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An *in vivo* Evaluation of Induction of Abnormal Sperm Morphology by Ivermectin MSD (Mectizan®)

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Abstract: The *in vivo* effects of orally administered ivermectin (Mectizan®) on sperm head morphology of albino mice were evaluated. Four different dose levels of 0.25, 0.5, 1.0 and 1.5 x the human therapeutic dose of 150 g kg⁻¹ body weight, were administered to the animals. The animals were exposed to a single oral treatment. The sperm of the mice from the cauda epididymes were examined 5 and 7 weeks after treatment. Ivermectin (Mectizan®) induced sperm head abnormalities; however, the induction was not significantly elevated above the negative control value. Furthermore, the induction of the sperm head abnormalities was not strictly dose-dependent and there was also no correlation between dose level of administered drug and incidence of abnormal sperms. This indicates that the drug might not be mutagenic.

Key words: Ivermectin, mectizan, mutagenicity, sperm abnormality, onchocerciasis, *in vivo*

INTRODUCTION

Onchocerciasis or river blindness is a debilitating disease of public health importance in sub-Saharan Africa, being the leading cause of blindness. Ninety five percent of cases of the disease occur in Africa. The main tool in the global control of onchocerciasis is the long term mass annual or bi-annual administration of ivermectin (Mectizan®). The African Programme on Onchocerciasis Control (APOC) is involved in control through community directed treatment (WHO, 1995). The coverage, compliance and sustainability of drug distribution and administration are thus vital to accomplishing the goals of APOC.

Ivermectin is distributed free under the auspices of the giant pharmaceutical company, Merck, Sharp and Dohme. It is a broad spectrum antiparasitic agent of medical and veterinary importance (Campbell *et al.*, 1983; Campbell and Benz, 1983). Treatment is usually administered as a single oral dose given with water. The suggested dose interval for most patients is 12 months; at some sites, it may be preferable to use a 6 month interval depending on such considerations as density or prevalence of skin microfilariae. Certain studies have recommended quarterly or half yearly administration of ivermectin in the hyperendemic communities (Idowu, 2004). The World Health Organization supervises the mass administration of the drug in endemic areas. It is

therefore an issue of interest and consequently desirable that this drug be safe to use due to the widespread exposure to it.

Recently, however, serious side effects and complications in the use of ivermectin have been reported in areas with endemic loaisis (Chippaux *et al.*, 1996; Gardon *et al.*, 1997; Boussinesque *et al.*, 1998). Although adverse events to ivermectin in some communities affect ivermectin compliance (Oyibo and Fagbenro-Beyioku, 2003), community awareness, participation and compliance to ivermectin administration are on the increase.

Ivermectin, a microfilaricide is believed to be a safe, well tolerated and non-toxic, producing mild or transient side effects in the human body (Desole *et al.*, 1989; Alexander *et al.*, 1993). The efficacy and tolerance of ivermectin in onchocerciasis is documented (Aziz *et al.*, 1992). Ivermectin at repeated doses of 0.4 mg kg⁻¹ was found to have no effect on reproduction in cattle, sheep, horses, pigs, dogs and rats. A single 0.4 mg kg⁻¹ dose of ivermectin given to bulls, rams and ewes and 0.6 mg kg⁻¹ given to stallions and boars had no ill-effects on breeding performance or on semen quality (Campbell and Benz, 1983). Ivermectin administered orally at 600 µg kg⁻¹ (0.6 mg kg⁻¹) monthly over 8 treatments had no adverse effects on spermatogenesis, fertility or reproductive performance of Beagle dogs (Daurio *et al.*, 1987).

The effects on embryogenesis have not been observed in higher animals in pre-clinical safety assessment studies. Retrospective studies in Mali of pregnant women accidentally treated with ivermectin produced no malformations, utero mortality and new born mortality (Duombo *et al.*, 1992). Single oral doses of 200 $\mu\text{g kg}^{-1}$ (0.2 mg kg^{-1}), every few months are therefore expected to be relatively safe for both men and women. However, the drug was not recommended for pregnant women, nursing mothers with 1 week old infants and children under 5 years of age because its safety had not been ascertained. Lankas *et al.* (1989) reported the effects of ivermectin on reproduction and neonates in rat.

Most endemic areas of the world, including Nigeria and most of sub Saharan Africa, are characterized by abject poverty and large teeming populations with few trained medical personnel. Consequently, most medical ailments cannot possibly be brought under direct medical supervision. A large percentage of the population resorts to self-medication with complete ignorance of the correct prescriptions (Otubanjo and Mosuro, 2001). One cannot, completely rule out the possibility of indiscriminate use and abuse of drugs and the attendant over exposure to drugs. This long standing indiscriminate use of drugs is indeed a habit in many developing countries; many drugs being purchased without prescription from chemist.

