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Some effects of chronic administration of chloroquine on the deoxyribonucleic acid (DNA) of the intracranial relay centres of adult Wistar rats

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ABSTRACT: The present study was carried out to assess some of the effects of chronic administration of chloroquine commonly used for prophylaxis or treatment of malaria, rheumatoid arthritis and lupus erythematosus on the deoxyribonucleic acid (DNA) of the inferior colliculus and medial geniculate body of adult Wistar rats.

Adult male and female Wistar rats (n=17) with average weight of 184g were randomly assigned into treatment (n=10) and control (n=7) groups. The rats in the treatment group received 2mg/kg body weight of chloroquine base dissolved in distilled water daily for fourteen days through orogastric tube administration. The control rats received equal volume of distilled water daily through the same route for fourteen days. The rats were fed with rat pellets purchased from Ladokun Feeds Ltd., Ibadan, Nigeria and given water liberally. The rats were sacrificed on day fifteen of the experiment and the inferior colliculus and medial geniculate body were carefully dissected out, and quickly fixed in 10% formal saline for histochemical procedures.

The histochemical findings showed that the sections of the inferior colliculus and medial geniculate body of the treated group exhibited less intense DNA staining and appearing pachychromatic. There were also observation of neuronal enlargement in the treated sections, while the histological findings showed pyknotic nuclei and microcystic changes appearing in the neutrophil.

These findings indicate that chronic administration of chloroquine had an adverse effect on the DNA of the inferior colliculus and medial geniculate body in adult wistar rats. Chloroquine may therefore have some adverse effects on the intracranial auditory relay sensibilities by these effects. It is therefore recommended for further studies aimed at corroborating these observations.

Key words: Chloroquine, DNA; Inferior colliculus; Medial geniculate body.

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Introduction

Chloroquine is given for malaria prophylaxis and treatment, but used by the rheumatologist for treating rheumatoid arthritis, systemic/discoid lupus erythematosus and other connective tissue disorders[1]. In malaria endemic areas like Africa, chloroquine has been greatly misused especially in cases of self-medication. In Nigeria, self-medication and drug misuse is on the increase due to prevailing economic conditions and deteriorating health care delivery system. An indication for chronic administration of chloroquine is in the prophylaxis of malaria for which the drug is administered at a dosage of 300 – 600mg weekly to adults[2].

Chloroquine crosses the blood brain barrier and has been reported to accumulate in the brain and other tissues.[2] Chloroquine intoxication leads to an increase in gangliosides in the nervous system with the largest effect in the dorsal root ganglion and retina as reported in pigs[3], rats[4], and rhesus monkey[5]. Rarely, neurologic symptoms such as vertigo, tinnitus, irritability, cranial nerve palsies and myasthenia-like muscle weakness, may also manifest following chloroquine treatment.

The inferior colliculus and medial geniculate body constitute the intracranial auditory relay centres. The inferior colliculus is the obligatory midbrain synaptic target of the ascending auditory pathway, in which the contralateral ear is represented primarily[6]. Inferior colliculus is essential for normal hearing and for the startle reflex; receives its ascending input mainly from the contralateral cochlear nucleus and the superior olive nucleus, and sends axons to the medial geniculate body[6]. The medial geniculate body is target of ascending projection from the inferior colliculus and descending input from the auditory cortex, this is the obligatory synaptic target in the thalamus for hearing[6]. It contains interleaved and overlapping tonotopic and aural bands, the most beautiful structure in the brain[6]. The cerebral cortex strongly affects the medial geniculate body through descending projections. These projections were thought to consist primarily of small areas with slow conduction velocities[7]. It has been demonstrated that neurons of auditory cortex showed great physiological plasticity when rats were exposed to specific stimuli coupled with concurrent stimulation of a forebrain sub-cortical structure in the nucleus basalis. Changes include massive expansion of frequency-specific representation[8].

