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An *in vivo* Evaluation of the Induction of Abnormal Sperm Morphology by Sulphamethoxypyridazine: Pyrimethamine (Metakelfin®)

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Abstract: The effects of sulphamethoxypyridazine: Pyrimethamine (Metakelfin®) a combination antimalarial drug on mouse sperm head morphology were evaluated in University of Ibadan Veterinary F_1 mice. Five different dose levels of 3.85:0.19; 7.7:0.38; 15.4:0.76; 23.1:1.13 and 30.8:1.54 mg kg^{-1} body weight of sulphamethoxypridazine:pyrimetamine, respectively were administered to the animals by a schedule of 5 consecutive daily intraperitoneal (i.p.) injections. The sperm of the mice from the cauda epididymis were examined 5 weeks after treatment. Metakelfin® induced sperm head abnormalities; however, of the 5 doses sampled; only the 7.7:0.38 mg kg^{-1} dose, corresponding to 0.5x the human therapeutic dose (HTD0, gave a statistically significant ($p>0.05$) increase over the negative control value of 2.53% abnormality. All the other dose level treatments did not yield statistically significant increase over the negative control value. The 15.4:0.77 mg kg^{-1} dose showed 3.5% abnormality and fewer abnormalities than the preceding lower dose. The drug is probably not mutagenic as induction of sperm head abnormality was not dose ependent.

Key words: Sperm morphology, Metakelfin, antimalarial drug

INTRODUCTION

Malaria continues to be the world's gravest killer disease (Ogutu *et al*., 2000). The most effective current means of malaria prevention and control is chemotherapy using potent antimalarial agents. It is therefore an issue of interest and consequently desirable that these agents be safe to use due to the widespread exposure to the agents in the malaria endemic areas. This is further accentuated by the fact that majority of the people in the under-developed countries of the world with teeming populations, do not have access to adequate trained medical personnel and consequently resort to self medication with complete ignorance of the correct prescriptions. One cannot, therefore, rule out the possibilities of over exposure due to the indiscriminate use and abuse, of these antimalarial drugs, as the drugs are purchased or obtained over-the-counter in many of such countries including Nigeria.

Diverse efforts, programmes and tools have been utilized to control the disease. Chemotherapy, which has long been used as a means of prevention and control, is an essential element of the Roll Back malaria programme with the objective to halve the burden of malaria for the world's people by the year 2010 (WHO,1993).

There are only a limited number of effective drugs which can be used to treat or prevent malaria; including the quinine and its derivatives, the antifolate and the recent artemisinin/artemether combination drugs. Historically, chloroquine as the drug of choice for malaria treatment has been gradually replaced with combination therapy due to drug resistance. Malaria drug resistance has been documented by Bloland (2001) and it has been reported that *Plasmodium falciparum* has developed resistance to all antimalarials in current use. However, the geographical distribution of resistance to any single antimalarial drug varies greatly. Still, drug resistance continues to reduce the efficacy and usefulness of monotherapy agents such as the 4-aminoquinolines like chloroquine and the anti metabolite antimalarials such as proguanil (WHO, 2001). The monotherapeutic treatment of malaria has been gradually replaced by combination therapy in the malaria treatment policy of many malaria endemic countries.

Due to the worldwide reports of drug resistance, particularly to falciparum malaria, many countries are increasingly adopting a new policy involving the use of combination drugs for prevention and treatment of malaria. Currently, the combination therapy incorporating artemisinin/artemether has been advocated as the first line therapy in many African countries, south of the Sahara Fansidar® is recommended for the intermittent preventive treatment of malaria.

