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Some histological effects of chronic administration of chloroquine on the medial geniculate body of adult wistar rat

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Summary

Some histological effects of chronic administration of chloroquine commonly used for prophylaxis or treatment of malaria, rheumatoid arthritis and lupus erythematosus on the medial geniculate body (MGB) of adult wistar rats was carefully studied. The rats of both sexes (n=18), 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 the orogastric tube administration while the control rats received equal volume of distilled water daily through the same route. The rats were fed with rat pellets purchased from Topfeed Ltd, Sapele, Delta State, Nigeria and given water liberally and were then sacrificed on day fifteen of the experiment. The MGB were carefully dissected out and quickly fixed in 10% formal saline for routine histological study after H & E and thionin methods. The histological findings after H & E methods indicated that the treated sections of the MGB showed faintly reduced nuclei size, with the presence of many autophagic vacuoles and degenerative neurons when compared to the control sections. On the other hand, the thionin method indicated that the treated sections showed sparsely distributed neurons, which stain less intensely when compared with the control. The nissl substance in some of the neurons appeared degenerative while some hypertrophied with some vacuolations. These findings indicated that chronic administration of chloroquine has a deleterious effect on the neurons and nissl substance of the MGB. Chloroquine may probably have adverse effects on auditory sensibilities by its deleterious effects on the nerve cells and nissl substances of the MGB of the adult wistar rats. It is recommended that further studies aimed at corroborating these observations be carried out.

Keywords: *Chloroquine, histological effects, medial geniculate body and wistar rats*

Résumé

Cette étude avait pour but d'évaluer certains effets histologiques de l'usage régulier et chronique de la chloroquine en prophylaxie ou pour le traitement du paludisme, du rhumatisme arthrite et l'erythromatose lupus des genicules médiales aux rats adulte. Au total 18 rats, de poids moyenne de 148g étaient choisi au hasard et groupes en 10 traites et 8 contrôles. Chaque rat recevait 2mg/kg de chloroquine par poids coporel dissous dans de l eau distille et administre pour 14 jours, et sacrifie au 15ieme jour. Par la voie orogastrique. Les rats étaient nourris avec des cubes manufactures par Topfeed Ltd, Sapele, Delta State, Nigeria. Les Géniculés médiale du corps étaient dissectés, fixés avec 10% de sel pour l'étude histologique après les méthodes de H & E et thionine. Les données histologiques après les méthodes H et E indiquaient que les sections traites du MGB demonstraient une réduction nucléaire avec la présence de plusieurs vacuoles autophagiques et les neurones degenerative comparant avec les mêmes sections chez le groupe de contrôle. La substance de Nissl de certains neurones apparaient degenerative alors que certaines hypertrophiaient avec des vacuolisations. Les résultats indiquaient que l usage chronique de la chloroquine a des effets dérisoires sur les neurones et la substance de Nissl aux genicules médiales du corps et probablement des effets adverses sur la sensibilité auditive chez les rats adulte. Il est recommandé de faire des études approfondies pour élucider ces observations.

Introduction

Chloroquine is a commonly used antimalaria drug that belongs to the quinolone family. It was given for malaria prophylaxis and treatment, but used by the rheumatologists for treating rheumatoid arthritis, systemic/discoid lupus erythematosus and other connective tissue disorders [1]. In Nigeria, self-medication and drug misuse is on the increase due to the prevailing economic conditions and deteriorating health care delivery systems. 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].

It has been reported that some patients with retinopathy may be asymptomatic following chloroquine treatment. When they are asymptomatic, visual acuity

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initially remains excellent despite complaints of parafoveal metamorphopsia and difficulty in reading or performing fine visual task due to central or paracentral scotomas. Neurologic symptoms, such as vertigo, tinnitus, irritability, cranial nerve palsies and myasthenialike muscle weakness, may also manifest following chloroquine treatment [1].