Cortical structures such as the medial and lateral geniculate bodies, inferior and superior colliculi have higher glucose utilization than other structures[9]. There is a correlation between functional activity and metabolic rate such as in the visual and auditory system[9]. Since chloroquine crosses the blood brain barrier, it is relevant to investigate its effect on the intracranial auditory relay centres. It is probable that the adverse effects of chloroquine on hearing such as tinnitus may be due to direct effect of chloroquine on the auditory relay centres. Neurons and glia cells showed the presence of deoxyribonucleic acid (DNA), which are the transmitters of genetic information. The DNA is also involved in protein synthesis where information stored in them is transferred to RNA. Feulgen reaction specifically stained nuclear chromatin, the aldehyde liberated during hydrolysis is from the deoxypentose but not from ribose. The integrity of nuclear DNA is one of the most extensively used biochemical markers for cell death[10]. This present study was to elucidate the effects of chronic administration of chloroquine on the DNA of the intracranial auditory relay centres.

Materials and Methods

Animals

Seventeen adult Wistar rats of both sexes, weighing 180-200g were randomly assigned into two groups, control (n=7) and treated (n=10). The rats were maintained in the animal holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Osun State. They were fed with rat pellets purchased from Ladokun Feeds Ltd., Ibadan, Nigeria and given water liberally. Chloroquine phosphate tablets were obtained from the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Drug Administration

The rats in the treatment group received 2mg/kg body weight of chloroquine base dissolved in distilled water for fourteen days. The rats in the control group received equal volume of distilled water using

orogastric tube. The rats were sacrificed by cervical dislocation on the 15th day. The skulls were opened using bone forceps to expose the brain of the rats. The inferior colliculus and medial geniculate body were quickly dissected out and fixed in 10% formal saline for routine DNA staining techniques.

Histochemical study

The tissues were dehydrated in an ascending grade of alcohol (ethanol) cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffused sections were stained routinely for DNA using Feulgen and Rosenbach (1924) staining procedures. The sections were then rinsed in distilled water, dehydrated through ascending grades of alcohol, cleared in xylene and mounted in DPX for DNA observation. The digital photomicrographs of the desired sections were then made for further observation.

Results

The control sections of the inferior colliculus (IC) and medial geniculate body (MGB) stained deep magenta colour. The stained nuclei were distinct, numerous, widely distributed and did not appear enlarged (Plate 1A and 2A).

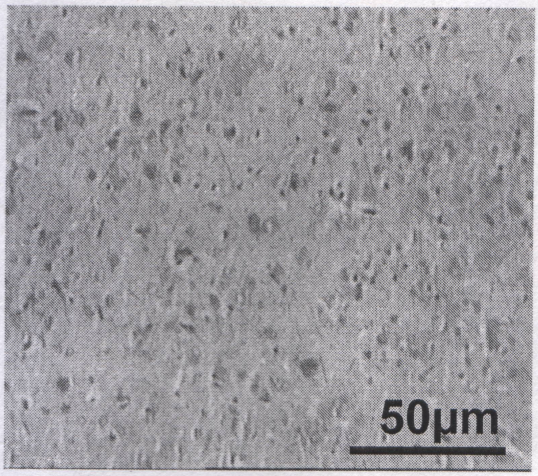
The treated sections of the inferior colliculus (IC) and medial geniculate body (MGB) showed less intense staining and appearing pachychromatic. There were microcystic changes appearing in the neuropil and an observable neuronal enlargement (hypertrophy) in the sections of the treated inferior colliculus and medial geniculate body. The H and E histological staining showed the presence of some pyknotic nuclei and some microcystic changes (Plate 1B and 2B).