During the past two decades, increasing attention has been directed towards the determination and evaluation of the mutagenic and carcinogenic potentials of drugs even though their useful chemotherapeutic efficacy cannot be underestimated. It is desirable then that these indispensable chemotherapeutic agents are free from any deleterious effects, particularly when the drug is distributed free and also considering its long term proposed usage to reduce transmission and thereby control the disease. The consequence of long term proposed usage of the drug in the control of onchocerciasis and the multiple annual administration of ivermectin in onchocerciasis control need to be evaluated for mutagenicity potential.

In this study, the sperm of ivermectin-treated mice were analyzed to determine whether the drug is mutagenic. An increase in the incidence of abnormal sperm morphology, according to the criteria of Wyrobek and Bruce (1975 and 1978) is indicative of mutagenicity.

MATERIALS AND METHODS

Experimental animals: Male albino mice were obtained from the animal breeding unit of the National Institute of Medical Research, Lagos (NIMR), (3H/HeJ strain). Mice

(12-14 weeks old) were acquired and quarantined in a pathogen-free, well ventilated room in order to enable the mice to acclimatize to their environment and also to avoid the transitory increases in abnormal sperm seen at the onset of mouse spermatogenesis in young mice. The mice were maintained in the same room throughout the study. Only mice of 14 weeks and above were tested. Drinking water and food (pelleted feeds) were supplied *ad libitum*. The study was undertaken in the Parasitology Laboratory at the University of Lagos, Lagos.

Drug: Ivermectin MSD (Mectizan[®]) was supplied by Merck and Co. Inc. The drug was dissolved in distilled water which was used as the solvent vehicle. The drug readily dissolved in distilled water. Doses used in this study were selected according to the Human Therapeutic Dose (HTD) of 150 $\mu\text{g kg}^{-1}$ body weight based on the average human weight of 65 kg.

Assay of sperm abnormalities: Induction of sperm-head abnormalities was tested according to the criteria of Wyrobek and Bruce (1975 and 1978). Four different dose level treatments were considered for the drug. Four doses were used to treat the mice, 1.25, 2.5, 5 and 7.5 μg , corresponding to 0.25, 0.5, 0.1 and 1.5x the human therapeutic dose. The drug was administered as a single oral dose.

A single oral dose was given. Two exposure periods of 5 and 7 weeks from drug treatment were considered. Four mice were treated for each dose level and each exposure period. Two mice, for each exposure period, were treated with the solvent vehicle alone as a negative control. The positive control of the study was 100 mg of methyl methane sulphonate/kg per day, i.p., for a period of 5 days.

Sperms were sampled and analyzed from the cauda epididymes at 5 and 7 weeks following the end of exposure to the 4 different dose levels of the drug. The implication of abnormal sperm heads observed 5 and 7 weeks after drug treatment is that the drug may have had an effect on sperm which had arisen from exposed spermatogonial cells, causing damage to the pre-meiotic stages of spermatogenesis. Thus, the abnormally shaped sperm heads observed for these exposure periods may have been due to induced point mutations in the early spermatocytes and spermatogonia at the pre-meiotic stages of spermatogenesis. This view is in support of those of earlier workers (Bruce *et al.*, 1974; Wyrobek and Bruce, 1975; Soares *et al.*, 1979).

The mice were sacrificed by cervical dislocation. The epididymes were excised and minced with fine scissors in physiological saline. Smears were prepared on clean,

grease-free slides after staining the cells with a mixture of normal saline and 1% eosin-Y (9:1) for 45 min. The slides were air dried and coded for subsequent examination under oil. Cytological evaluation for sperm-head abnormalities was carried out using a binocular microscope at 1000×magnification. Four separate slides were prepared for each mouse, i.e., two for each epididymes out of which two were randomly selected for scoring. The slides were read blinded to treatment. The sperms were assessed for morphological abnormalities of sperm head shape according to the criteria of Wyrobek and Bruce (1975). For each animal, 750 sperms were assessed for morphological damage.

Statistical analysis: Differences between the control and experimental groups were analyzed by means of the student's t-test. The test was considered positive when the frequency of abnormal sperm heads was at least double the negative control level, with $p < 0.05$ as the criterion of significance. Furthermore, the test must have yielded statistically significant increases at a minimum of two consecutive dose levels, be reproducible in separate experiments and finally, show evidence of a dose-related increase in abnormalities.

