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Morphological effects of chronic administration of zidovudine on the intracranial auditory relay centers of adult Wistar rats

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Abstract:

BACKGROUND: Zidovudine is one of the very active antiretroviral treatments which have been effective in several combination regimens for the treatment of HIV disease. Widely accepted theories have recognized that such treatment regimens agents are capable of penetrating the blood brain barrier and may continue to exert damage to the brain, after antiviral treatment.

AIMS AND OBJECTIVES: The objective of this study is to investigate zidovudine-induced morphological changes in auditory relay centres of the brain. It is likely that the adverse effects of zidovudine as reported by several researches may be due to some underlying effect on the microanatomy of the intracranial auditory relay centres.

MATERIALS AND METHODS: Forty rats with an average weight of 200g were randomly assigned into treatment (n1=20) and control (n2=20) groups. Zidovudine was dissolved in distilled water and a dose of 300mg/70kg (0.857mg/200g) was calculated and administered twice daily to the rats of the treatment group orally by the use of an orogastric tube for thirty days. The control group received equal volume of distilled water as placebo. The rats were later sacrificed by cervical dislocation and the inferior colliculi and medial geniculate bodies were dissected for morphometric and histological analysis.

RESULTS/DISCUSSION: Results from this study revealed a significant increase ($p < 0.05$) in weight of the inferior colliculus of the treated rats but a significant decrease ($p < 0.05$) in weight of the medial geniculate body of the treated rats, as compared to their corresponding control groups. Histologically, the treated tissues revealed similar necrotic and cellular degenerative changes in the stroma when compared to tissues from the control group. These observations confirm the adverse effects of Zidovudine on the inferior colliculus and medial geniculate body of adult Wistar rats. However, it was also observed in this study that the effect of zidovudine was not similar on the two intracranial relay centres as previously believed.

CONCLUSION: Further studies are needed in corroborating these observations, especially to determine the mechanism of hearing loss that is associated with zidovudine therapy..

Keywords:

Inferior colliculus, medial geniculate body, morphological effects, Wistar rats, zidovudine

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Introduction

The inferior colliculus (IC) and medial geniculate body (MGB) constitute the intracranial auditory relay centers (Winer *et al.*, 2002; Müller-Preuss and Mitzdorf, 1984; Razak *et al.*, 2009). It has been acknowledged that MGB is an obligatory synaptic target for hearing in the thalamus and receives input from neurons

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of the IC as well as from the auditory cortex, whereas the IC is where the contralateral ear is represented (Winer *et al*., 1998; 1999; 2002). In a previous study on this series, it was observed that efavirenz, which may cross the blood–brain barrier less than zidovudine (AZT), has adverse effect on the chromatophilic substances of the IC and MGB of adult Wistar rats (Adjene and Igbigbi, 2012).

AZT is one of the "highly active antiretroviral therapies" (HAART), which has been effective in many combination regimens for the treatment of HIV infection (Gulick *et al*., 2006; Rutherford *et al*., 2003). However, there is still the notable two-sanctuary concept (Varatharajan and Thomas, 2009; Wynn *et al*., 2002), whereby it is recognized that HAART agents capable of penetrating the blood–brain barrier may continue to exert damage to the brain tissues, after antiviral treatment. Given that AZT could penetrate the blood–brain barrier (Ene *et al*., 2011), the hypothesis has been based on the association of AZT with hearing loss. Marra *et al*. and Buriti *et al*. observed a significant association between hearing loss and antiretroviral therapy (Marra *et al*., 1997; Buriti *et al*., 2013), as well as potential oxidative stress changes (Teranishi *et al*., 2012; Angeli *et al*., 2012). On the contrary, a Schouten *et al*. report does not support the notion that AZT treatment could cause damage to hearing (Schouten *et al*., 2006).

The objective of this particular piece of study is to investigate morphological changes on the IC and MGB AZT attributable to AZT therapy. It is probable that the adverse effects of AZT concerning abnormal vivid dreams, confusion, and tinnitus among others may be due to some underlying effect on the microanatomy of the intracranial auditory relay centers (Marra *et al*., 1997).

Materials and Methods

Animals care and ethics

Before the start of experimentation, ethical approval was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka with reference number DELSU/BMS/ANA/13/14/0005 dated February 2, 2014. Forty adult Wistar rats of both sexes, weighing between 180 and 220 g, were randomly assigned into two groups: Control ($n_1 = 20$) and treatment ($n_2 = 20$). The rats were obtained and maintained in the Animal Holdings of the Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka. The rats were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given water liberally. AZT was obtained from the President Emergency Plan for AIDS Relief (PEPFAR) Unit, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria and manufactured by GlaxoSmithKline of the United State of America.

Drug administration

The rats in the treatment group received 300 mg/70 kg (0.857 mg/200 g) body weight of AZT being the dosages required twice daily. The drug was dissolved in distilled water and administered twice daily for 30 days through the orogastric tube while the control rats received equal volume of distilled water through the same route and for the same period.

