

An *In Vivo* Assay of the Mutagenic Potential of Praziquantel (PZQ) Using Sperm Head Abnormality Test

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ABSTRACT The mutagenic potential of praziquantel (PZQ) 2-(Cyclohexylcarbonyl) – 1, 2, 3, 6, 7, 11b-hexahydro-4H-pyrazino-(2,1-*a*) isoquinolin-4-one, an antihelminthic was evaluated *in vivo* using sperm head abnormality assay of about 15 weeks-old isogenic mice. For 3, 5, 7 weeks of drug exposure period, three different dose levels 0.02; 0.04; and 0.08 (mg/gbw) of PZQ (the calculated equivalence of human therapeutic dose-HTD) were intraperitoneally administered. Observations revealed that PZQ had no significant effect on the body weights of the tested animals. Seven types of sperm head morphological abnormalities in varying degrees were observed. Sperm head abnormality was 5.14%, 5.13% and 5.03% in the control mice and 5.62%, 6.56% and 6.72% in the experimental models for the 3, 5 and 7 weeks exposure period respectively. The abnormality was lower in the control as compared to the experimental mice and this represents a difference of 0.48% (3 weeks); 1.43% (5 weeks) and 1.69% (7 weeks). The difference in the abnormal sperm heads between the control and experimental model is statistically insignificant ($P < 0.05$). The induction was slightly dose-dependent with 0.08mg which was the highest dose used inducing the highest number of sperm head abnormalities for each exposure period. PZQ is probably not mutagenic because the values of aberrant sperm heads that were present in the lower dose levels are close to those of the control. PZQ, therefore, may be a mutagenically safe drug for mass therapy and the control of schistosomiasis in Africa.

INTRODUCTION

Human schistosomiasis, a water-borne parasitic infection caused by two main species of schistosome (blood flukes), namely *Schistosoma mansoni* and *S. haematobium*, is one of the socio-economically important diseases in sub-Saharan Africa (Mafe, 1999). It affects over 200 million people in tropical and sub-tropical regions where it is a hazard particularly to individuals who enter fresh water emanating from irrigation and water resource development schemes (WHO, 1997; TDR, 2000).

Control of Schistosomiasis

The primary objectives of chemotherapy in schistosomiasis control are the reduction and prevention of schistosome-attributable morbidity King et al. (1990), WHO (1993) and Ross et al. (1998). Most recently, however, there has been a global and increasing concern that drugs, as well as environmental chemicals, may be potentially hazardous to mankind by causing gene mutations or chromosomal aberrations (Odeigah, 1997). One major drawback in this situation is that most

parasitic infections often require chemotherapeutic agents in large doses. Thus, these may not be suitable for mass treatment because of toxicity and cost; certain individuals also showed contraindication for the drug and ought to be excluded before mass treatment (Ukoli, 1990 and Mduluzi et al., 2001), therefore over-exposure to such agents is not desirable. However, of the very four compounds available for chemotherapeutic control is the renowned drug praziquantel (PZQ) the most therapeutically efficacious chemical suitable for use on a large scale according to Doenhoff (1987), Hatz et al. (1990), WHO (1995). Most endemic areas of the world, Nigeria inclusive, are characterized by poverty and large teeming populations with few trained medical personnel, as a result of which all medical ailments cannot possibly be brought under direct medical supervision. Furthermore, with increasing awareness of the genotoxic potential of a wide variety of drugs and chemicals, Otubanjo and Mosuro (2001) reported that a large percentage of the populations resort to self-medication with complete ignorance of the correct prescriptions. One cannot completely, then, rule out the possibility of indiscriminate use, and the attendant over-exposure to fake drugs as sold in commuter buses and over-the-counter.

A sizeable number of antiparasitics and

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anthelmintics commonly used as therapeutic agents in certain chronic diseases have been found to exert mutagenic effects on humans. In this present investigation, sperm head abnormality test is employed in carrying out the *in vivo* evaluation of the mutagenic potential of praziquantel (PZQ). This test is one of the quickest, simplest and least expensive methods for identifying mutagens and carcinogens (Wyrobek and Bruce, 1975).

Sperm Morphology Tests

Wyrobek (1983) reported that large reductions in sperm number or motility or large increases in sperm with abnormal shapes are associated with reduced fertility. Sperm tests provide a direct measure of the quality of sperm produced in chemically treated animals such as in the use of formaldehyde (Odeigah, 1997).

Studies in Mice: In the sperm morphology test, assessment of chemical effects on exposed mice is based on visual scoring for the percentage sperm with abnormal head forms and shapes in smears of sperm from epididymis according to the works of Wyrobek and Bruce (1975), Krzanowska (1981), Odeigah (1997).