RESULTS

Analyses of sperm head abnormalities were made 5 and 7 weeks following the end of exposure to 4 different dose levels of ivermectin. Sperms observed at these times were presumably exposed to the drug while they were spermatocytes and spermatogonia. Figure 1 shows normal sperm and the different sperm-head abnormalities recorded from the prepared slides from the treated animals during microscopic study. Photomicrographs of each type of sperm head shape were taken and reproduced. In the course of scoring the abnormalities, it was observed that no specific type of abnormal sperm head was predominant as they all occurred with different frequencies in both treated and control mice.

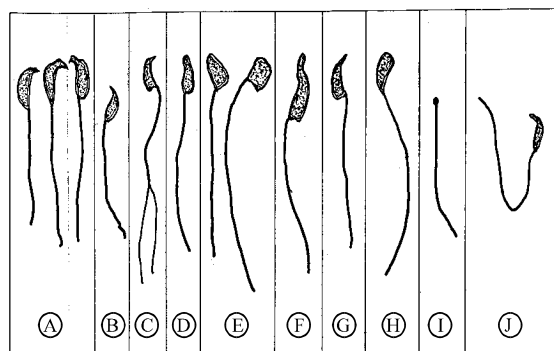


Fig. 1: Observed shapes of abnormal sperm head.

A- sperm with normal head (the morphology of a normal sperm head of a mouse consists of a definite head shape accented by a marked hook, a rectangular mid-piece attachment site and single tail); B- No hook; C- 2-tails; D- knobbed hook; E- Amorphous head; F- Bent hook; G- Hook at wrong angle; H- Tail folded over head; I- Pin-head; J- Banana-shaped head

Table 1 shows the effect of different dose levels of ivermectin on sperm head morphology after 5 and 7 weeks exposure. The negative controls showed 2.53 and 2.30% abnormalities, respectively. The positive control gave a statistically significant elevation of abnormal sperm heads (data not shown). Figure 2 presents the effects of different exposure periods of ivermectin on sperm head abnormality.

Ivermectin did not induce statistically significant increases in sperm-head abnormality over the controls as the criteria for positive response were not satisfied. There was an increase in the frequency of abnormal sperm heads but the increase was not significant at the $p < 0.05$ level. While Fig. 2 shows increase in percentage sperm abnormality with increase drug concentration for the different exposure periods. The range in frequencies of sperm abnormality recorded were 2.40-3.27, 2.53-3.50, 2.67-3.77, 2.97-4.16 for 1.25, 2.5, 5.0 and 7.0 mc g/bwt

Table 1: Effects of ivermectin on sperm morphology in NIMR 3H/HeJ mice after 5 and 7 weeks exposure ^aIvermectin dose

Ivermectin dose (mg kg ⁻¹ body weight)	(xHTD)	Exposure periods (weeks)			
		5 weeks		7 weeks	
		Total abnormality	(%)	Total abnormality	(%)
Control	0.00	76	2.53	70	2.30
1.25	0.25	82	2.73	72	2.40
2.5	0.50	91	3.03	76	2.53
5.0	1.00	107	3.57	58	2.67
7.5	1.50	114	3.80	89	2.97

