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Biochemical effects of chronic administration of chloroquine on some vital tissue of Swiss albino mice

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Abstract: The present study was conducted to investigate the influence of chloroquine on some vital tissues of mice. Healthy adult male Swiss albino mice weighing between 35-45 gm were used for this study. Treated group was exposed to 200 mg/kg body weight/day of chloroquine phosphate given orally for 24 days. Control animals were given distilled water for the same period. Gastrocnemius muscle, spleen and brain tissues were biochemically investigated post-treatment. The results obtained showed a significant increase in protein levels of the gastrocnemius muscle ($P<0.001$), brain ($P<0.001$) and spleen ($P<0.05$). The total lipid content of both muscle and brain showed a highly significant increase ($P<0.001$) while the cholesterol level was increased significantly ($P<0.05$) only in spleen. Ascorbic acid also exhibited a significant increase ($P<0.001$) in muscle. Thus the use of choloroquine for longer periods requires strict monitoring as chronic usage may lead to the development of many detrimental effects in the host.
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1-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 antimalarial 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. Once in a food vacuole, chloroquine is thought to inhibit the detoxification of heme. Chloroquine then becomes protonated (to CQ2+) 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 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.

2-Material and Methods:

Twenty four adult male Swiss albino mice weighing between 35 and 45 gm 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 LD50, 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 24 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.

Data collection:

At the end of treatment, on the 25th day, animals were sacrificed. Spleen, brain and gastrocnemius muscle of control and treated animals were dissected out and blotted free of blood, weighed on a Roller Smith Torsion balance to the nearest milligram and used for the determination of a variety of different parameters.

Assays:

Protein estimation in brain (cerebral hemisphere), spleen, and muscle homogenate in both control and treated groups was done by the method of Lowry et al. 1951). Level of cholesterol in brain (cerebral hemisphere), spleen, and muscle was estimated by the method of Zlatkis et al.1953). Assessment of total lipid level was done in brain and muscle by using the method of Zoellner and Krisch 1962. Total Ascorbic Acid was assessed in gastrocnemius muscle by the method of Roe and Kuether 1943.

Statistical analysis

Test for statistical significance between control and chloroquine treated animals was done using Student's t test and significance was determined at the 0.05 % level.

3-Results:

In the brain (Table 1) of treated mice a significant (P<0.001) increase in protein level was registered after 45 days of exposure to chloroquine as compared with the control group. However, the cholesterol level did not increase in the treated group. The most markedly affected parameter was the total lipid content where a highly significant increase (P<0.001) was recorded after 45 days of exposure when compared with the control group.

In spleen (Table 2), chloroquine treatment significantly increased protein content (P<0.05) compared with the control group. The spleen cholesterol level was also affected and it increased significantly (P<0.05) compared with the control group after 45 days of treatment.

Oral administration of chloroquine for 45 days produced a significant increase (P<0.001) in the protein content, total lipid and total ascorbic acid levels and an insignificant increase (P<0.05) in cholesterol in the muscle (Table 3) of treated mice compared with the control group.

Table 1- Effect of chloroquine on protein, cholesterol and total lipid content in brain (cerebral hemesphere)

Groups	Protein (mg/100mg frozen tissue wt.)	Cholesterol (mg/100mg frozen tissue wt.)	Total Lipid (mg/100mg frozen tissue wt.)
Control group I	11.68±0.71	2.09±0.81	22.08±4.25
Treated group II	21.19±0.55***	2.37±0.67	51.08±7.11***

Values are mean± SD, ***P≤0.001,+ not significant n=15

Table 2- Effect of chloroquine on protein and cholesterol in spleen

Groups	Protein (mg/100mg frozen tissue wt.)	Cholesterol (mg/100mg frozen tissue wt.)
Control group I	22.54±0.44	2.06±0.10
Treated group II	31.19±6.69**	4.42±2.35**

Values are mean± SD, ***P≤0.001, P≤0.05 n=15

Table 3- Effect of chloroquine on protein, cholesterol, total lipid and total ascorbic acid(TAA) content in gastrocnemius muscles

Groups	Protein (mg/100mg Frozen tissue	Cholesterol (mg/100mg Frozen tissue)	Total Lipid (mg/100mg Frozen tissue)	TAA (mg/100mg Frozen tissue)
Control group I	6.08±0.31	0.29±0.003	41.54±0.51	2.39±0.15
Treated group II	19.71±2.41***	0.71±0.013	81.94±4.5	4.39±0.05***

Values are mean± SD, ***P≤0.001, P≤0.05 n=15

4-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 the 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 in results which could be clearly attributed to the toxicity of this drug. The lipid content was found to be increased in all of the tissues examined i.e. brain, spleen and muscle. The histological results also 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 Mc Donald and Engel (1970) have shown that extensive phospholipids accumulation takes place in muscle fibres. Such an increase was noted earlier in the liver and kidney of miniature pigs (Lullmann et al, 1978 and Mastuzawa & Hostetler, 1980). Nilsson et al 2005 also showed that there was a three fold increase in the concentration of total lipid in the skeletal muscle of chloroquine treated miniature pigs. The mechanism for the increased lipid content was likely due to accumulation of chloroquine in lysosomes. The probable inactivation of lysosomal enzymes due to increased chloroquine level in lysosomes would in turn reduces the activity of lysosomal enzymes involved in the degradation of polar lipids which would then lead to the storage of lipids. This is further substantiated by the fact that the activities of several lysosomal enzymes have been shown to be reduced by lysomotropic drugs (de Duve et al, 1974).

Chloroquine administration also increased the protein content in all the three organs. Chloroquine is known to be concentrated in lysosomes which play important role in the conformational conversion of protein. Shyng et al (1993) showed that during these conformational changes, deletion of nucleic acid occurs which results in conversion of the α-helical structure into β-pleated sheets. The pathological isoform of these proteins are heat resistant and protease resistant and are known as prion proteins. Kitamoto et al (1992) reported that these protease resistant proteins accumulate in muscle fibre and this pathological condition is called chloroquine myopathy which is characterized by degenerated muscle fibre with numerous autophagic, rimmed vacuoles. Matsunga et al (2002) reported that the pH is the crucial factor in determining the conformational state of some amyloidgenic proteins and an increase in pH might be responsible for the biosynthesis of prion molecules that are accumulated. Thus chloroquine also inhibits proteolysis leading to increased protein levels.

The cholesterol content showed a significant (P<0.05) increase in the spleen of the treated group compared with the control group. Fredman et al. (1982) documented a similar increase in chloroquine treated miniature pigs. In the present work an insignificant increase in muscle cholesterol and no increase in brain cholesterol content was found in the treated group compared with the control group. Similar results were reported by Klinghardt et al (1981) where chloroquine

treatment did not alter the concentration of cholesterol in the cerebrum, spinal cord and dorsal root of ganglia.

Ascorbic acid is an important biologically active antioxidant which is widely distributed in animal cells (Chinoy, 1978). After treatment the ascorbic acid level was found to be increased significantly (P<0.001) in the chloroquine exposed group. The level of ascorbic acid is affected by a large number of drugs which are unrelated pharmacologically or chemically and induce the synthesis of ascorbic acid. In the present study the increase in the level of ascorbic acid post treatment could be an active inbuilt ameliorative character of the metabolite. Ascorbic acid has been well documented as an ameliorative agent and is actively involved in recovery from the damage caused by the treatment to different tissues(Chinoy, 1993).

In conclusion 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 such studies in future which could throw light on other aspects of antimalarial drug toxicity and its therapeutic treatments.

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