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Full Length Research Paper

Studies on chronic administration of chloroquine on gastrocnemius muscle and spleen of Swiss albino mice

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The aim of the present study was to investigate the influence of chloroquine on some vital tissues of mice (gastrocnemius muscle and spleen). Healthy adult male Swiss albino mice weighing between 30 and 40 g were used for the study. Treated group was exposed to 200 mg/kg body weight/day of chloroquine phosphate given orally for 45 days. Control animals were given distilled water for the same period. Microscopic examination of muscle and spleen revealed concomitant changes in histology. Thus, the use of chloroquine for longer periods requires strict monitoring as chronic usage may lead to many detrimental effects in the host.

Key words: Malaria, Chloroquine, toxicity, gastrocnemius muscle, spleen.

INTRODUCTION

Malaria is a major global health problem, with an estimated 300 to 500 million clinical cases occurring annually and 1.5 to 2.7 million deaths, predominantly in children, mostly among the children of Sub-Saharan Africa (Breman, 2001).

Chloroquine was first synthesized in Germany, but was not recognized as a potent antimalarial drug until the 1940s as part of the US World War II military effort. By 1946, it was found to be far superior to other contemporary synthetic antimalarial drug (Coggeshall and Craige, 1949). It became the corner stone of anti-malarial chemotherapy for the next 40 years. Chloroquine quickly became the main drug of choice globally to treat uncomplicated *Plasmodium falciparum* infections, for instance as part of the Global Malaria Eradication Campaign, launched by the WHO in 1955. It is one of the least expensive antimalarial drugs available and is still in widespread use. Chloroquine can be taken both as prophylactic and as a treatment.

Despite much research during the last 40 years, the exact mechanism by which chloroquine kills the malaria parasite remains controversial (Foley and Tilly, 1997; Foote and Cowman, 1994; Peters, 1998). The drug chloroquine inhibits DNA and RNA biosynthesis and produces rapid degradation of ribosomes and dissimilation of ribosomal RNA. Inhibition of protein synthesis is also observed, evidently as a secondary effect. Inhibition of DNA replication is proposed as a general mechanism of the antimicrobial action of chloroquine. Chloroquine accumulates in very high concentrations in the parasite food vacuole (Geary et al., 1990). Once in a food vacuole, chloroquine is thought to inhibit the detoxification of heme. Chloroquine then becomes protonated (to CQ^{2+}) as the digestive vacuole is known to be acidic (pH 4.7); chloroquine then cannot leave by diffusion. Chloroquine caps hemozoin molecules to prevent further biocrystallization of heme, thus leading to heme buildup. Chloroquine binds to heme (FP) to form what is known as the FP-Chloroquine complex; this complex is highly toxic to the cell and disrupts membrane function. Action of the toxic FP-Chloroquine and FP results in cell lyses and ultimately parasite cell auto digestion. In essence, the parasite cell drowns in its own metabolic products.

Earlier studies show many adverse effects of chloroquine on tissue (Okpako and Aziba, 1989; Warhurst and Robinson, 1996; Ebong et al., 1999). Chloroquine is a potent autophagic drug that may lead to cellular degradation of hepatocytes in the liver with the concurrent production of vacuoles (Abraham et al., 1968). Observed increases in the number of lysosomes suggest