Dissection of inferior colliculi and medial geniculate bodies

On day 31st of the experiment, the rats were reweighed and sacrificed by cervical dislocation. The heads were harvested by a small craniotomy prepared rostral to the lambdoidal suture about 1.5–2 mm lateral to the midline, thereby exposing the posterior aspect of the thalamus. Consequently, the inferior colliculi and MGBs were carefully exposed and dissected out, dried and weighed using Toledo weighing balance (METTLER TOLEDO, UK), and were quickly fixed in 10% formal saline for further routine histological techniques.

Histological study

The inferior colliculi and MGBs were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene, and embedded in paraffin wax. Serial sections of about 6 microns thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with hematoxylin and eosin method (Drury *et al*., 1976). Photomicrographs of the desired results were obtained using research photographic microscope (LABOMED, LX400, UK) in the Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Results

Effects on weight

The results indicated that there was a significant increase ($P < 0.05$) in the relative weight (%) of the treated IC and a significant decrease ($P < 0.05$) in the relative weight (%) of the treated MGB as compared to their corresponding control [Table 1].

Table 1: The mean weights of the inferior colliculi and medial geniculate bodies

	Group of animals	
	Control (n=20)	Treatment (n=20)
Brain weight (g)	1.65±0.03	1.81±0.04
IC weight (g)	0.12±0.01*	0.18±0.03*
Relative IC weight (%)	7.10±0.60*	10.10±1.41*
MGB weight (g)	0.20±0.02*	0.16±0.01*
Relative MGB weight (%)	12.44±1.23*	8.57±0.65*

*Significant (P<0.05). IC - Inferior colliculus, MGB - Medial geniculate body

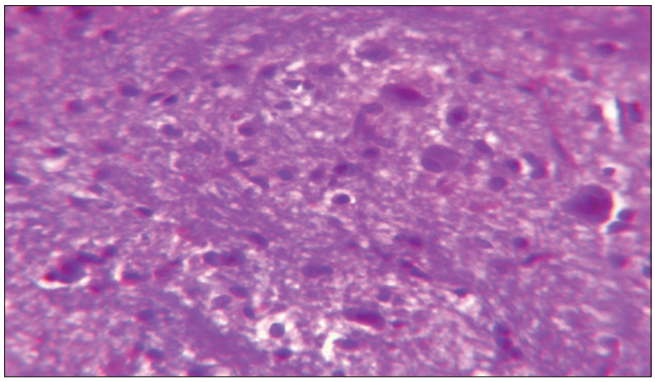

Figure 1: The desired sections of the inferior colliculus from the control animals showed normal histological features with the neurons appearing distinct and of various sizes. The neuron and glial cells appeared normal and no vacuolations in the stroma of the sections (Plate A)

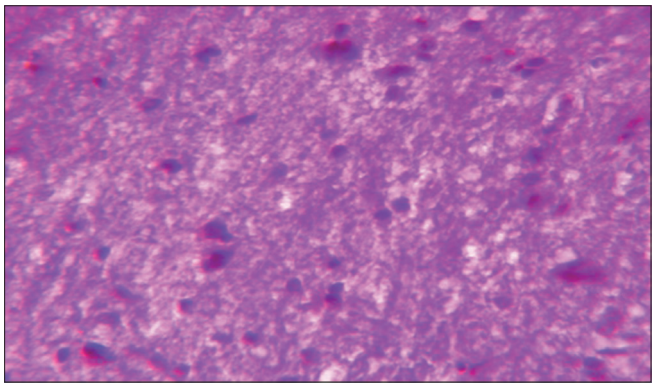

Figure 3: The desired sections of the medial geniculate body from the control animals showed normal histological features with the neurons appearing distinct and of various sizes. The neuron and glial cells appeared normal and no distinct vacuolations in the stroma of the sections (Plate A)

Effects on morphology

The desired sections of the IC and MGB from the control animals showed normal histological features with the neurons appearing distinct and of various sizes. The neuron and glial cells appeared normal, and there were no vacuolations in the stroma of the sections [Figures 1 and 2]. The histological sections of the IC and MGB of the treated group revealed some necrotic and cellular degenerative changes such as pyknotic nuclei, sparse cellular population, and some vacuolations in the stroma of the IC and MGB as compared to the control group [Figures 3 and 4].

Discussion

Confounding nature of observation

The morphometric result of this research revealed that chronic administration of AZT showed a significant increase in the relative weight of the IC but significantly reduced the relative weight of the treated MGB [Table 1]. This portends that long-term AZT therapy can significantly increase the weight of IC while simultaneously significantly reducing the weight of MGB.

