Journal of Experimental Research

March 2020, Vol 8 No 1

Email: editorinchief.erjournal@gmail.com editorialsecretary.erjournal@gmail.com

Received: 02/05/2020
Accepted for Publication: 02/06/2020

ANXIOLYTIC AND ANTI-INFLAMMATORY CO-TREATMENT EFFECTS ON ISONIAZID-INDUCED SEIZURE.

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ABSTRACT

This study investigated the adjuvant effect of anxiolytic and anti-inflammatory co-treatment on Isoniazid-induced seizure. A total of twenty (20) healthy adult Wistar rats, with average weight of 210kg were used, and divided into five (5) groups of four(4) rats per a group. Seizures were induced in the animals in groups 2, 3, 4, 5 using 300mg/kg isoniazid single dose p.o, while animals in group 1 served as control group and were given 0.1ml of normal saline (placebo). Group 2 was the untreated (positive) seizure group, while animals in group 3 were treated with 5mg/kg diazepam and group 4 were treated with 50mg/kg hydrocortisone, those in group 5 were treated with combination of 5mg/kg diazepam and 50mg/kg hydrocortisonefor 5days. Sections of the hippocampus were immunohistochemically stained for astrocyte expression using Glial fibrillary acidic protein (GFAP) marker. The seizure group exhibited positive immunoreactivity to GFAP with astrogliosis. Hydrocortisone and diazepam groups revealed restored normal astrocytes expression. The combined therapy provided a synergist effect depicted as retraction of most astrocytes. So, a combination of anxiolytic and anti-inflammatory agents should be considered in seizure management.

Keywords: Anxiolytic, Anti-inflammatory, Seizure, Astrocytes, Steroids

INTRODUCTION

Status epilepticus is a life-threatening neurological emergency with high mortality and common in the management of tuberculosis infection (Pitkanen et al. 2005).Tuberculosis (TB) is an airborne disease caused by Mycobacterium tuberculosis (Mtb), that usually affects the lungs (Nicole, 2015).M. tuberculosis infection occurs through inhalation of infectious aerosol released from close contact (Cruz-Knight and Blake-Gumbs, 2013; Berry et al., 2013; Nicole, 2015). Although, many inherent factors interplay in the surveillance of Mtb, including the difficulty in obtaining the right drug for treatment, drug resistance and comorbidity, with infection, anxiety, inflammation and seizure (Toossi, 2000; Smitha and Jordi, 2011; Grab et al. 2019). The seizure episode is often due to the secondary effect of drug treatment particularly with Isoniazid.

Isoniazid (INH) is one of the potent antimycobacterial agents that inhibit cell wall synthesis of *Mycobacterium tuberculosis* (Mtb) and it is used in both therapeutic and prophylactic regimens of tuberculosis (Toossi, 2000; Smitha and Jordi, 2011; Blise et al. 2017; Grab et al. 2019). Resistance to INHis common and has been linked to risk of acute neurotoxicitywith single high therapeutic dose, which manifests as neurological side effects, including peripheral neuritis, dizziness, and insomnia and seizures (Fekit et al. 2011; Cruz-Knight and Blake-Gumbs, 2013; Nicole, 2015). The important mechanism is a deficiency of gamma-aminobutyric acid (GABA) and pyridoxine induced by INH leading to reduced production of GABA, as it is usually a product of pyridoxine-dependent decarboxylation reaction (Finbarrs-Bello et al. 2019). The hallmark of GABA deficiency manifest as seizures which can be managed with the use of benzodiazepines, and pyridoxine in humans and rodents, this helps with rapid restoration of GABA stores and resolution of the seizure (Yasudishi et al., 2005; Finbarrs-Bello et al. 2019).

Anxiety disorders represent another

common psychiatric co-morbidity in patients with epilepsy, affecting prognosis and quality of life (Kroenke et al. 2007). The associated sleep deprivation also increases the risk of seizures attacks (Mato et al. 2011). Anxiolytics, or anti-anxiety drugs are recommended to prevent and treat anxiety, which targets key chemical messengers in the brain such as dopamine, serotonin, and GABA, to help decrease abnormal excitability (Beldum, 2008; Durant et al. 2010; Mula, 2016). One of the frequently prescribed anxiolytics (benzodiazepine), diazepam suppresses generalized seizures and effectively stops absence, infantile, and other myoclonic seizures and even restores normal sleep patterns (Ravindran et al. 2010; Mula, 2016). They are the drug of choice used for arrest of status epilepticus and all kinds of eclamptic convulsions (Ravindran et al. 2010).

