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Effect of chronic oral administration of chloroquine on the histology of the heart in Wistar rats

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Abstract
The effect of chronic oral administration of chloroquine, an antimalarial and antirheumatic drug on the histology of the heart in Wistar rats was investigated. Ten Wistar rats were randomly grouped into two, control and treated. The treated group rats were administered 20mg/kg body wt, weekly of chloroquine for 4 weeks while the control group rats were given distilled water for 4 weeks. On day 29 of the experiment, the rats were weighed and sacrificed. The hearts were carefully dissected out and quickly fixed in 10% formal saline for routine histological study after H&E method. The histological findings indicated that the treated sections of the hearts showed moderate hypertrophy of the cardiomyocytes when compared with the control. Thus, our result suggests that though chloroquine may be a widely used antimalarial and antirheumatic drug, its chronic administration may result in cardiotoxicity. Therefore, it is recommended that the drug be prescribed with caution in patients with cardiac abnormality, such as cardiomyopathy and further studies to corroborate this observation should be carried.

Keywords: Antimalarial; chloroquine; cardiotoxicity; histology; cardiomyopathy.

Introduction

Malaria is a parasitic disease of great epidemiological importance in the tropics (Sitprija, 1988). It remains one of the most important and widespread diseases in the world (Sánchez-Chapula et al., 2010). Chloroquine is one of the drugs of first choice for treatment of malaria (Sánchez-Chapula et al., 2010). It is also used as an anti-inflammatory agent in rheumatoid arthritis and in lupus erythematosus (Webster, 1992). Availabe data show that chloroquine is concentrated in the liver and many other tissues following its administration (Adelusi and Salako, 1982). In toxic doses, it is known to cause appreciable cellular damage to liver, kidney and heart muscle (deGroot et al., 1981; Ngaha, 1982). The use of chloroquine has been associated with toxic cardiovascular effects, including a fall in blood pressure (Olatunde, 1970), rhythm abnormalities (Williams, 1966; Guedira et al., 1998). Prolonged therapy can lead to cardiomegaly and cardiac failure (Hughes et al., 1971; Izunya, et al., 2010) and electrocardiographic changes, including T-wave depression or inversion, and prolonged QRS and QTC intervals (Sanghvi and Mathur, 1965; Bustos et al., 1994). Acute poisoning by chloroquine can cause death by failure of myocardial contraction and cardiac arrest (Don-Michael and Aiwazzadeh, 1970).

The heart is a muscular organ present in all vertebrates, and responsible for pumping blood through the blood vessels by repeated rhythmic contractions (Heath et al., 1999). The heart of a vertebrate is composed of cardiac muscle (myocardium), an involuntary muscle tissue which is found only within this organ. The myocardium is the heart's muscular wall (Heath et al., 1999). It contracts to pump blood out of the heart and then relaxes as the heart refills with returning blood. Its outer surface is called the epicardium. Its inner lining is the endocardium (Heath et al., 1999). This study was considered important since rheumatoid arthritis and malaria are common ailments in the tropics and the need to avoid the risk of cardiomyopathy resulting from prolonged oral administration of chloroquine. Moreover, it has been suggested that chloroquine has the potential to induce hypertrophic cardiomyopathy (Baguet et al., 1999; Guedira et al., 1998; Teixeira et al., 2002). Thus, the aim of this study is to investigate the effect of prolonged oral administration of chloroquine on the histology of the heart in Wistar rats, in view of the fact that the effect of chloroquine on the morphology of the heart has already been determined (Izunya et al., 2010).

Materials and Methods

Location and duration of study

This study was conducted at the histology laboratory of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The preliminary studies, animal

acclimatization, drug procurement, actual animal experiment and evaluation of results, lasted for a period of two months (February and March, 2010). However, the actual administration of the drug to the test animals lasted for one month.

Animals

Experiments were carried out on ten (10) Wistar rats (150g) procured and maintained in the Animal Holdings of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The animals were housed under a controlled room temperature of about 25–28 °C, relative humidity of about 60–80% and photo-periodicity of 12 h day / 12 h night, and fed with rat pellets (Bendel Feeds and Flour Mills, Ewu, Nigeria) and water ad libitum. They were randomly assigned into two groups, the control (n = 5) and treated (n = 5) groups.

Drug administration

The chloroquine phosphate tablets used for this experiment were manufactured by Emzor Pharmaceutical Industries, Lagos, Nigeria and were purchased from Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria. Rats in the treatment group received 20mg/kg body weight of chloroquine phosphate dissolved in distilled water weekly for 4 weeks. Rats in the control group received equal volume of distilled water using orogastric tube. At the end of the experiment, on the 29th day, the rats were sacrificed using humane killing with chloroform and the hearts were harvested.

Histological study

For light microscopic examination, heart tissues from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5µm thickness and stained with haematoxylin and eosin (Drury et al., 1967). Photomicrographs of the relevant stained sections were taken with the aid of a light microscope.