A well tolerated combination antifolate, sulphadoxine and pyrimethamine (Fansidar®), has been extensively used for chloroquine-resistant falciparum malaria. However,

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Fansidar®-resistant *P. falciparum* now exists in a number of areas (Meek *et al*., 1986). The discovery of multi-drug resistance in various strains of *P. falciparum* led to the search for new potent antimalarial drugs. The search culminated in the combination drug containing sulphamethoxypyridazine and pyrimethamine (Metakelfin), a highly active blood schizonticide against all the 4 human species of malaria and an anti-protozoan agent used in the treatment of toxoplasmosis in immuno-compromised HIV patients (Parfitt, 1999). It is used as a single dose therapy for malaria treatment. The combination of antifolate drugs for malaria treatment act synergistically against the parasite specific enzymes. The efficacy of the sulpha-pyrimethamine has been compromised by drug resistance (Biswas, 2001); however there exist fewer cases of Metakelfin resistance (Irare *et al*., 1991). Pyrimethamine has been known to show potentiation when in combination with sulphonamides, exerting antimalarial activity by inhibiting plasmodial dihydrofolate reductase, thereby indirectly blocking the synthesis of nucleic acids in the malaria parasite. It is readily absorbed and slowly, but steadily, excreted from the body. It is usually combined with sulphonamides, with sulphadoxine as Fansidar®; dapsone as Maloprim, sulphamethoxypridazine as Metakelfin and mefloquin. Sulphamethoxypyridazine is a long-acting sulphonamides that is readily absorbed and distributed throughout the body. It is protein- binding and is found in most body fluids, including saliva, sweat, cerebrospinal fluid, occular, pleural and peritoneal fluids.

Metakelfin is a typical antifolate combination drug which, like Fansidar®, has been extensively and exclusively used for treatment of complicated falciparum infections. Fansidar® resistance has been established in East and central Africa (Ogutu *et al*., 2000; Triggs *et al*., 1997) and parts of West Africa (Onyiorah *et al*., 1996). While the artemisinin/artemether combination drugs are presently efficacious, their prohibitive cost and availability have not allowed their widespread use in the poorer nations. Antifolate combination drugs are both widely available and relatively inexpensive. It has been reported that their continued use should be encouraged (Bloland, 2001; WHO, 2001). Presently, Fansidar® is the antimalarial recommended for the intermittent preventive treatment of malaria in pregnancy (IPT). However, the drug may not be a suitable prophylactic especially for travelers because of the risk of severe skin reactions.

MATERIALS AND METHODS

Laboratory animals: Male albino mice which had been inbred for more than 20 generations were obtained from the animal breeding unit of the Veterinary Department, University of Ibadan (UI VET strain). Mice (12-14 weeks old) were acquired and quarantined in a pathogen-free, well ventilated room for 1 week in order to enable the mice 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*.

Drugs: Sulphamethoxypyridazine:pyrimethamine, (Metakelfin®) was supplied by Farmitalia Carla Erba, Italy. The drug was dissolved in normal physiological saline, which was used as the solvent vehicle. The drug readily dissolved in physiological saline.

Doses used in this study were selected according to the therapeutic dose used in humans for each drug, which is calculated based on the average human weight of 65 kg. The human therapeutic dose (HTD) for Sulphamethoxypyridazine is 15.4 mg kg^{-1} and for pyrimethamine is 0.77 mg kg^{-1}. The highest dose utilized in this study corresponded to double (2x) the human therapeutic dose. Other doses utilized were 0.25x, 0.5x ,1.0x and 1.5x HTD.

Assay of sperm abnormalities: Induction of sperm-head abnormalities was tested according to the criteria of (Wyrobek *et al*., 1983). Five different dose level treatments were considered for the drug. The doses tested were 3.85:0.19; 7.7:0.38; 15.4:0.77; 23.1: 1.13 and 30.8:0.19, kg body weight of Sulphamethoxypyridazine: pyrimethamine, respectively, corresponding to 0.25x; 0.5x; 1.0x; 1.5x; and 2x the human therapeutic dose (HTD)

Single intraperitoneal (i.p.) injections of 0.5 mL of the different dose levels of the drug were administered to the mice for five consecutive days, as suggested by Wyrobek *et al*. (1983). A 5 week exposure period was considered from the first administration. Five mice were treated for each dose level. Five mice were exposed to the solvent vehicle only as a negative control. The positive control was 100 mg of methyl methane sulphonate/kg/day, i.p., for a period of five days. The positive control gave a statistically significant elevation of abnormal sperm heads in the mice (data not shown).