It is known that during spermatogenesis DNA synthesis occurs before the pre-meiotic phase and no further synthesis occurs throughout the duration of spermatogenesis in the cell cycle. Since once the sperm head develops its shape it becomes extremely stable. Thus, sperm-head morphological abnormalities may be as a consequence of a naturally occurring level of mistakes in the spermatozoon differentiating process and a chemical mutagen might increase the frequency of these mistakes according to the work of Bruce and Heddle (1979). The abnormalities may be as a result of the mistakes made in packaging the genetic material in the sperm head or perhaps as a result of an abnormal chromosome complement. However, it is probable that sperm with abnormal shapes would contain abnormal genetic material according to Wyrobek and Bruce (1978); Otubanjo and Mosuro (2001).

MATERIALS AND METHODS

Isogenic strains of male albino mice (*Mus musculus*) 13-15 weeks old, obtained from the animal breeding unit of the Federal Vaccination Production Laboratory (FVPL) and NAFDAC

collaboration Yaba, were used as experimental models. The mice acquired were quarantined for 2 weeks in the biological garden, small animal colony of the University of Lagos in order to enable the mice acclimatize to their new environment. The mice were divided at random into 4 groups, and all randomly assigned to different dose of the drug under evaluation.

All the mice were housed in conventional plastic cages respectively. These standard cages were bedded with dry wood shavings, which were changed every 2 days to prevent maggoty. They were maintained in the same room throughout the study and sustained on pelleted food (mice cubes) obtained from Ladokun Farms, Ibadan. Body weight of all the mice at the beginning of the drug therapy and at the start of the dissection was recorded. The mean weight of 24g was considered for calculation.

The Drug, Preparation and Administration

This drug was obtained from the public department of the Nigerian Institute of Medical Research (NIMR), Yaba. The required dose was calculated based on the human therapeutic dose (HTD) of 40mg/600mg (Reynolds, 1996) and then dissolved in physiological saline, which was used as the solvent vehicle. Three different dose level treatments were considered: 0.48mg, 0.96mg (as normal dose for the mean mice weight of 24gbwt), 1.92mg corresponding to 0.16ml, 0.32ml and 0.63ml respectively.

Drug Therapy: This was carried out according to the method of Ehling et al. (1968) and Odeigah (1997). The intraperitoneal injection route, as suggested by Wyrobek et al. (1983), was favourably used because it is the fastest and most efficient means of delivery to reach the germ cells even though this may not be accurately compared with human oral treatment. The drug was administered within a week. All the model mice were injected once in a day for 5 days. This was slowly and gently done and their reactions were observed and checked intermittently throughout the injection duration so as to arrest any possible form of metabolic shock. Three different exposure periods were considered namely 3, 5 and 7 weeks from the first day of drug therapy. Three mice were treated for each dose level and each exposure period respectively. Similarly, three mice for each exposure period were treated with the solvent vehicle only as a negative control.

Animal Sacrifice: The animals (mice) were sacrificed euthanasially using local anaesthesia. The anaesthetized mice were quickly dissected to remove the testes and the epididymis.

Sperm Head Abnormality Assay: The sperm cells were sampled 3, 5 and 7 weeks from the last injection so as to coincide with the period of spermatocytes. Spermatogenesis in mice takes about five weeks (35 days) to complete according to Bruce and Heddle (1979).

The caput and cauda epididymis excised from the male mice were placed in a petridish containing 1ml of physiological saline and then minced and teased carefully well with fine scissors and forceps to release the spermatozoa. After gentle pipetting, the suspension is separated from the tissue fragments and to this suspension was added a drop of 1% Eosin Y solution (10: 1) for 30 mins. Air-dried smears were prepared on clean, grease-free glass slides using another clean slide angularly positioned at 30° to spread the drop through the whole length of the slide. The slides were then coded, randomized and examined cytologically under 40x binocular light microscopy. Eight separate slides were prepared for each mouse out of which four were randomly selected for scoring using the tally counter. For each mouse 800 sperm cells were assessed for morphological aberration according to the criteria of Wyrobek and Bruce (1975). The aberrant ones were later photographed with a photomicroscope at 800x magnification.

Statistical Analysis: Differences between the

control and experimental groups were analyzed by means of the Wilson Rank-Sum test or the student's t-test.

RESULTS

Mice Survival: All the mice virtually survived the duration of the experiment.

Sperm Structure and Types of Abnormalities: Seven different forms of sperm head abnormality were observed in the mice during the *in vivo* evaluation of the drug -praziquantel. These include pin head (most prominent), long hook, short hook, ribbon shape, knobbed head, hammer head, and hook at wrong angle.

The pin head sperm abnormality appeared predominantly in both the control and treated models.