The effects of chloroquine on the intracranial auditory pathway is very little and few literatures had been documented. The MGB and inferior colliculus form the intracranial auditory relay centres. The MGB is the target of the ascending projections from the inferior colliculus and descending input from the auditory cortex. The MGB is the obligatory synaptic target in the thalamus for hearing and it contains interleaved and overlapping tonotopic and aural bands [6]. The cerebral cortex strongly affects the MGB through descending projections thought to consist primarily of small areas with slow conduction velocities [7].

Cortical nuclei such as the medial and lateral geniculate bodies, inferior and superior colliculi have higher glucose utilization than other structures [8]. There is also a correlation between functional activity and metabolic rate such as in the visual and auditory system [8]. Nissl substances are reported to play key roles in cellular metabolism [9]. They are nodal points in the endoplasmic reticulum, which permeates the cell body and dendrites, but are absent at the axon and axon hillock [10]. Tissue enzymatic activities prove reliable for interpretation purposes, since they condition the rate of cerebral biochemical reactions in which modifications in substrate intermediates and end products represent the effect of their action [8].

Since chloroquine crosses the blood brain barrier, it is relevant to investigate its effect on the histology of the MGB. It is probable that the adverse effects of chloroquine on hearing, such as tinnitus may be due to direct effect of chloroquine on this auditory relay center. The present study was to elucidate some histological effects of chloroquine on the MGB of adult wistar rat.

Materials and methods

Animals

Seventeen adult wistar rats of both sexes, weighing 180-200g were randomly assigned into two groups, control (n=7) and treatment (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 pellets purchased from Topfeed Ltd., Sapele, Delta State, Nigeria (nutrient value of the feed – Crude Protein 16%, Energy 11.1MJ, Calcium 1.0%, Phosphorus 0.7%, Fibre 7.0%, Oil 5.0%) and given water liberally. Chloroquine phosphate tablets were obtained from the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife.

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 MGB was quickly dissected out and fixed in 10% formal saline for routine histological techniques.

Histological 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.

Some of the deparaffinised sections were stained for 1-6hrs in 0.2% thionin and monitored microscopically and then rinsed in distilled water, differentiated in 95% ethanol, dehydrated in one change of absolute alcohol, cleared in xylene and mounted in DPX for nissl substance observation [12], while other deparaffinised section were stained routinely with haematoxylin and eosin (H&E) method [13]. The digital photomicrographs of the desired sections were made for further observation.

Results

The sections of the MGB from the controls showed normal histological features with the neurons appearing distinct and the glial cells normal without vacuolations in the stroma (Fig.1A), while the nissl substance stained intensely with uniform distribution (Fig.2A).

The sections of the MGB from the treatment group showed some cellular degenerative changes, vacuolations appearing in the stroma and with some autophagic vacuoles (figure 1B), while the nissl substances in the treatment sections of the MGB appeared sparse and unevenly distributed when compared with the control sections (Fig. 2B). The nissl substance in some of the neurons also appeared degenerative while some hypertrophied. There were more spaces in between the neurons in the treatment group than the control (Fig.2B).

Fig. 1a. Photomicrograph of the medial geniculate body (MGB) of the control section (H & E method x 100)

Fig. 1b: Photomicropgraph of the medial geniculate body (MGB) of the treatment section (H & E method x 100)

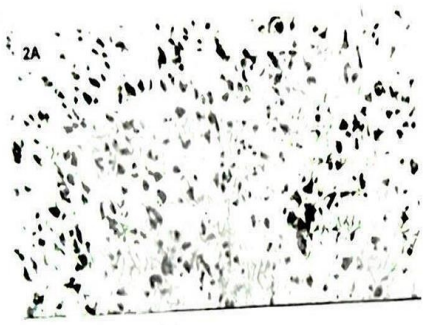

Fig. 2a: Nissl substance in the medial geniculate body (MGB) of the control section (Thionin stain x 100)

Fig. 2b: Nissl substance in the medial geniculate body (MGB) of the treatment section. (Thionin stain x 100)

Discussion

The result (H&E) revealed that chronic administration of chloroquine caused some cellular degenerative changes with vacuolations appearing in the treatment group compared with the control sections of the MGB. The nissl substance stained less intensely and some of the neurons appeared degenerative while some hypertrophied in the treatment sections of the MGB. There were more spaces in between the neurons of the MGB in the treatment group than that of the control group.