Results

Histological examination of heart tissue of control rats showed normal myocardial fibers and muscle bundles with normal architecture (Plate 1). Histological examination of heart tissue of treated rats showed moderate hypertrophy of cardiomyocytes (Plate 2).

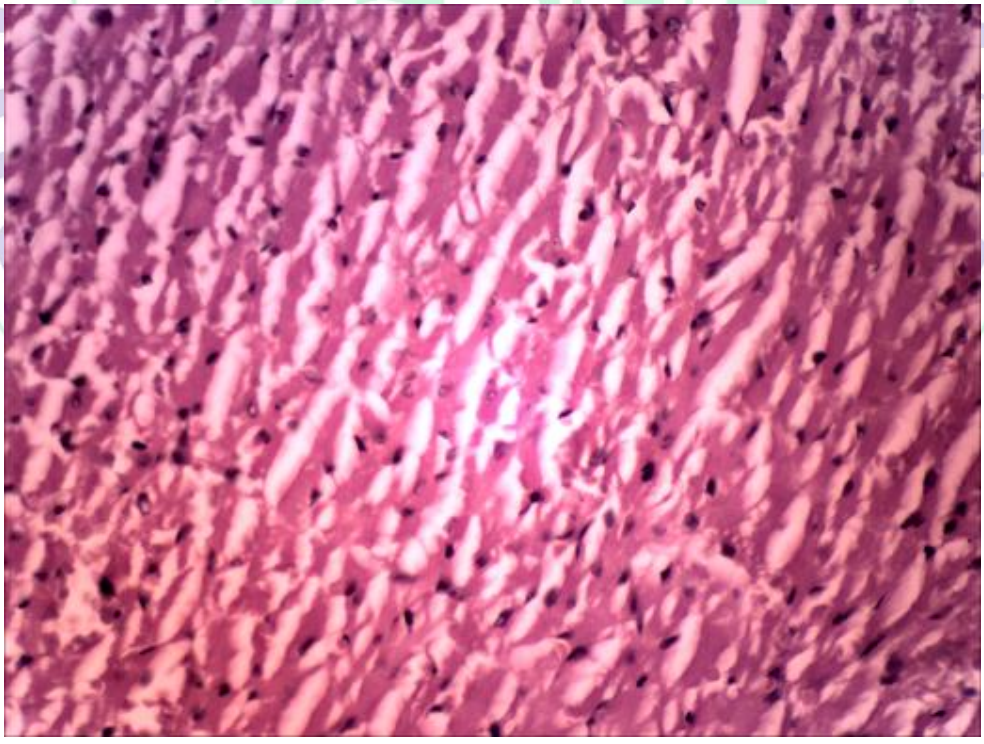

Plate 1 (Control Group): Control section of the heart showing normal histological features (X400).

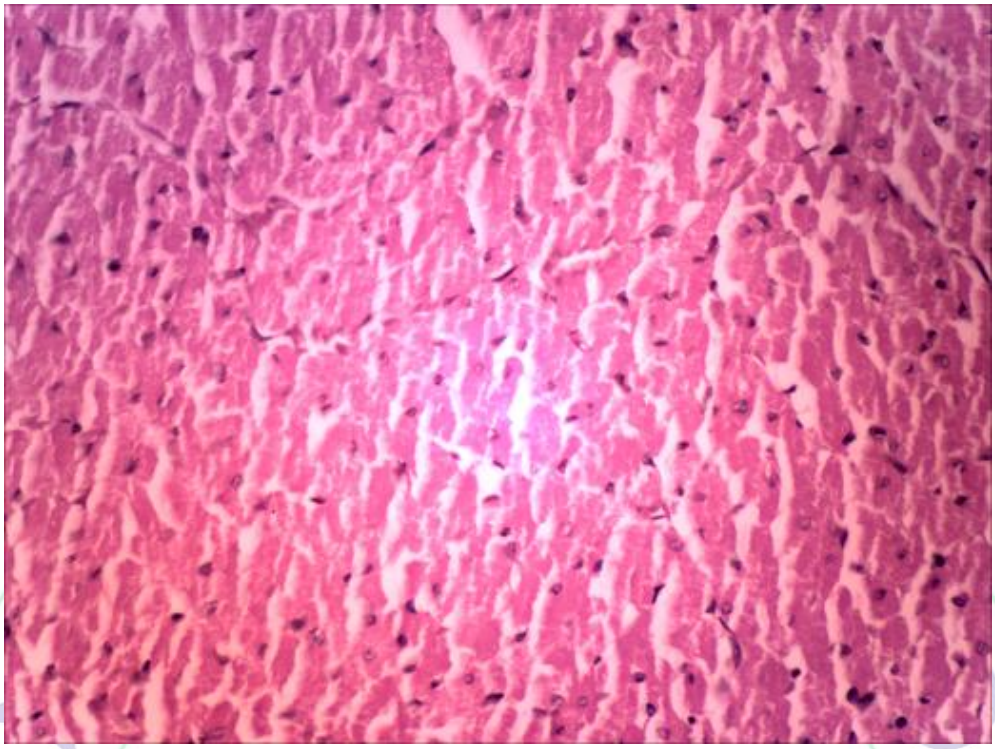

Plate 2 (Experimental Group): Treatment section of the heart that received 20mg/kg of chloroquine for 28 days, showing moderate hypertrophy of cardiomyocytes (X400).