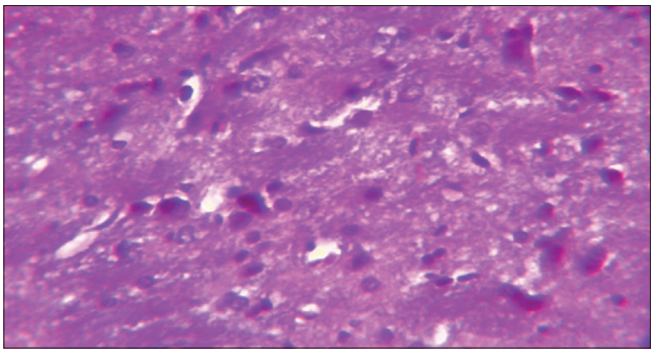

Figure 2: The inferior colliculus of the treated group revealed some cellular degenerative changes such as sparse cellular population, hypertrophy, and some vacuolations in the stroma of the inferior colliculus as compared to the control group (Plate B)

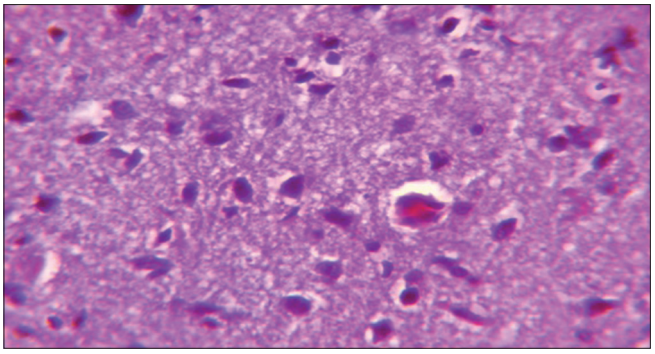

Figure 4: The medial geniculate body of the treated group revealed some necrotic and cellular degenerative changes such as pyknotic nuclei and autophagic vacuoles in the stroma of the medial geniculate body as compared to the control group (Plate B)

On one hand, this is quite a confounding observation; though on the other hand, it could be explained in simplistic terms that oxidative damage in the brain presents with varying degrees of morphological changes (Bremner, 2006; Bremner *et al*., 2008; Lucassen *et al*., 2014; Landgrebe *et al*., 2009). Indeed, previous results had indicated the possibility of oxidative stress-inducing damage (Adjene and Igbigbi, 2012; Igbigbi *et al*., 2013).

The observation of necrotic and cellular degenerative changes including sparse cellular population and varying degree of vacuolations in the stroma of the treated tissues should ideally amount to less weight. This supports the concept of brain atrophy being associated with stress (Bremner, 2006) and our finding of reduced weight in MGB. On the contrary, the observation of increased weight in IC seems confounding. It has been speculated that pharmacologic disruption of cellular systems, especially the blood–brain barrier, can cause swelling of the brain parenchyma which may lead to a net shift of water from the extracellular space to the interior of the brain cells (de Vries *et al*., 2009) and this could possibly explain the observed findings above. That is, cytotoxic edema may involve intracellular swelling of glial, endothelia, and neurons. It has also

been recently reported that cerebral edema in the brain hemispheres and cerebellum correlates with the tissue levels of oxidative stress indices as well as necrotic signs of degenerative changes (Cretu *et al*., 2010). Even if these effects (i.e., weight being increased in IC but decreased in MGB) may be explained by separate theories, it is still confounding that AZT has different levels of effect on the two intracranial auditory relay centers.

Corroboration with previous reports and potential implication

These different levels of effects reported in this research were observed in the measurement of oxidative stress indices, whereby malondialdehyde and superoxide dismutase were lower in the IC but higher in MGB of AZT-treated tissues (Adjene and Igbigbi, 2012). The differences were also observed in efavirenz treatment study (Adjene and Igbigbi, 2012). It is known that AZT therapy is associated with hearing loss (Marra *et al*., 1997; Buriti *et al*., 2013; Sagwa *et al*., 2013), and this was reflected histologically by the presence of necrotic and degenerative changes in the nuclei of both the IC and MGB. The mechanism of the hearing loss is likely due to interruptions at the midbrain level of ascending auditory connections. Most of the ascending and descending fibres of the auditory pathways synapse at the IC; therefore, bilateral lesions of inferior colliculi will definitely affect hearing loss. Contrarily, in a study on novel tinnitus generators, it was observed that only the MGB showed consistent activity pattern of increased activity in ablated rats and noted that the IC may not be involved (Bauer *et al*., 2013).

It is probable that the degree of loss of hearing attributable to pharmacologic adverse effect may be dependent on the impact on only one of the two auditory relay centers. Hence, the follow-on hypothesis from these findings is that since the MGB is known to be the major auditory nucleus of the thalamus, the IC and MGB may contribute unequally to hearing loss. This is because the AZT-induced and pharmacologic adverse effects are restricted to the MGBs only without the IC; the fibers going to the superior olivary nucleus will affect only one side hearing and thus could be a possible explanation for the observation in this study. This hypothesis supports the suggestion that elucidation of such mechanism could benefit pharmacologists in developing specific therapies either for hearing loss (Bauer *et al*., 2013) or with limited adverse effects on the auditory function.

Conclusions

Chronic administration of AZT has adverse effects on the microanatomy of IC and MGB of adult Wistar rats. However, there is a confounding difference in the effects of AZT on the two intracranial relay centers. The observation of dissimilar effect in relative weight corroborates with different levels of effect on some oxidative stress indices. Further studies are important to elucidate which of these effects has more impact on hearing loss.

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Conflicts of interest

There are no conflicts of interest.

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