Effect of Chronic Oral Administration of Chloroquine on the Histology of the Liver 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 liver in wistar rats was investigated. Ten wistar rats were randomly grouped into two, control and treated. The treated group rats were administered 20 mg/kg body wt, weekly of chloroquine for 4 weeks while the control group rats were given distilled water for 4 weeks. On day 29th of the experiment, the rats were weighed and sacrificed by cervical dislocation. The livers were carefully dissected out and quickly fixed in 10% formal saline for histological studies. The histological findings after H and E methods indicated that the treated sections of the liver showed cytoplasmic vacuolation; nuclear enlargement and vesiculation of the hepatocytes 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 have a deleterious effect on the liver of wistar rats and by extension may affect its function. It is therefore recommended that the drug be prescribed with caution in patients with history of liver disease.

Key words: Antimalarial, chloroquine, hepatotoxicity, histology, wistar rats

INTRODUCTION

A number of research studies have described the deleterious effect of commonly prescribed anti-malarials on the liver. Amodiaquine - formerly widely used as a chemoprophylactic against Plasmodium spp. - produces significant hepatocellular dysfunction (Larrey et al., 1986; Neftel et al., 1986; WHO, 1990; Pero and Taylor, 2002; Ajani et al., 2008), it is now rarely used due to a causative association with bone-marrow depression (Cook, 1994). 'Fansidar' (pyrimethamine + sulphadoxine) has been extensively used in chemoprophylaxis, and remains an effective chemotherapeutic agent; it also produces significant hepatocellular dysfunction (Reisinger et al., 1989). Mefloquine, a compound now widely used both in chemoprophylaxis and chemotherapy, can also produce significant changes in liver-function tests (Reisinger et al., 1989); it has not, however, been associated with significant histological abnormality (Cook, 1994). Quinine, again the first-line agent against P.falciparum infection, is also hepatotoxic (Wernsdorfer and McGregor, 1988; Okonkwo et al., 1997; Debra and Megan, 1999), albeit rarely (Wernsdorfer and McGregor, 1988). Halofantrine which is widely prescribed for the treatment of infections with chloroquine-resistant strains of Plasmodium falciparium has also been shown to be hepatotoxic (Nwanjo et al., 2007; Obi et al., 2004). Amongst the artemisinins, artesunate used as antimalarial against multidrug- resistant strains of plasmodium falciparum (Hien and White, 1993) has also been found to be hepatotoxic (Ngokere et al., 2004; Nwanjo and Oze, 2007; Izunya et al., 2010).

Chloroquine is a widely used antimalarial agent (Sharma and Mishra, 1999). In most endemic areas, chloroquine use was the main first line therapy for malaria (Olanrewaju and Johnson, 2001) until recently when WHO succeeded in promoting the combination treatment for malaria infection (Nosten and Brasseur, 2002). It is also used to treat rheumatoid arthritis and systemic lupus erythematosis (Ducharme and Farinotti, 1996; Dubois, 1978).

Available 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 liver is the largest solid organ in the body. It is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and
inactivated in the liver and is therefore susceptible to the
toxicity from these agents. Certain medicinal agents when
taken in overdoses and sometimes even when introduced
within therapeutic ranges may injure the liver.

Reports regarding the effects of chronic oral
administration of chloroquine on the histology of the liver
are scanty in existing literatures. There is however a
report which showed that chloroquine treatment for 12
weeks in mice causes cytolysis in hepatocytes (Okonkwo
et al., 1997).

This study was considered important since
rheumatoid arthritis and malaria are common ailments in
the tropics and the need to avoid the risk of hepatitis
resulting from prolonged oral administration of
chloroquine. In view of this, the present study was
carried out to investigate the effect of chronic oral
administration of chloroquine on the histology of the liver
in wistar rats.

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 (150 g) 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 preparation and administration: The chloroquine
phosphate tablets used for this experiment were
manufactured by Emzor Pharmaceutical Industries,
Lagos, Nigeria and certified by National Agency for Food
Drug Administration and Control (NAFDAC). They were
purchased from Irrua Specialist Teaching Hospital, Irrua,
Edo State, Nigeria. Rats in the treatment group received
20 mg/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.

The animals were sacrificed using humane killing
with chloroform 24 h after the last dose on the 29th day
and the livers were harvested.

RESULTS

Histological analysis of the livers of rats in control
group showed normal morphological appearance
(Plate 1).

Histological analysis of the liver of rats in treated
group showed cytoplasmic vacuolation; nuclear
enlargement and vesiculation (Plate 2).
DISCUSSION

Histological results suggested degeneration of the liver cells of the wistar rats upon chronic oral administration of chloroquine. This was shown by the cytoplasmatic vacuolation, nuclear enlargement and vesiculation of the hepatocytes. The findings in this study agree with the work of Okonkwo et al. (1997) in which chloroquine administration for 12 weeks caused cytolysis in hepatocytes in mice.

