaks in all experiments indicate a rapid clearance of PZQ from the blood. Within 12 hours of a single dose (A and B experiments), most of the PZQ has been cleared from the blood whereas for the twice-a-day dosing (C experiment), it took 14 to 16 hours for most of the drug to disappear from the blood. This study demonstrated that the elimination of unchanged PZQ is essentially complete within 24 hours whether it was administered as a single dose of 25 mg/kg BW or as a twice-a-day dose (2 × 25 mg/kg BW) given 4 hours apart.

The results of this experiment also suggest that the current practice in endemic areas of giving the second dose of PZQ 4 hours after the first dose is rational. By the fourth hour after the first dose, there was a drastic drop in the PZQ serum level. There seems to be no evidence of PZQ accumulation with a repeat dose. The rapid elimination phase of the concentration-time graphs gave approximately $t_{1/2}$ values ranging from 1.00 to 2.50 hours.

The time to maximum serum concentration of PZQ (2 - 3 hours) followed by the continuous decline to 12 hours and the half-life values of 1.00 - 2.50 hours agree with the results of Patzschke *et al* (1979) done in normal human volunteers. With regards to PZQ half-life, there is also agreement between the results of this study and that of Leopold *et al* (1978) and Westhoff and Blaschke (1992). These observations are consistent with the concept that in both normal volunteers and in mildly infected patients without por-

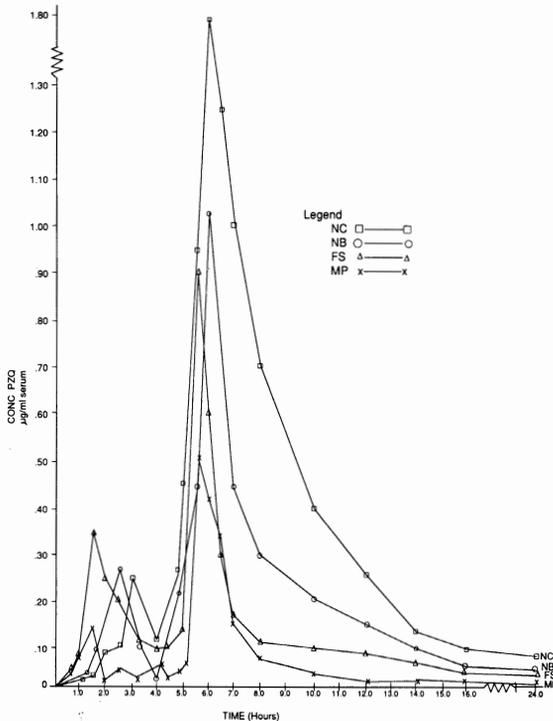


FIG. 4 Concentration - time curve with twice-a-day dosing with PZQ (C Experiment)

Fig 3—Concentration-time curve of PZQ with twice-a-day dosing with PZQ (C Experiment)

tal hypertension or advanced schistosomal disease there is no prolongation of the elimination half-life of PZQ. Watt *et al* (1988) studied the pharmacokinetics of PZQ in *S. japonicum* -infected patients with and without severe liver disease and found an increase in PZQ plasma concentrations with severe disease. Interestingly however the half-life was not significantly prolonged in their study.

Based on HPLC analysis, the concentration-time course of praziquantel after oral administration in mildly schistosomal-infected patients showed that absorption was fairly rapid and complete within 24 hours whether given as a single dose or as a twice-a-day dose.

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