Sperm were sampled from the cauda epididymis at 5 weeks from the first injection. The mice were sacrificed by cervical dislocation. The epididymis were excised and minced with fine scissors in physiological saline. Smears were prepared on clean, grease-free slides after staining 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 1000x magnification. Six separate slides were prepared for each mouse i.e., three for each epididymis out of which four 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 Wyrobeck and Bruce, 1975). For each animal, 600 sperm were assessed for morphological damage.

Statistical analysis: Differences between the control and experimental groups were analyzed by means of the Wilcoxon Rank sum test or the students 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, shown evidence of a dose-related increase in abnormalities.

RESULTS AND DISCUSSION

Figure 1 illustrates normal sperm and the different sperm-head abnormalities observed from the prepared slides from the studied animals.

Sulphamethoxypyridazine: Pyrimethamine, (Metakelfin®) did not induce statistically significant increases in sperm head abnormalities over the controls and therefore may not be adjudged a positive inducer of abnormal sperm heads in treated UI Vet. F1 mice after 5 weeks exposure, (Table 1). Of the 5 doses sampled only the 7.7:0.38 mg kg^{-1} dose, corresponding to 0.5xHTD, gave a statistically significant increase. The negative control mice showed 2.53% abnormality. Furthermore, the induction of morphological aberrations in sperm heads by the different concentrations of Metakelfin®, after 5 weeks exposure, was not strictly dose-dependent.

The mice did not exhibit any adverse reactions to exposure to the drug and quite tolerated the drug well; there was no post-administration death recorded.

Analyses of sperm-head abnormalities were made 5 weeks following the end of exposure to the drugs. Sperm observed at these times were presumably exposed to the drugs while they were spermatocytes and spermatogonia.

There are no available reports on the mutagenicity of sulpha drugs and pyrimethamine. In this present study, using the sperm head abnormality test, the combination antimalarial drug, sulphamethoxypyridazine and pyrimethamine (Metakelfin®) was not able to induce

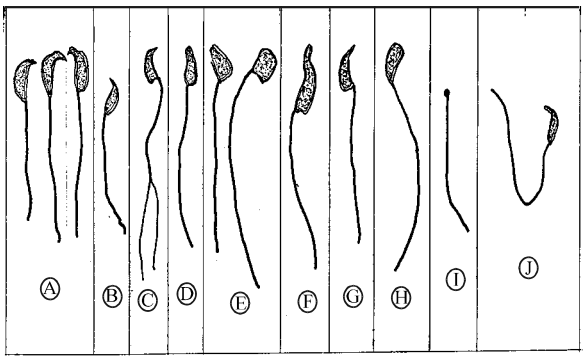

Fig. 1: Observed shapes of normal and abnormal sperm heads. A-sperm with normal head (the morphology of a normal sperm head of a mouse consists of a definite head shape accented by a marked hook, a rectangular mid-piece attachment site and single tail); B-no hook; C-2-tails; D-knobbed hook, E-amorphous head; F-bent hook; G-hook at wrong angle; H-tail folded over head; I-pin-head; J-banana-shaped head

Table 1: Effect of sulphamethoxypyridazine:pyrimethamine (Metakelfin®) on sperm head morphology in UI Vet F1 mice after 5 weeks exposure

Daily dose (mg kg^{-1} b.w.t)	No. of treated animals	Total abnormality	%frequency of sperm with abnormal head shapes
Negative control	5	76	2.53
3.85:0.19	3	85	2.83
7.7:0.38	5	236	7.87*
15.4:0.77	5	105	3.50
23.1:1.15	5	147	4.90
30.8:1.34	5	166	5.53

*Statistically significant at $p<0.05$

statistically significant increases in morphologically aberrant sperm heads in albino mice. One plausible reason for the inability of Metakelfin® 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 spermatozoa. There have been no reports of the drugs binding to DNA which can lead to the faulty differentiation of spermatozoa in the testes (Wyrobeck and Bruce, 1978). It may be plausible to suggest that this fact together with the relatively poor tissue retention of drugs, sulphamethoxypyridazine and pyrimethamine and their relatively rapid metabolism and excretion, have resulted in their inability to induce sperm-head abnormalities. There is also no evidence of a compromise in the blood-testis barrier. However, these negative findings do not preclude the possibility of the occurrence of genetic effects of a different nature.