Degenerative changes have been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death (Cohen, 1993; Vaux et al., 1994). These two types differ morphologically and biochemically (Bose and Sinha, 1994). Apoptosis or Programmed Cell Death (PCD) is a non-inflammatory response to tissue damage characterized by a series of morphological and biochemical changes (Sakkas et al., 1999; Sinha and Swerdloff, 1999; Shen et al., 2002; Grunewald et al., 2005). Apoptosis can be triggered in two principal ways: by toxic chemicals or injury leading to damage of DNA or of other important cellular targets, and activation or inactivation of receptors by growth-regulating signal factors in the organism (Schulte-Hermann et al., 1999).

Initiation of apoptosis can result from multiple stimuli, including heat, toxins, Reactive Oxygen Species (ROS), growth factor withdrawal, cytokines such as transforming growth factor-beta, loss of matrix attachment, glucocorticoid, nitric oxide, and radiation (Thompson, 1995; Pollman et al., 1996). These stimuli work in conjunction with other intrinsic factors that determine the cell's potential to undergo apoptosis (McConkey and Orrenius, 1991). However, high levels of ROS disrupt the inner and outer mitochondrial membranes, inducing the release of the cytochrome-C protein and activating the caspase cascade which ultimately results in the fragmentation of a cell's DNA (Wyllie, 1980; Green, 1998; Makker et al., 2009).

Pathological or accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects (Farber et al., 1981). The process of cellular necrosis involves disruption of the membranes structural and functional integrity. Cellular necrosis is not induced by stimuli intrinsic to the cells as in apoptosis or Programmed Cell Death (PCD), but by an abrupt environmental perturbation and departure from the normal physiological conditions (Martins et al., 1978).

Chloroquine is an aminoquinolinic membrane-penetrable agent capable of intercalating into double-stranded DNA without causing physical damage to the DNA (Mitscher, 2005). The DNA intercalation is non-selective for malarial parasites as it occurs also with mammalian DNA. Thus protein synthesis and enzyme activities may be diminished or disrupted in sensitive tissues (Oforah et al., 2004). Owing to its weak base properties, chloroquine also accumulates in lysosomes and may trigger apoptosis via the inhibition of autophagic protein degradation (Amaravadi et al., 2007; Boya et al., 2005; Fan et al., 2006; Shacka et al., 2006; Maclean et al., 2008).

As an antimalarial, chloroquine acts by inhibiting hemoglobin 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 however 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). 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 (Kumar and Bandyopadhyay, 2005; Balla et al., 1991, 1993).

Interestingly, there are reports indicating that high levels of free heme cause severe toxic effects to kidney, liver, central nervous system and cardiac tissue (Kumar and Bandyopadhyay, 2005; Dhalla et al., 1996). Moreover, 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.

ROS generation is a normal component of oxidative phosphorylation and plays a role in normal redox control of physiological signaling pathways (Sawyer et al., 2002; Giordano, 2005; Murdoch et al., 2006). However, excessive ROS generation triggers cell dysfunction, lipid peroxidation, and DNA mutagenesis and can lead to irreversible cell damage or death (Sawyer et al., 2002; Giordano, 2005; Murdoch et al., 2006), and other ROS mediated alterations in chromatin structure may significantly affect gene expression (Konat, 2003; Rahman, 2003). Modification of proteins by ROS can cause inactivation of critical enzymes and can induce denaturation that renders proteins nonfunctional (Lockwood, 2000; Stadman and Levine, 2003). Moreover, there are also reports that cadmium toxicity in
liver may be mediated by the production of reactive oxygen species known to induce necrosis in various rat organs (Hsu et al., 2007; Razinger et al., 2008), lipid peroxidation (Borges et al., 2008) and a decrease in antioxidant enzymes (El-Sharaky et al., 2007).

ROS are small, oxygen-based molecules that are highly reactive because of unpaired electrons (Papa and Skulachev, 1997). ROS can react with cellular components, especially membrane lipids, and lead to cell damage (Rikans and Hornbrook, 1997). The most prominent ROS are the superoxide anion (O$_2^{•–}$), hydrogen peroxide (H$_2$O$_2$), 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 nonenzymatic antioxidants, such as vitamins E, C, beta carotene, ubiquinone, lipotic acid, and urate (Nordberg and Arner, 2001; Giordano, 2005).

In a normal liver, the level of ROS is low, and antioxidant defenses are adequate to protect the liver from oxidative damage (Fernandez et al., 1997). 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 Gutterer, 1999; Livingstone, 2001). However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Lennon et al., 1991). However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart (Lelli et al., 1998; Lee et al., 1999).

In this study, chloroquine may have acted directly through generation of high levels of free heme or ROS on the hepatocytes, affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis. In cellular necrosis, the rate of progression depends on the severity of the environmental insults. The greater the severity of the insults the more rapid the progression of neuronal injury (Ito et al., 2003). The principle holds true for toxicological insult to the brain and other organs (Martins et al., 1978). Thus, it may be inferred from this result that chronic oral administration of chloroquine is toxic to the liver in wistar rats.

CONCLUSION

Our study revealed that chronic oral administration of chloroquine causes cytoplasmic vacuolation; nuclear enlargement and vesiculation of the hepatocytes. These results have established the hepatotoxic potential of chronic oral administration of chloroquine in wistar rats. It is therefore recommended that the drug be prescribed with caution in patients with history of liver disease.

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REFERENCES