Neuronal degeneration has been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death. These two types differ morphologically and biochemically [14]. 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 [15]. It was reported that cell death in response to neurotoxins might trigger an apoptotic death pathway within brain cells [16]. Cell death in response to neurotoxins occurs as a controlled event involving a genetic programme in which caspase enzymes are activated [16].

The process of cellular necrosis involves disruption of membrane's structural and functional integrity. Cellular necrosis is not induced by stimuli intrinsic to the cells as in programmed cell death, but by an abrupt environmental perturbation and departure from the normal physiological conditions [17]. Extensive cell death in the central nervous system is present in all neurodegenerative disease [16]. The type of nerve cell lost and the particular part of the brain affected dictate the symptoms with an individual disease [16].

In this study, chloroquine phosphate could have acted as toxins to the cells of the MGB affecting their cellular integrity and causing a defect in membrane permeability and cell volume homeostasis. Chloroquine is known to cross membranes by simple diffusion thus getting access to the cells [18]. This property of chloroquine could have been one of the causes of the degenerative changes observed in this study. In cellular necrosis, the rate of progression depends on the severity of environmental insults. The greater the severity of insults, the more rapid the progression of neuronal injuries [19]. The principle is true for toxic logical insults to the brain and other organs [17]. The prime candidates for inducing the massive cell destruction observed in neurodegeneration are neurotoxins [16]. It may be inferred from the present results that prolonged administration of chloroquine resulted in increased toxic effects on the MGB.

The decrease in neuronal density of the treatment group as reported in this study may have been as a result of cell death caused by the toxic effect of chloroquine. The degenerative processes caused by chloroquine have been reported to lead to the formation of

dense bodies, myelin figures and electron dense whorls in nervous tissues [3,4,5].

Nissl substances are reported to play key roles in cellular metabolism [9]. Nissl bodies are nodal points in the endoplasmic reticulum, which permeates the cell body and dendrites and they are absent at the axon and axon hillock [10]. Under the control of nuclear deoxyribonucleic acid (DNA), cytoplasmic RNA is concerned with protein synthesis. The protein synthesized is transported down the axon by axoplasmic flow or transport. Nerve cells require large amounts of protein to maintain their integrity and perform their functions [10].

Degeneration of Nissl substance is usually characterized by disintegration resulting in powdering remains that are confined to the periphery of the cell. The nissl substances have been reported to be altered by chemicals, toxins, certain drugs and oxygen-lack causing loss of function or interference in normal metabolism [20]. Neuronal degeneration is reported to cause a decrease in nissl bodies as chromatolysis occurs [17]. During necrotic cell death, ribosomes are dispersed from the rough endoplasmic reticulum and polyribosomes disassociate resulting in a number of monomeric ribosomes that are found free in the cytoplasm [17]. In this study, neuronal degeneration was observed in sections of the MGB in rats treated with chloroquine. The decrease in the staining intensity of the nissl substance in the treatment group may be due to the deleterious effects of chloroquine on the nissl substances and consequently cellular metabolism.

In this study, chloroquine was observed to cause disintegration of Nissl substances in the MGB of the treatment group. This underlines a possible interference in protein synthesis in this auditory relay centre. It is probable that this consequence of chloroquine on the cellular metabolism of neurons and the neuronal degenerations on medial geniculate body in the treatment group may cause damage to the nervous system (Brain and Spinal cord) of the fetus, including damage to hearing reported following chloroquine administration.

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