

ORIGINAL ARTICLE

Effect of chronic administration of quinine on the myocardium of mice

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Received 3rd July 2008.

Revised 30th October 2008.

Published online 12th December 2008.

Summary

This study was designed to evaluate the effect of chronic administration of quinine on the myocardium of Swiss albino mice. 20 or 30 mg quinine/kg b.w. were administered for 30 consecutive days, while the control mice received equal amounts of normal saline. The mice were sacrificed 24 hours after the last administration. Some of the hearts were excised and processed for light microscopic study while some were assayed for malondialdehyde (MDA) and catalase activities. The histological findings indicated that the treated sections of the heart were at variance with the control. The myocardium in the control section presents well organized myofibrils with long cylindrical mononucleated cells in contrast to a dose dependent distorted arrangement of myofibrils and scanty cellular components observed in the treated sections. There was a statistically significant decrement in the weight difference and relative percentage weight of the heart in a dose dependent manner just as the activities of MDA and catalase significantly increased in the treated groups. These findings indicate that chronic administration of quinine may have some deleterious effects on the heart of mice and by extension may affect its function.

Key words: quinine – heart – myocardium – histology – chronic administration – malaria

INTRODUCTION

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).

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Malaria is an infectious disease caused by protozoan parasites (Krotoski et al. 1982, Snow et al. 2005). It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Malaria parasites are transmitted by female *Anopheles* mosquitoes (Joy et al. 2003). The parasites multiply within red blood cells, causing symptoms that include anaemia (light headedness, shortness of breath, tachycardia etc.), as well as chills, fever, and, in the most severe cases, coma leading to death (Krotoski et al. 1982, Joy et al. 2003, Snow et al. 2005). Malaria infections are treated through the use of antimalarial drugs, such as quinine or artemisinin derivatives, although drug resistance is increasingly common (Trager and Jensen 1976). One drug currently being investigated for possible use as an anti-malarial, especially for treatment of drug-resistant strains, is the beta blocker propranolol. Propranolol has been shown to block both *Plasmodium*'s ability to enter red blood cell and establish an infection, as well as parasite replication (Trager and Jensen 1976, Sachs and Malaney 2002, Trampuz et al. 2003).

Since the 17th century malaria has been treated with quinine, an extract from the bark of the *Cinchona* tree that suppresses the parasite stages in the blood (Woodward and Doering 1944). Quinine is still used today but there are now number of improved drugs. Other widely used anti-malarials include pyrimethamine (Kaufman and Rúveda 2005). Quinine (Fig. 1) is an alkaloid and derived principally from the bark of the cinchona tree (Barennes et al. 1996). It is an efficient antipyretic (fever-reducing agent) and is used to reduce fever in many diseases. It was the only known remedy for malaria until the development in recent years of synthetic drugs (Barennes et al. 1996). It has been one of the most successful and widely prescribed drugs for the management of malaria due to its efficacy (Barennes et al. 2006). It contains two major fused-ring systems: the aromatic quinoline and the bicyclic quinuclidine.

The efficacy of quinine was probably discovered by Jesuit missionaries in Peru, who introduced the drug into Europe in about 1640 (Barennes et al. 2006). The increase in its use through the years threatened the exhaustion of the South American trees, and efforts to cultivate cinchona trees in other countries finally succeeded in the Netherlands Indies (now Indonesia) in the late 19th century. The drug acts by inhibiting the hemozoin biocrystallization, thus facilitating an aggregation of cytotoxic heme. Toxic free heme accumulates in the parasites, leading to their death

(Barennes et al. 2006).

Although quinine is gradually being replaced by synthetic drugs (Wyllie 1980), we cannot shy away from the fact that it is still in our pharmaceutical market and quite a number of people still adopt it in the management of malaria in tropical Africa and beyond. We therefore set to determine the effect of chronic administration of quinine on the myocardium of Swiss albino mice. This could reduce the abuse of this drug as well as self medication which could result in cardiac complications such as myocarditis.

MATERIALS AND METHODS

Animals

Experiments were carried out on twenty four (24) Swiss albino mice (26–30 g) procured and maintained in the Animal Holdings of the Department of Anatomy and Cell Biology Obafemi Awolowo University, 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 mice pellets (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. Eight mice in each group were examined.

Drugs

Quinine was procured from Sigma Chemical Company Inc., St Louis, Mo, USA, and each animal was administered daily with a single dose of quinine or control saline intraperitoneally between 07:00 to 8:00 hours for a period of 30 consecutive days.

Experimental design

The drug was administered intraperitoneally for 30 consecutive days at a dose of 20 and 30 mg/kg to groups B and C respectively. The control group (group A) received an equivalent volume of normal saline. Twenty four hours after the last administration, the animals were sacrificed and the heart excised, perfused with normal saline and blotted dry on a filter paper. All animals were treated in accordance with the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (1985).

