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ORIGINAL ARTICLE

Testing the cytotoxicity and genotoxicity of the antimalarial drug mefloquine

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Abstract Numerous drugs show significant behavioral, developmental, teratogenic and mutagenic effects. This study investigates the cytotoxic and genotoxic effects of two doses (20 and 40 mg/kg body weight) of the antimalarial drug mefloquine (MQ), in male mice. The parameters included the assessment of: mitotic index, cell cycle kinetics, and frequency of chromosomal aberrations in bone marrow cells, translocation frequency in spermatocytes and head and tail abnormalities in sperm. A high dose of mefloquine resulted in a decreased mitotic index, suggesting a dose–response correlation. A notable recovery in mitotic activity was observed during the first and third week post treatment, which approached the mitotic index of control. Chromosomal abnormalities including gaps, breaks, and fragments also increased with the higher dose of mefloquine. Spermatids and secondary spermatocytes were moderately sensitive to the drug; whereas primary spermatocytes were more sensitive, and spermatogonia were highly sensitive to both doses of MQ.

Conclusion: Mefloquine (MQ) at the doses used can be considered as a drug with a potential mutagenic effect. The results obtained from the spermatocyte diakinesis-metaphase 1 test and sperm abnormalities suggest that MQ may contribute to high incidence of birth defects and congenital abnormalities.

1. Introduction

Mefloquine, a 4-quinolinemethanol (MQ), is the only drug proven to be effective against multiple drug-resistant malaria. Experimental studies indicated that mefloquine does not significantly bind to DNA (Davidson et al., 1975). Schupbach (1979) showed no mutagenic activity of mefloquine in three tester strains TA 1535, TA 1537 and TA 1538 of *Salmonella typhimurium*. Grisolia and Takahashi (1994) showed that the administration of MQ did not lead to any clastogenic activity in the bone marrow of Wistar rats, when given in doses of 150, 300, 600 and 1200 mg/kg, equivalent to the human therapeutic doses. However, *in vitro* studies showed that mefloquine at doses (100 and 50 μg/ml) significantly altered poly-morphonuclear neutrophils (PMN) while, amodiaquine and chloroquine did not (Labor and Babin-Chevaye, 1988). The genotoxic effect of amodiaquine (AQ), mefloquine (MQ) and halofantrine (HF), investigated in rat liver cells

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using alkaline Comet assay. AQ, MQ and HF at doses between 0 and 1000 μmol/L significantly increased DNA strand breaks of rat liver cells illustrating dose-dependent relationship in the order of AQ > MQ > HF (Farombi, 2005, 2006). However, the study of clastogenic activity in human lymphocytes has not been done, *in vitro* or in patients undergoing therapy. Chatterjee et al. (1998), who used similar groups of three antimalarial drugs to examine the genotoxic and mutatoxic potential of MQ revealed that these drugs are weak mutagen, but capable of inducing significant sister chromatid exchange (SCE) and chromosomal aberrations (CA) in the bone marrow cells of mice. Karbwang and White (1990) detected the induction of lymphocytopenia at doses of 30 mg/kg. Genotoxicity tests by Akerele and Obaseiki-Ebor (2002) indicated that MQ was generally not genotoxic, but had the same potential mutagenicity as chloroquine phosphate.

The current study was undertaken to examine the long term treatment on cytotoxic and mutagenic potential of MQ in male mice, administered at 20 and 40 mg doses. The mitotic index of bone marrow cells, chromosomal aberration in metaphase cells and cytogenetic evaluation diakinesis-metaphase 1 in mouse spermatocytes were the focus of the study. Morphological abnormalities of the sperm too, were studied over 3 weeks. Following the last day of the drug treatment, sperms were analyzed at weekly intervals for 6 weeks, to study the effect of the drug on each stage of the spermatogenic cycle, which is completed in about 5 weeks (Okberg, 1956; Wyrobek, 1978).

2. Material and methods

2.1. Experimental animals

Swiss albino mice, strain SWR/J, were used in this study. Adult male mice were obtained from the Central Animal House of King Saud University (Women's Branch – Malaz, Riyadh, KSA). The animals were housed in well ventilated, hygienic conditions under standardized temperatures. Food and water were provided *ad libitum*. Male mice were used in order to avoid hormonal interference of the estrous cycle in females. All animals were 10–12 weeks of age, each weigh about 25 g. Animals were randomly selected for experimental control.

2.2. Dosage and treatment

Mefloquine (trade name Mephaquine [Mepha Ltd., Aesch-Basel, Switzerland]) was purchased from a local pharmacy in Riyadh, KSA, and was suspended in Tween 80 (1%) to the desired concentrations (1 mg/0.5 ml, and 0.5 mg/0.5 ml). These doses correspond to human doses of 40 and 20 mg/kg body weight (Preston et al., 1981; Fryauff et al., 2007). The stock solution was freshly prepared before each dose was administered.

For studying cytotoxic and cytogenetic effects of bone marrow (mitotic index, cell cycle kinetics, and chromosomal aberrations in metaphase cells), animals were divided into three large groups of 30 animals each: two treated with a different dose of MQ and the third served as the control. Each large group was divided into six small groups of five animals each.

For spermatocyte diakinesis metaphase-1 test and for studying the morphological abnormalities of sperm head and tail, animals were similarly divided into three groups of 35 male animals each. Two groups were treated with a different dose of MQ and the third group served as control. Each large group was subdivided into seven small groups of five animals each.

2.3. Sampling time

Cells were obtained from bone marrow of both control and treated mice. Mitotic indices, cell cycle kinetics, and analysis of chromosomal aberrations were performed at 24, 48, 72 h and at weekly intervals for 3 weeks following the last dose. For the spermatocyte metaphase-1 test, samples were tested at 24 h and at weekly intervals for 6 weeks following the last dose of drug, which covered a complete spermatogenic cycle. Morphological head and tail abnormalities of the spermatozoa were recorded for 6 weeks at weekly intervals following the last dose.

2.4. Techniques for the study of mitotic index, cell cycle kinetics, and metaphase chromosomes from bone marrow cells

Bone marrow from treated and control mice was flushed out from the two femurs into saline solution. The aspirated cells were divided into two samples; one was processed immediately for the study of mitotic index and cell cycle kinetics (Cho et al., 2011). The second sample is treated with Colchicine for 2 h for the study of metaphase chromosomal aberrations followed by hypotonic treatment with 0.075 M KCl for 20 min at 37 °C. Cells were then fixed in Carnoy's fixative (3:1 methanol:1 acetic acid). Air dried slide preparations were stained with 5% Giemsa stain (Evans et al., 1964; Adler, 1984). A total diploid number of chromosomes were counted in 100 metaphases and different types of CA were determined and tabulated according to Savage (1975). The effect of MQ on cell cycle kinetics of the bone marrow cells was also examined.

2.5. Analysis of germinal cell tissue, spermatocyte diakinesis-1, and morphological abnormalities of head and tail regions of spermatozoa

To evaluate the effect of MQ on meiotic chromosomes from germinal cells in different stages of spermatogenesis, treated animals and control groups were dissected and testes were removed from the seminiferous tubules. The cytogenetic analysis of diakinesis metaphase-1 spermatocytes involved the detection of reciprocal translocations and other chromosomal aberrations induced in spermatogonia of the treated males (Leonard, 1977).

To examine any morphological abnormalities of sperm, the animals caudae epididymides were removed, dissected and sperm were processed for fixation and stained with 1% eosin for 10–15 min. Preparation of spermatozoa was performed according to Wyrobek (1978).

2.6. Photography

All photography was performed using a photographic research microscope (Olympus Bx41).