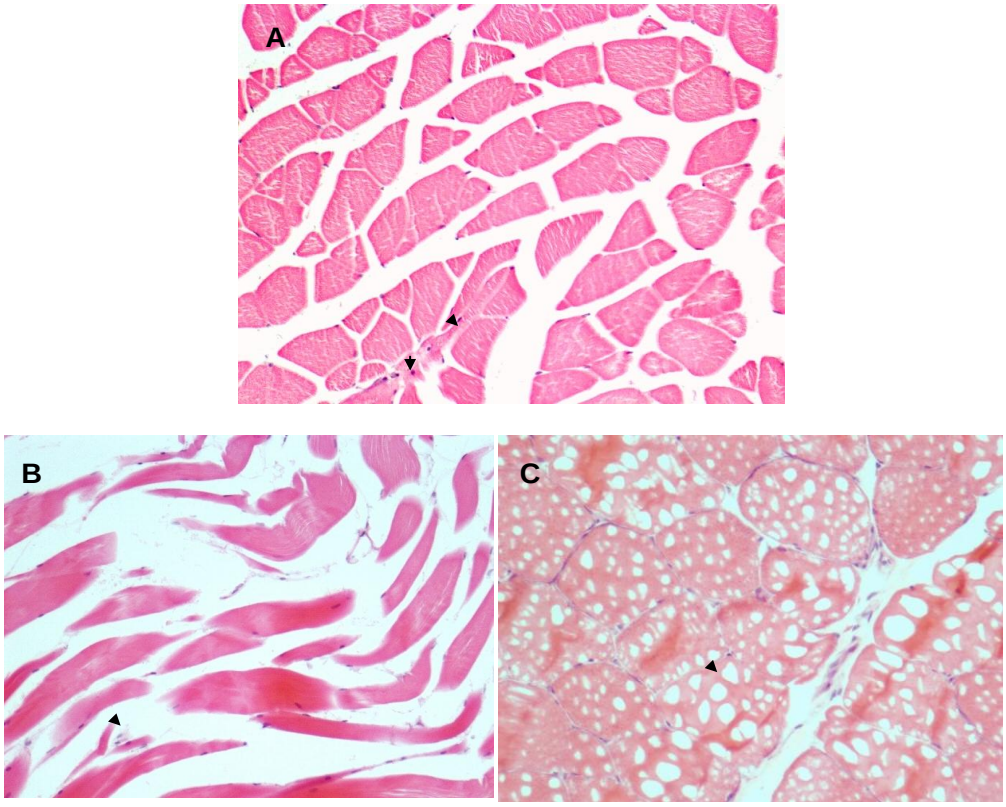

Figure 1. L.S. of control muscle and treated muscle under high magnification (40×). (A) Control muscle: showing distinct nuclei on peripheral and parallel fibers; (B) Treated muscle: showing disorganization of muscle fibers with nuclear pycnosis and nuclear proliferation at the periphery; (C) Treated muscle: showing nuclear disorganization with evidence of nuclear pycnosis and vacuolation of muscles fibres.

further cellular degradation. This is accompanied by fusion of lysosomes with autophagic vacuoles resulting in the biogenesis of new lysosomes (Ericsson, 1969). The reported accumulation of chloroquine in lysosomes has an apparent destabilizing effect on lysosomal membranes. Toxic manifestations appear rapidly within 1 to 3 h after ingestion (Jaeger and Flesch, 1994). Thus, information is needed about its effects on organs where the drug accumulates so as to gain insight into the impact of the long term administration of this drug.

MATERIALS AND METHODS

Twenty four adult male Swiss albino mice weighing between 30 and 40 g were selected for the study. Animals were housed in well ventilated wire meshed cages, exposed to a 12 h light cycle in an air conditioned atmosphere at a temperature of 26±2°C and provided with food and water *ad libitum*. Animals were divided into two groups, Groups I and II; where Group I served as an untreated control and Group II as the chloroquine treated test group.

Chloroquine phosphate (99.3% Pure) and other chemicals were obtained from Sigma Aldrich (UK). The prophylactic drug chloroquine phosphate was dissolved in single distilled water. The dose of the drug was selected on the basis of its oral LD_{50}, that is, 500 mg/kg body weight for mice (Walum, 1998). The test drug was administered orally at a dose level of 200 mg/kg body weight for 45 days. A dose less than 200 mg/kg body weight did not produce significant results in other tissues while a higher dose resulted in significant toxicity (Dattani et al., 2009). Hence, to evaluate the impact of an intermediate dose in the present study, the aforementioned dosage was selected.

At the end of treatment on the 46th day, animals were sacrificed. Spleen and gastrocnemius muscle of control and treated animals were dissected out. Histological studies of spleen and muscle were carried out by using the standard technique of haematoxylin and eosin staining.

RESULTS

Histological examination of the muscle of treated animals under low magnification showed disorganized muscle bands with increased muscle fibre diameter, disrupted muscle fibres in the fasciculae bundle with alteration and broadening of the fibre diameter. Under high magnification, nuclear disorganization and nuclear pycnosis were evident in transverse section (T.S) and alteration of the diameter with a distinct nucleus was exhibited in the longitudinal section (L.S). (Figure 1; A to C). Disorganization and vacuolation of muscle fibres with