Biochemical Analysis

Assay for Malondialdehyde (MDA)

Samples of the heart (left ventricle) were excised,

blotted dry on a filter paper and weighed. A 10% homogenate of each heart in chilled phosphate buffer was immediately prepared with a Potter-Elvehjem homogenizer. The homogenates were centrifuged (3000 rpm for 10 min) and the supernatant immediately stored in the freezer (-20°C) and assayed within 48 h. MDA an index of lipid peroxidation was determined using the method earlier described by Ofusori et al. (2007).

Catalase activity

This was determined adopting the methods of Aksenes and Njaa (1981). Hydrogen peroxide was prepared with phosphate buffer; 0.2ml of the sample was added to 1.8 ml of 30 mM of hydrogen peroxide (H_2O_2) substrate in a 2 ml cuvette. The phosphate buffers were used as a blank. The absorbance for the test sample, blank and standard was read against a blank at 240 nm at 30 s interval for 1 min. The enzyme activity was calculated using the molar extinction coefficient of 40.00 per M per CM expressed as unit per ml.

Total protein

This was determined using the Biuret method (Gonall et al. 1949). 5.0 ml of blank Biuret reagent prepared by dissolving $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystal in 500 ml of distilled water was added to a sample blank. These were mixed well and allowed to stand for 20 min at room temperature $25\text{--}27^{\circ}\text{C}$. Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using: optical density for standard \times concentration of standard.

Histological Procedure

Samples of the left ventricle from the heart of some of the sacrificed animals were fixed in 10% formol saline and processed for light microscopic study. These processing methods included dehydration through graded ethanol (50%, 70%, 90% and 100%), clearing in xylene and infiltration in paraffin wax for 2 hours at 56°C and embedding of the tissues in paraffin wax for 48 hours. Sections were then obtained using a rotary microtome at $5\mu\text{m}$ thickness. The sections were finally subjected to a haematoxylin and eosin staining procedure.

Statistical Analysis

All the data were expressed as the mean \pm standard error of mean (SEM). Data were statistically evaluated with SPSS/10 software. One-way analysis of variance (ANOVA) at the significance level $2\alpha=0.05$ was used.

RESULTS

There was an alteration in the cytoarchitecture of the treated sections as compared with the control (Figs 2A–C). The myocardium in the control section presents well organized myofibrils with long cylindrical cells with one nucleus centrally positioned as against a dose dependent derangement of myofibrils and scanty cellular components observed in the treated sections (Figs 2A–C). There was a dose dependent statistically significant body weight loss when the weight difference was compared (Table 1). Also, the weight of the heart and the relative weight (%) present a dose dependent decrement (Table 2). Table 3 shows activities of MDA (marker enzymes of lipid peroxidation) and catalase in the heart of control and experimental mice. There was a dose dependent statistically significant increment in the activities of MDA and catalase in the treated groups when compared with the control group.

DISCUSSION

We investigated the effect of chronic administration of quinine used as anti malaria therapy on the myocardium of Swiss albino mice. This was an attempt to provide useful information on the application of this drug. Our investigation revealed that the treated sections of the heart showed some histological changes that were at variance with what obtained in the control sections. The result of this experiment presents a dose dependent derangement of myofibrils and scanty cellular components of the heart which could be associated with functional changes that may be detrimental to the health of the mice. Cellular degeneration has been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death. These two types differ biochemically and morphologically (Farber et al. 1981). Pathological cell death is regarded as necrotic and could result from extrinsic insults to the cell such as toxic and traumatic effects (Waters et al. 1994). Cell death in response to toxins occurs as a controlled event involving a genetic programme in which cascade enzymes are activated (Johnson 1995). Johnson (1995) reported in his work that drug poisoning, water intoxication, hypoxia from asphyxia and acute hyponatremia could result in cellular distortion. Chronic administration of quinine in this present investigation may have acted as poison to the myocardium, thus affecting the myofibril

Table 1: Mean values of body weight (g) of the animals before and after quinine treatment (mean ± SEM)

| | Control | 20 mg/kg | 30 mg/kg |
|------------|------------|-------------|--------------------------|
| Initial | 26.1 ± 4.1 | 30.0 ± 3.9 | 28.0 ± 4.2 |
| Final | 28.0 ± 4.2 | 26.2 ± 3.1 | 21.1 ± 4.1 |
| Difference | 1.9 ± 0.0 | -3.8 ± 0.8* | -6.9 ± 0.1* [§] |

*Statistically significant as compared with control.

[§]Statistically significant when animals treated by the dose 30 mg/kg vs animals treated by the dose 20 mg/kg.

Table 2: Mean values of heart weight and relative weight of the heart to the absolute (initial) body weight (mean ± SEM)

| | °Control | 20 mg/kg | 30 mg/kg |
|---------------------|-------------|--------------|---------------------------|
| Weight of Heart (g) | 0.14 ± 0.01 | 0.12 ± 0.00* | 0.08 ± 0.00* [§] |
| Relative Weight (%) | 0.58 | 0.40 | 0.29 |

Symbols as in Table 1.