^aThe percentages are the means for groups of four mice for each point

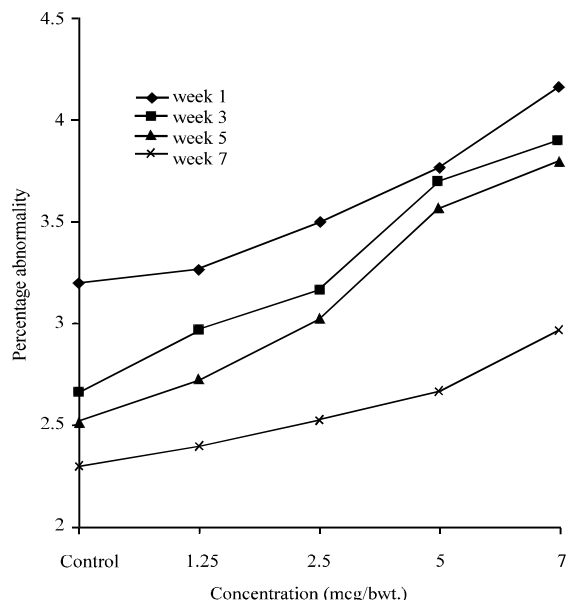


Fig. 2: Relationship between percentage abnormality and different dose levels

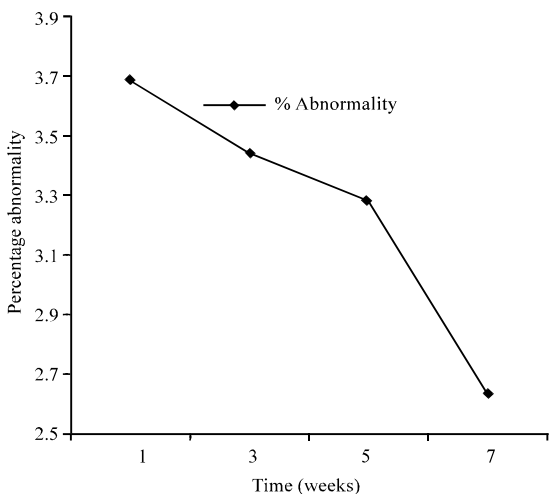


Fig. 3: The effect of different exposure periods if ivermectin on head of mice

dose levels, respectively over the 7 weeks period. Figure 3 shows that abnormality decreased with length of drug exposure.

DISCUSSION

Onchocerciasis afflicts over 17.7 million individuals in sub Saharan Africa, where the disease is endemic (WHO, 1987). In the absence of a viable anti-onchocerciasis vaccine, the only effective means of

prevention and control lies in chemotherapy. Ivermectin is a potent microfilaricide, effective in a single dose therapy and has been widely adopted as the drug of choice to treat and control onchocerciasis. Indeed, control and prevention campaigns in endemic areas of the world involve the free distribution of the drug, annually or bi-annually, to afflicted populations.

Certain percentage of sperm shows abnormal morphology in mice of different strains and age. The frequency of sperm abnormality varies among different inbred strains of mice, being constant for adult mice, with incidence ranging from 2-5% (Soares *et al.*, 1979). Age influences the degree and frequency of abnormality. By the 13th week of age the percentage of morphologically abnormal sperm in mice would have reached the level characteristic of its strain (Krzanoskwa, 1981). The control mice used in the present study showed 2.30-2.53% sperm abnormality.

Spermatogenesis in mice takes about 5 weeks to complete. 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 (Monesi, 1962). Also sperm-head abnormality has been indicated to result from diverse factors which include, errors in the differentiation process during spermateleosis, errors in the packaging of the genetic material, induced mutations in the spermatogenic cells, the occurrence of point mutations during spermatogenesis and exposure to irradiation or chemical mutagens (Bruce *et al.*, 1974; Topham, 1980a, b; Wyrobek *et al.*, 1983).

There are no available evaluation reports on the mutagenicity of ivermectin in mice. In this study, using the sperm-head abnormality test, orally administered ivermectin induced an increase in sperm head abnormalities in albino mice over the negative controls, although the increase was not statistically significant at $p < 0.05$. The criterion for a positive response or mutagenicity is based on evidence of statistically significant occurrence of abnormal sperm at $p < 0.05$. Furthermore, there should be evidence of a dose-related increase in abnormalities and reproducibility in separate experiments. Ivermectin may thus not be considered to be a mutagen as the criteria for mutagenicity were not strictly satisfied.

Although an increase in the frequency of abnormal sperm heads was observed in more than two consecutive dose levels except the highest dose level for both exposure periods, this was not enough to confer a positive response on the drug. The drug may therefore not be adjudged a positive inducer of abnormal sperm heads and may not be mutagenic.

One plausible reason for the inability of ivermectin to have induced statistically significant increases in abnormal sperm in mice is that the testes of the treated animals probably did not accumulate enough concentrations of the drugs, so as to alter the differentiation of the spermatozoa. Ivermectin is readily metabolized and excreted from the body; the maximum duration of plasma concentration of the drug is 6-7 h (Mectizan®-Ivermectin MSD product Monograph). Furthermore, the incidence of sperm head abnormalities tended to decrease with exposure period, as more abnormalities were recorded with the 5 week exposure period than the 7 week exposure period.

There have been no reports of ivermectin binding to DNA, which can ultimately lead to the faulty differentiation of spermatozoa in the testes (Bruce *et al.*, 1974). It may be plausible to suggest that this fact together with the relatively poor tissue retention of the drug and its relatively rapid metabolism and excretion, have resulted in their inability to induce sperm-head abnormalities. However, these negative findings do not preclude the possibility of the occurrence of cytogenetic effects of a different nature.

However, interpretations of findings obtained with the sperm morphology test must be made with caution until all available test systems have confirmed the drug to be non-mutagenic.

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