In addition, the core of immune response to every infection is inflammation; there are some reports on the development of inflammatory reactions during seizure attacks, including increased release of chemokines, pro-inflammatory cytokines and prostaglandins in the brain of rodents (Vezzani et al. 2002; Vezzani and Tiziana, 2005). It is well established that inflammatory mediators are also produced by brain parenchymal cells (microglia, astrocytes, and neurons) and by cells of the blood-brain barrier (BBB) and choroid plexus (Vezzani, 2012). Also, there is evidence that inflammation has significant effects on blood brain barrier (BBB) integrity, affects normal brain function and contributes to the pathophysiology of seizures (Marchi et al. 2007). Insight into the inflammatory response supports the use of anti-inflammatory agents as adjuvants in the treatment of tuberculosis (Toossi, 2000; March et al. 2011; Zumla et al. 2014). As such, corticosteroid (e.g. hydrocortisone) treatment has been proven to be beneficial to survival of TB patient even with seizure (Senderovits and Viskum, 1994; Grosso et al. 2008; Schutz et al. 2018; Grab et al. 2019).

These commonalities highlight the possibility that both anti-inflammatory and anxiolytics or even their combination may play a potential role in treatment regimens for seizure. In this study we targeted brain resident innate immune cell, the astrocytes, which are involved in promoting neuronal survival and drug metabolism in the brain, using an animal model of chemical-induced status epilepticus seizure that can be seen in infections like tuberculosis.

MATERIALS AND METHODS

Drugs

Isoniazid PubChem CID; 3767 (Seizure inducing drug), Diazepam PubChem CID; 3016 (Anxiolytic agent), Hydrocortisone (Solu-cortef) PubChem CID; 5754(Anti-inflammatory drug) were procured from the registered pharmacist at Enugu state.

Animals

The experiment was performed on 20 inbred adult Wistar rats (11 weeks old) weighing 210±20g and age-matched. The rats were housed in the animal facility of the animal house of ESUT College of Medicine, Enugu, Nigeria. The rats were housed (4 per cage) under standardized conditions (25^{o}C, 40–50% humidity; 12/12-h light/dark cycle, with light on at 6:00 a.m.) and habituated for a week before experiments. Food and water were available ad libitum throughout the study. The experimental procedures and techniques used in this study were in accordance with accepted principles for laboratory animal use and care by NIH, 1985 and EU directive of 1989:86/609/EEC. The protocol used was reviewed and approved by the Research Ethics Committee of Faculty of Basic Medical Science (REC-FBMS), Enugu State University of Science and Technology.

Experimental Design

Group 1: 0.1ml normal saline
Group 2: 300mg/kg/bw Isoniazid (single dose, p.o)
Group 3: 300mg/kg/bw Isoniazid +5mg/kg/bw Diazepam for 5days
Group 4: 300mg/kg/bw Isoniazid +50mg/kg/bw Hydrocortisone for 5days
Group5: 300mg/kg/bw Isoniazid+ 5mg/kg/bw Diazepam +50mg/kg/bw Hydrocortisone for 5days

Tissue processing and Immunohistochemistry

At the sixth day, the animals were deeply anesthetized with ether anesthesia and sacrificed, except the group 2 which was sacrificed on the day 1 post- induction of seizure. The brains were fixed in 10% neutral formal saline solution and embedded in paraffin.Sections of 10um thickness of each representative group were deparaffinized and further processed. Immunoperoxidase was used to label astrocytes using glial fibrillary acidic protein (GFAP) (Novocastra, LEICA Germany) as a marker. Endogenous peroxidase activity was blocked with pre-incubation in 0.3% H_2O_2. After washing, the sections were pre-incubated for 1 hour at room temperature in the appropriate normal serum before incubation in primary antibodies overnight at 4°C. The sections were then rinsed and incubated in secondary antibodies at1:200 dilution for 2 hours at room temperature, and then reacted in avidin biotin complex solution (Novocastra, LEICA Germany) for 1.5 hours using 30-30-diaminobenzidine (DAB) as chromogen. The sections were then mounted on slides, dried, dehydrated, cleared and cover slipped with Dibutylphthalate polystyrene xylene (DPX)**.** The slides were interpreted and photographed. Star shaped cells with specific dark brown colors in the cytoplasm or nuclei depending on the antigenic sites are considered to be positive. The haematoxylin stained cells without this form are scored negative. Nonspecific binding/brown artifacts on cells and connective tissue were disregarded.