Dose-Relation to Frequency of Abnormalities

3 Weeks Exposure Period: The abnormal sperm head recorded was 5.62% out of the total number of sperm scored (Table 1) and this was not significant at $P < 0.05$ level ($t_2 = 11.03$) t_2 tabulated 2.353. The controls show 5.14% abnormality; at the lowest dose level of 0.02mg/ gbw 5.55% of the sperms were abnormal which is even 0.41% higher than the control value.

5 Weeks Exposure Period: For this exposure period, the total abnormality was 6.56% (Table 2) and this was insignificant at $P < 0.05$ level ($t_2 = 3.12$) t_2 tabulated 2.353. The control value was 5.13%, which was lower than all the values of the dose levels.

Table 1: Summary of morphologically abnormal sperm heads induced by different dose levels of pzq in mice after 3 weeks exposure period

S. No.	Dose levels (MG/G.BWT)	Total no. of sperms scored	Total av. of abnormal sperm heads	% Abnormal sperm heads	% Difference from control	t_2
	Control	2400	123.4 $\pm$ 9.7	5.14		
I	0.02	2400	133.3 $\pm$ 10.7	5.55	0.41	
II	0.04	2400	133.9 $\pm$ 7.6	5.58	0.44	
III	0.08	2400	137.4 $\pm$ 7.4	5.73	0.59	
	Total	7200	404.6 $\pm$ 25.7	5.62	0.48	11.03

Table 2: Summary of morphologically abnormal sperm heads induced by different dose levels of pzq in mice after 5 weeks exposure period

S. No.	Dose levels (MG/G.BWT)	Total no. of sperms scored	Total av. of abnormal sperm heads	% Abnormal sperm heads	% Difference from control	t_2
	Control	2400	123.0 $\pm$ 5.9	5.13		
I	0.02	2400	143.8 $\pm$ 11.4	5.99	0.86	
II	0.04	2400	144.0 $\pm$ 13.8	6.00	0.87	
III	0.08	2400	184.4 $\pm$ 9.0	7.68	2.55	
	Total	7200	472.2 $\pm$ 34.2	6.56	1.43	3.12

Table 3: Summary of morphologically abnormal sperm heads induced by different dose levels of pzq in mice after 7 weeks exposure period

S. No.	Dose levels (MG/G.BWT)	Total no. of sperms scored	Total av. of abnormal sperm heads	% Abnormal sperm heads	% Difference from control	t ₂
	Control	2400	120.7± 9.5	5.03		
I	0.02	2400	151.5± 3.7	6.31	1.28	
II	0.04	2400	165.2± 13.4	6.88	1.85	
III	0.08	2400	167.3± 10.8	6.97	1.94	
	Total	7200	484 ± 27.9	6.72	1.69	10.67

The effects of the different dose levels of PZQ were also observed.

7 Weeks Exposure Period: For this exposure duration, 6.72% was the total abnormality and this was not significant at $P < 0.05$ level ($t_2 = 10.67$) t_2 tabulated 2.353.

The effect of different dose levels of PZQ was noted. The control was 5.03% abnormality. The difference in the number of abnormal sperm heads induced in the control and the lowest dose level 0.02mg/g.bwt was not significant at $t, 0.05$ (Table 3).

The relationship between percentage abnormality and different dose levels shows that the percentage of abnormalities induced by 0.08 (mg/gbwt) increases slightly over that of 0.02 and 0.04 (mg/gbwt) during the exposure periods. However, during the exposure periods of 3, 5, and 7 weeks, the mean number of sperm head abnormalities tend to increase with the dosage of the drug.

DISCUSSION

The *in vivo* assay of the drug – Praziquantel using sperm abnormality test recorded the incidence of seven different forms of sperm malformations. The incidence of pinhead sperms is proportionally higher and consistently dominant over all other varying types of sperm head abnormality in the treated mice as well as in the control. This abnormality could have been the result of heads that cut off from the tail during storage and or preparation at the time of experimental procedure. Other types include sperms with long and short hooks, ribbon-like shape, hook at wrong angle, hammer head, and knobbed head. These could be due to the induction of point mutations in the early spermatocytes and spermatogonia at the pre-meiotic stages of spermatogenesis (Hugenholtz and Bruce, 1983). It suggested, therefore, that the sperm cells observed at both the 5 and 7 weeks were presumably exposed to the tested drug. This is because spermatogenesis in mice normally lasts a period of 5 weeks (Bruce and Heddle, 1979).

Taylor (1980) showed that mutation in germ cells prior to or during the reproductive period can be transmitted to later generations resulting in reproductive defects. This may lead to carcinogenicity or teratogenicity in somatic cells. It may also alter a gene so that it contains a wrong code. The results of this present work indicate that test for mutagenicity of PZQ is negative in animal models. Thus, since the PZQ efficacy is unparalleled in comparison with other antischistosomal drugs it is thus safe for mass chemotherapy in schistosomiasis control.

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