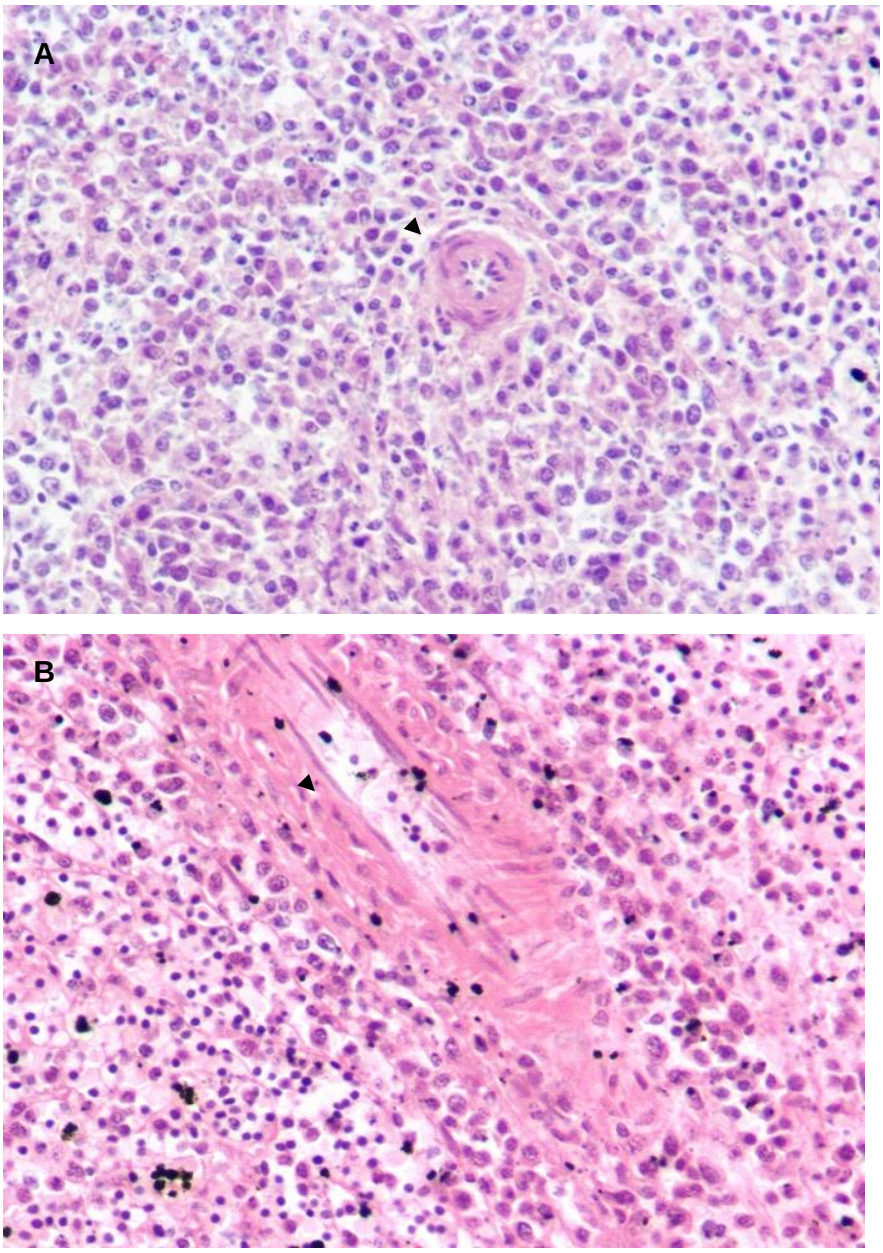

Figure 2. L.S. of control spleen and treated spleen under high magnification (40×). (A) Control spleen: showing normal organization of red pulp with megakaryocytes; (B) Treated spleen: showing red pulp disorganization and an increased number of megakaryocytes with an increased number of mast cells.

nuclear pycnosis and nuclear proliferation at the periphery was also visible in the L.S. under high magnification. However, no significant changes were observed in the sarcolemma of muscle from treated animals when compared with the control group. The spleen of treated animals showed disorganization of megakaryocytes with cells around the trabaculae, disorganization of red pulp and an elevated number of mast cells under high magnification. Corpuscles were also seen to be spread uniformly (Figure 2; A and B).

DISCUSSION

Malaria is a disease that was on the verge of eradication once, but has recently returned with greater vigor. This calls for greater preventive and curative treatments and better procedures for disease control. Widespread use of antimalarial drugs further demands a critical evaluation of drug toxicity as well as damage to the tissues. In the present study, administration of chloroquine for 45 days revealed anomalies which could be clearly attributed to

the toxicity of this drug. The histological results confirmed the lysomotropic nature of chloroquine, where disarray of muscle fibre with alteration and broadening of the fibre diameter and nuclear pycnosis were observed, which is attributed to lipidosis. Histochemically, McDonald and Engel (1970) have shown that extensive phospholipid accumulation takes place in muscle fibers. Such an increase was noted earlier in the liver and kidney of miniature pigs (Lullmann et al., 1970; Mastuzawa and Hostelter, 1980). Histologically in spleen, corpuscle degradation around sinusoids, scattering of cells and degradation of red pulp was observed. The increased megakaryocytes, blast cells and mast cells suggest a possible alteration in haemopoiesis (Othman and Arowolo, 1998).

Conclusively, the results of our study suggest that prolonged exposure to the antimalarial drug chloroquine phosphate potentiates adverse effects on vital tissues of the host. Contemplating the risks to humans due to the widespread use of these quinoline derivatives, these findings suggest the necessity of proper instructions and careful monitoring by doctors when prescribing chloroquine for longer duration as it can produce a number of undesirable effects. Furthermore, this work also identifies the need for more of such studies in future which could throw light on other aspects of antimalarial drug toxicity and its therapeutic treatments.

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