Discussion

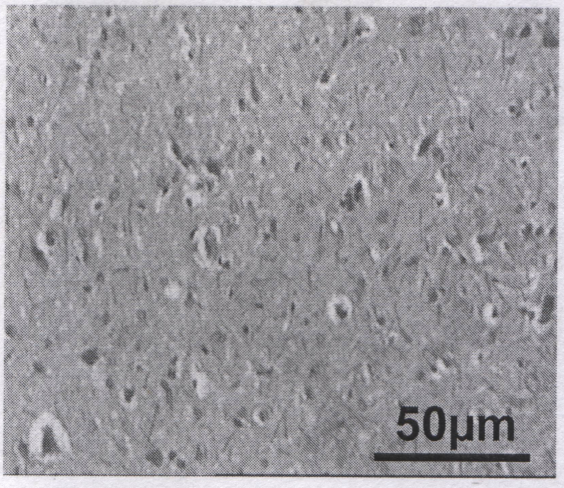
Neurons and glia cells showed the presence of deoxyribonucleic acid which are the transmitters of genetic information. The DNA is also involved in protein synthesis where information stored in them is transferred to ribonucleic acid. The neurons are differentiated from glia by the presence of a large sized nucleus, which stained more intensely. Feulgen reaction specifically stained nuclear chromatin, the aldehyde liberated during hydrolysis is from deoxypentose but not from ribose. DNA is important for normal maintenance of cellular integrity. Chloroquine acts on cell sites by binding to deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins. This binding alters the biological and physiological characteristics of DNA helix[10]. In this study, chloroquine administration was observed to affect the staining intensity of nuclei in the neurons and glia cells. The cells that stained following chloroquine administration had enlarge darkly stained nuclei among others. The enlarged darkly stained DNA in the neurons may indicate possible adverse effects of chloroquine on DNA integrity. Chloroquine has been reported to cause complete inhibition of DNA synthesis[10]. It interferes with protein synthesis through the inhibition of DNA replication[11]. This interference might be the probable cause of the hypertrophied nuclei observed in this experiment. Nuclei hypertrophy leads to cell death, which may underscore the reduction in cellular density following treatment with chloroquine.

The integrity of nuclear DNA is one of the most extensively used biochemical marker for the cell death[12]. This work has been shown to cause pyknotic nuclei and microcystic changes in the neurophil of the inferior colliculus and medial geniculate body using the H and E histological staining technique. In cellular necrosis, endonucleases cleave DNA cells selectively because proteaoses are coactivated, causing digestion of histones and increased accessibility of nucleosomal DNA producing random DNA digestion[13]. It has been reported that during cell death, calcium ion (Ca^{2+}) and magnesium ion (Mg^{2+}) activate endonucleases to digest DNA[14].