During the past two decades increasing attention has been directed towards the determination of the mutagenic

and carcinogenic potentials of drugs. The development of methodologies capable of detecting within short periods, mutagenic activities, has permitted the testing of a much larger number of compounds that has hitherto been impossible. It is pertinent, however, to state here that some of these drugs, have effective therapeutic activities and have not outlived their usefulness as chemotherapeutic agents, although they may be mutagenic.

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REFERENCES

Biswas, S., 2001. Plasmodium falciparum dihydrofolate Val-16 and Thr-108 mutation associated with *in vivo* resistance to antifolate drug: a case study. Indian J. Malariol., 38: 76-83.

Bloland, P.B., 2001. Drug Resistance in Malaria. WHO/CDS/CDS/DRS, 4: 27.

Irare, S.G., M.M. Lemnge and J.I. Mhina, 1991. Falciparum malaria fully cleared by amodiaquine, pyrimethamine-sulphadoxine and pyrimethamine-sulfalene in areas of chloroquine resistance in Dodoma, Tanzania. Trop. Geogr. Med., 43: 352-356.

Meek, S. R., E.B. Doberstyn, B.A. Gaurere, C. Thanapanich, E. Nordlander and S. Phiphaisan, 1986. Treatment of *falciparum malaria* with quinine and tetracycline combined mefloquine/ sulfadoxine/ pyrimethamine on the Thai-Puchean border. Am. J. Trop. Med. Hyg., 35: 246-250.

Ogutu, R.B., B.L. Smoak, W. Ndueti, A. Mbori-Ngacha, F. Mwathe and G.D. Shanke, 2000. The efficacy of Pyrimethamine-sulphadoxine resistance of *Plasmodium falciparum* malaria in Kenyan children. Trans. R. Soc. Top. Med. Hyg., 94: 83-84.

Onyiorah, E., M.B. van Hensbroek, M.S. Jah and B. Greenwood, 1996. Early clinical failures after pyrimethamine-sulphadoxine treatment of uncomplicated malaria. Trans. R. Soc. Trop. Med. Hyg., 90: 307-308.

Parfitt, K., 1999. Antimalarals. In: Martindale: The Complete Drug Reference, 32nd Edn., Parfitt, K. (Ed.)., Pharmaceutical Press, London, pp: 422-442.

Triggs, J.K., H. Mbrana, O. Chambo, E. Hills, W. Walkins and C.F. Curtis, 1997. Resistance to pyrimethamine/ sulphadoxine in *P. falciparum* in 12 villages in north east Tanzania and a test of chlorproguanil/dapsone. Acta Trop., 63: 185-189.

World Health Organization: Geneva, 1993. A Global Strategy for Malaria Control.

World Health Organization, 2001. The use of antimalarial drugs. Report of an Informal Consultation. WHO/CDS/RBM33, pp: 141.

Wyrobek, A.J. and W.R. Bruce, 1975. Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. (USA), 72: 4425-4429.

Wyrobek, A.J. and W.R. Bruce, 1978. The Induction of Sperm-shape Abnormalities in Mice and Humans, in Chemical Mutagens Principles and Methods for Their Detection. Hollaender A and de Serres EJ. Eds. Vol.5. Plenum Press, N.Y., pp: 255-285.

Wyrobek, A.J., L.A. Gordon, J.G. Burkhart, M.W. Francis, R.W. Kapp, G. Letz, H.G. Malling, J.C. Topham and M.D. Whorton, 1983. An evaluation of the mouse sperm morphology test and other sperm tests in non-human mammals. A report of the US, Environmental Protection Agency Gene-Tox Programme. Mutat. Res., 115: 1-72.