Table 3: Activities of MDA and catalase in the heart of Swiss albino mice (mean ± SEM)

| | Control | 20 mg/kg | 30 mg/kg |
|----------------------------------|-------------|--------------|---------------------------|
| MDA (mol/mg protein) | 0.78 ± 0.00 | 0.85 ± 2.31* | 0.94 ± 2.25* [§] |
| Catalase activity (U/mg protein) | 111 ± 0.00 | 117 ± 5.11* | 123 ± 5.13* [§] |

Symbols as in Table 1

derangement and cellular integrity and causing a defect in the ion channels of the heart: fast sodium and calcium sodium channels. In cardiac muscles, action potential is caused by the opening of the two channels which may be impaired by drug poisoning resulting in a decrease in the permeability of ions thus preventing early return of the action potential voltage to its resting level (Guyton and Hall 2000). 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.

The reported morphological alterations caused by the cytotoxic effect of chronic administration of quinine on the heart may be caused by oxidative stress. Oxidative stress can be caused by strenuous exercise, oxidation of food and other chemical reactions that occur in the cell (Coyle and Puttfarcken 1993). Oxidative stress leads to the

production of free radicals which damage and destroy cells (Anderson 2004) as evidenced in the dose dependent significant ($P<0.05$) increment in the activities of MDA observed in this study (Table

3). Our investigation has shown that chronic administration of quinine can lead to peroxidative injury.

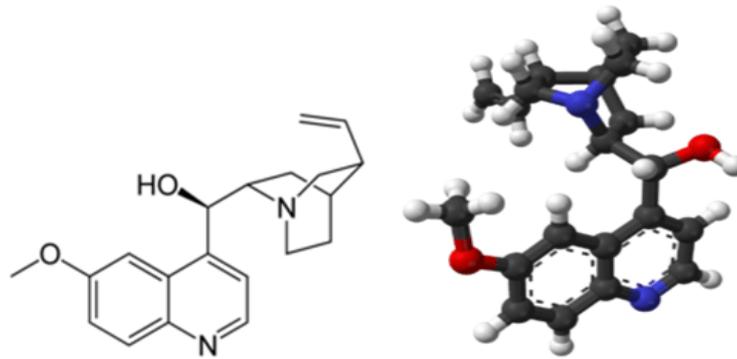


Fig. 1. **Chemical structure of quinine** (Barennes et al. 2006) (R)-(6-methoxyquinolin-4-yl)((2S,4S,8R)-8-vinylquinuclidin-2-yl) methanol.

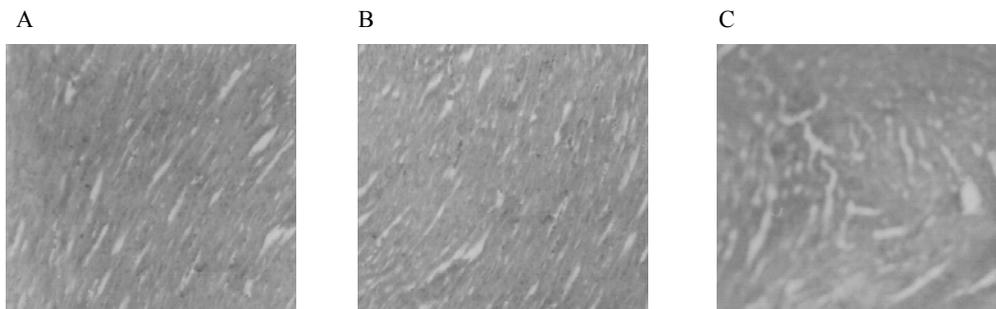


Fig. 2. **The myocardium:** (A) of the control , (B) after 20 mg/kg quinine, (C) after 30 mg/kg quinine. All tissue samples stained by haematoxylin and eosin.

Catalase is a major determinant of cardiac antioxidant status (Wohaieb and Godin 1987). It is known to be involved in detoxification of H_2O_2 concentrations (Yoshikawa et al. 1993, Manonmani et al. 2002). In toxicology, catalase, in particular, is important for the scavenging of hydrogen peroxides (Tatsuki et al. 1997). This may have been responsible for the dose dependent significant increment ($P<0.05$) observed in this study (Table 3) and believed to have been triggered by oxidative damage of cardiac tissue; most especially its membrane protein and lipid (Durdi and Timur

2007). Pieper et al. (1995) concluded that hydrogen peroxide is a major determinant of diabetic tissue damage. The increase in catalase activity under toxic conditions plays an important role in protection against oxygen stress in several tissues (Tatsuki et al. 1997).

The present investigation has shown that though quinine may be one of the most successful and widely prescribed drugs for the management of malaria, chronic administration may result in cardiac damage. It is therefore suggested that the drug be prescribed with caution in patients with

cardiac dysfunction. Self-medication involving quinine should also be discouraged as this may possibly lead to prolonged exposure or overdose and subsequent cardiac damage as observed in this investigation.

ACKNOWLEDGEMENT

The authors are grateful to E. Gbela of the Department of Morbid Anatomy and Forensic Medicine of the Obafemi Awolowo University, Nigeria, for his technical assistance.

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