PLATE 1

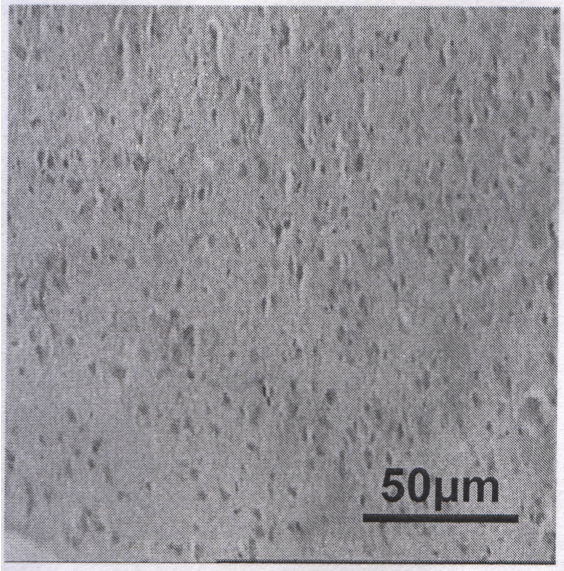


A - Control

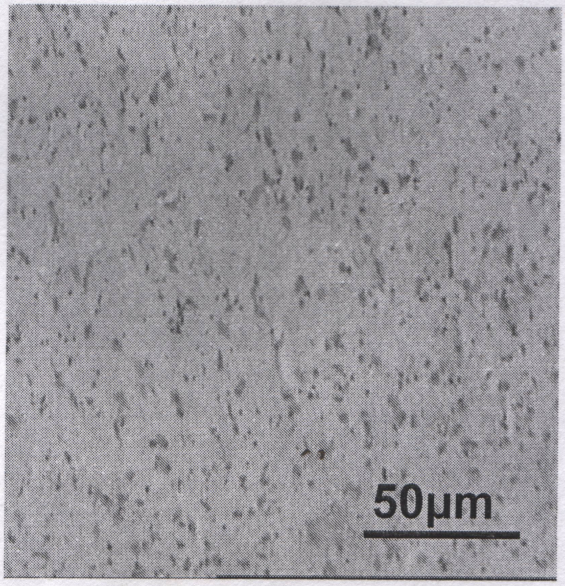


B - Treated

General histology of the inferior colliculus (H & E method)

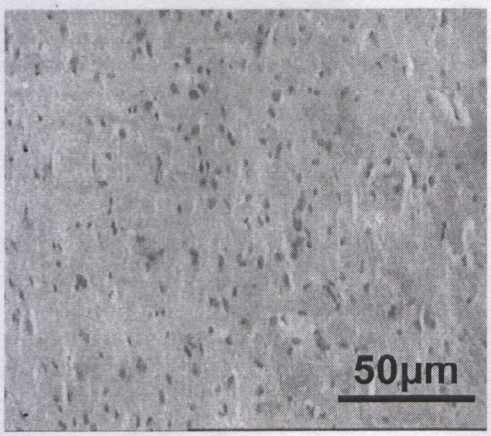


A - Control

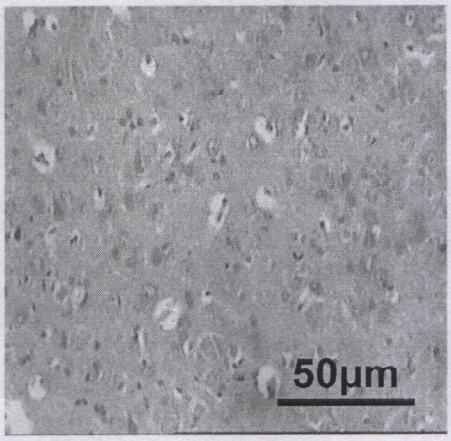


B - Treated

PLATE 2



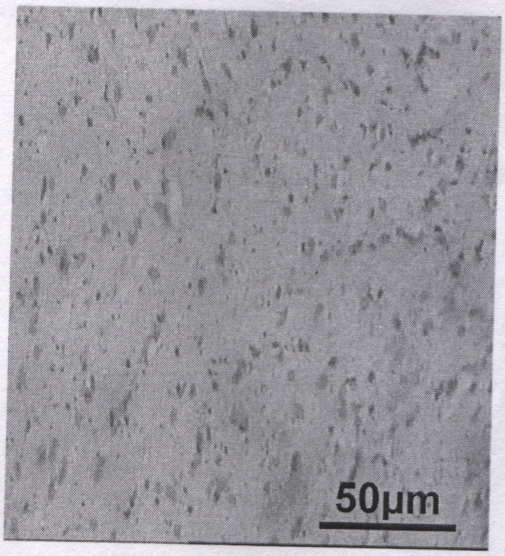
A - Control



B - Treated

General histology of the medial geniculate body (MGB) (H & E method)

A - Control



B - Treated

Deoxyribonucleic acid (DNA) in the medial geniculate body (MGB)

(Feulgen and Rossenbach method)

Conclusion

In conclusion, our study revealed that chronic administration of chloroquine may therefore have some toxic effects on the intracranial auditory relay centres such as the inferior colliculus and medial geniculate body in adult wistar rats. The chronic administration of chloroquine result in pyknotic nuclei and some microcystic changes in the neuropils of the inferior colliculus and medial geniculate body. The DNA of the inferior colliculus and medial geniculate body also show some evidence of hypertrophy and appearing pachychromatic. These results indicate that chronic administration of chloroquine may affect the functions of these two intracranial auditory relay centres in auditory sensibility. These effects could obviously affect the integrity and competence of the two intracranial auditory relay centres in regard to their activities mediated in auditory sensibility.

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