Discussion

Histological results suggested toxicity of the myocardial cells of Wistar rats upon chronic oral administration of chloroquine. This was shown by the moderate hypertrophy of cardiomyocytes. As an antimalarial, chloroquine acts by inhibiting hemozoin biocrystallization, which gives rise to toxic free heme accumulation that is responsible for the death of the parasites (Barennes et al., 2006). Heme (iron protoporphyrin IX) serves as the functional group of various proteins, including hemoglobin, myoglobin, nitric oxide synthase, and cytochromes (Beri and Chandra, 1993). Heme is therefore essential for diverse biologic processes (Beri and Chandra, 1993). It has been shown that heme is a potentially damaging species, which can directly attack and may impair intracellular targets including the lipid bilayer, the cytoskeleton, intermediary metabolic enzymes, and DNA (Wagener et al., 2003). Also, there are available reports indicating that high levels of free heme cause severe toxic effects to kidney, liver, central nervous system and cardiac tissue and that free heme catalyzes the oxidation, covalent cross-linking and aggregate formation of protein and its degradation to small peptides (Kumar and Bandyopadhyay, 2005).

Moreover, excess of free heme may constitute a major threat because heme catalyzes the formation of ROS, resulting in oxidative stress and, subsequently, cell injury (Balla et al., 1993; Balla et al., 1991). Free heme is highly lipophilic and will rapidly intercalate into the lipid membranes of adjacent cells (Beri and Chandra., 1993), where it catalyzes the formation of cytotoxic lipid peroxide via lipid peroxidation and damages DNA through oxidative stress (Kumar and Bandyopadhyay, 2005). Acworth et al. (1997) revealed that increased lipid peroxidation can negatively affect the membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors. In fact, reactive oxygen species have been implicated in the pathophysiology of a large number of diseases (Barp et al., 2002). Evidence from experimental as well as clinical studies suggests the role of oxidative stress in the pathogenesis of heart dysfunction (Singal et al., 1998; Manolio, 1991; Piano, 2002; Reinke et al., 1987). Furthermore, elevated ROS are implicated in the development of cardiac hypertrophy, reperfusion injury, remodelling and heart failure (Sorescu and Griendling, 2002). The mechanisms by which ROS can damage cardiac muscle are multiple and certainly involve direct toxicity by inducing both necrosis and apoptosis (Chesley et al., 2000), impairing myocardial function (Bolli et al.,

1987) and inducing cardiac arrhythmias (Beresewicz and Horackova, 1991). Studies in experimental animals have directly implicated ROS in cardiac injury secondary to anthracycline exposure (Doroshow, 1983) and tachycardia (Cesselli et al., 2001; Ukai et al., 2001).

ROS are small, oxygen-based molecules that are highly reactive because of unpaired electrons (Papa and Skulachev, 1997). The most prominent ROS are the superoxide anion (O_2•–), hydrogen peroxide (H_2O_2), and the hydroxyl ion (OH•) (Turner and Lysiak, 2008). Cells also have intrinsic antioxidant systems that counter ROS accumulation. These include enzymes such as catalase, glutathione peroxidases, and superoxide dismutase, and non-enzymatic antioxidants, such as vitamins E, C, beta-carotene, ubiquinone, lipotic acid, and urate (Giordano, 2005; Nordberg and Arner, 2001). Nevertheless, under several situations, the rate of generation of ROS exceeds that of their removal and oxidative stress occurs (Giordano, 2005; Di Giulio et al., 1995; Halliwell and Gutteridge, 2000; Livingstone, 2001). In excess concentrations, these ROS pose a risk of damage to cellular carbohydrates, proteins, lipids, and nucleic acids (Amici et al., 1989; Paradis et al., 1997). This increase in ROS triggers cardiomyocyte expression of the proto-oncogene c-fos, one of the first indicators of hypertrophy (Cheng et al., 1999; Laskowski et al., 2005). ROS also activate members of the mitogen-activated protein kinase (MAPK) family, protein kinase C, phosphatidyl inositol 3-kinase, and calcineurin, ultimately leading to increased cardiomyocyte protein synthesis, hypertrophic gene expression and increased cardiomyocyte volume (Sawyer et al., 2002; Xiao et al., 2002; Sabri et al., 2003; Ghosh et al., 2003). Based on these reports therefore, it is conceivable that the chloroquine used in this study may have acted through the generation of excess free heme or reactive oxygen species to induce the cardiomyocyte hypertrophy observed in the treated group.

Conclusion

The present investigation has shown that though chloroquine may be a widely used antimalarial and antirheumatic drug, its chronic administration may result in cardiac damage. It is therefore suggested that the drug be prescribed with caution in patients with cardiac abnormality, such as hypertrophic cardiomyopathy and further studies aimed at corroborating this finding should be carried out.

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