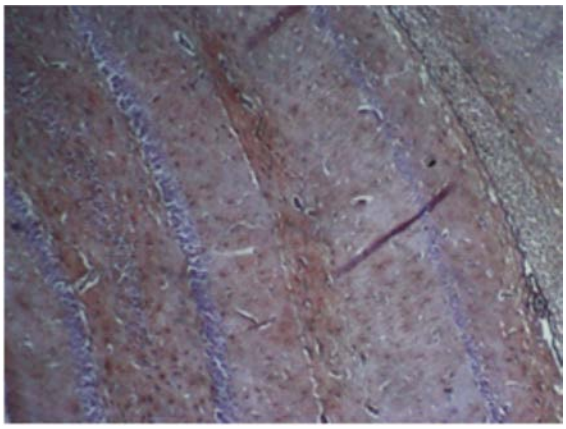

Figure 1: Sections of the hippocampus (GFAP. x200) of control group (group 1) showing normal astrocytes

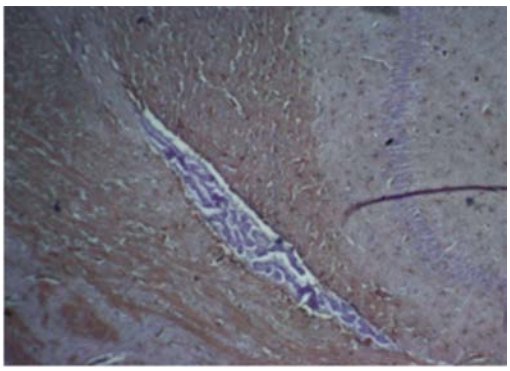

Figure 2: Section of the hippocampus (GFAP. x200) of the rat treated with Isoniazid 300mg/Kg (group 2), showing reactive astrocytes

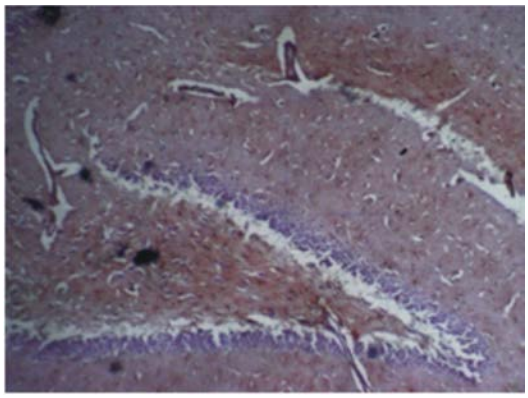

Figure 3: Sections of the hippocampus (GFAP. x200) of the rat treated with Isoniazid 300mg/Kg and Diazepam 5mg/Kg, showing mild expression of reactive astrocytes

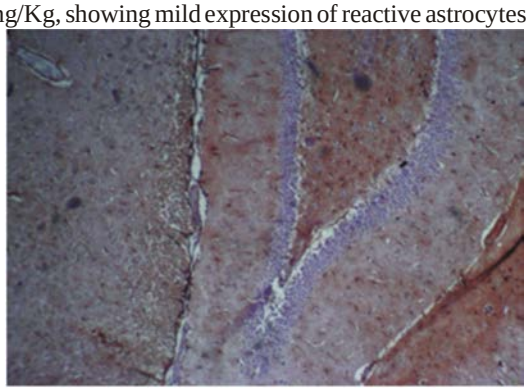

Figure 4: Sections of the hippocampus (GFAP. x200) of the rat treated with Isoniazid 300mg/Kg and Hydrocortisone 50mg/Kg, showing mild astrocytes retraction

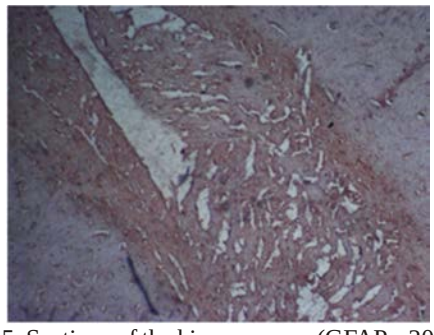

Figure 5: Sections of the hippocampus (GFAP. x200) of the rat treated with Isoniazid 300mg/Kg, Diazepam 5mg/Kg and Hydrocortisone 50mg/Kg, showing severe retraction of astrocytes.

DISCUSSION

Astrocytes are morphologically star shaped glial cells found in the brain and spinal cord. Astrocytes support neurons, endothelial cells of blood-brain-barrier, and provide nutrients and conducive environment for neuronal survivals (Nag, 2011). Astrocytes have been implicated in the maintenance of the extracellular ion balance, repair and scarring process of the central nervous system (CNS); particularly following injuries and disease conditions (Bylicky et al. 2018). This glial cell exhibits morphological diversity, plasticity and disease related deficits by changing their phenotypes and population (Zhou et al. 2019). In this study, astrocyte was demonstrated by Glia fibrillary acidic protein (GFAP) which showed dark star shaped astrocytes with various processes. The hallmark of astrocytic expression was seen in the seizure model (Fig 2: INH), marked by astrocytes that become reactive in expression with prominent processes. This is consistent with astrocytic morphological phenotypes in neurotoxicity (Finbarrs-Bello et al. 2016). It is a common occurrence to observe astrocytes in negative responses through their hyperreactivity and glial scar formation in excitotoxic and/or mechanical injuries (Becerra-Calixto et al. 2017).

Although, reactive astrocytosis may tend to have biphasic characteristic, one for cell death and one for pro-neuroprotection probably depending on the context (Becerra-Calixto et al. 2017). The morphological and physiological changes that astrocytes undergo in response to pathology is most often characterized by an up-regulation of the astrocytic intermediate filament glial fibrillary acidic protein (GFAP) and cellular hypertrophy, which may or may not be associated with cell proliferation (Rivera et al. 2019). This suggests that Isoniazid administration induces neuronal damage which resulted in the activation of astrocytes in order to curb injured neurons and promote their survival. Conversely, it could also be attributed to Isoniazid-induced reduction in GABAnergic neurotransmission of which astrocytes also play pivotal role in GABA synthesis and release at synaptic terminals (Carta and Luca, 2008).

Anxiolytic treatment resulted in the retraction of astrocytes which is a reversal of the seizure effect. By implication, diazepam facilitates the action of GABA, decreases the neuronal excitability and necessitates astrocytes retraction. Similarly, anti-inflammatory drug hydrocortisone was used to evaluate the possible modulatory effect of anti-inflammatory agent as adjuvant in seizure management. Hydrocortisone, exhibits a better promising effect by restoring astrocytes to relative normal morphology. Grosso et al. (2008) reported that hydrocortisone could be useful in the treatment of drug resistant, childhood epilepsies. Furthermore, a previous study demonstrated that corticosteroids, which include hydrocortisone are safe and efficient for treatment of epilepsy, particularly short therapy (Buzatu et al. 2009). This was evident in this study as hydrocortisone was able to ameliorate the astrogliosis induced by isoniazid.

The combination of anxiolytic and anti-inflammatory treatments reveals marked positive immunoreactivity and retraction of astrocytes as shown by their morphology. The above result demonstrated the beneficial effect of the combined therapy to enhance the management of status epilepticus. We opined that diazepam enhanced GABA release in the process while the hydrocortisone might have acted via the corticosteroid hormone receptor and/or exerted adjunct effect in the course of the treatment.

CONCLUSION

The combination of anxiolytic and anti-inflammatory therapy could be a promising treatment for seizure. Thus, we advocate the combination of both therapies in the management of seizure patients for better treatment outcome.

Conflicts of Interest

Authors have declared that they have no competing interest

Acknowledgment

We thank the staff of the histology laboratory for providing the technical assistance.

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