ORIGINAL ARTICLE

Official Journal of the Neuroscience Society of Nigeria (NSN)

https://doi.org/10.47081/njn2017.8.2/003
ISSN 1116-4182

Effect of Folic Acid on Duovir-N Induced Weight, Behavioural and Cyto-Cerebellar Changes in Adult Male Wistar Rats

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Received: ……. July 2016
Accepted: ……. March 2017

ABSTRACT

Duovir-N is a highly active antiretroviral therapy (HAART), a combination of three drugs (lamivudine, zidovudine and nevirapine) used for pre-exposure prophylaxis and management of human immunodeficiency virus infection in sub-Saharan Africa. This research investigated the potential ameliorative effect of folic acid on Duovir-N induced toxicity on the cerebellum. Forty adult male Wistar rats were divided into 4 groups of 10 rats each. They were administered with distilled water, 9.28 mg/kg of Duovir-N only, 9.28 mg/kg of Duovir-N and 0.07 mg/kg of folic acid, and 0.07 mg/kg folic acid. Drugs were administered twice daily for 30 days after which neurobehavioral test in the open field maze was perform. The rats were then sacrificed and their cerebellum harvested, processed and stained using haematoxylin and eosin method. Result showed a significant ($p<0.05$) decreased in weight of the Dourvir-N (HAART) groups compared to the control or folic acid groups. There was also a significant ($p<0.05$) reduction in the brain to body weight index between the HAART group compared with control and folic acid groups. There were no significant changes in all the parameters of the open field maze between the HAART group and the control. The cerebellum was affected with mild to moderate shrinkage of pyramidal cells and distortion of the granular cells. These results indicate that Duovir-N affects cerebella histology, and folic acid is able to ameliorate this, thus, may be beneficial to people taking Dourvir-N.

Keywords: *Duorvir-N, Cerebellum, Folic acid, Human Immunodeficiency Virus, Neurobehaviour*

INTRODUCTION

Antiretroviral therapy (ART) has changed human immunodeficiency virus (HIV) infection from a near-certainly fatal illness to one that can be managed chronically, and as a result HIV-infected patients live longer, thereby creating opportunities that leads to the encounter of chronic toxicity (Margolis et al. 2014). It is therefore pertinent that clinicians should be able to recognize, treat or prevent these toxicities to improve the patient's health.

Increasing the quality of and access to ART in Africa are important public health priorities. The World Health Organization's (WHO) guidelines for treatment of HIV infection in adults and adolescents aims to improve treatment effectiveness and reduce risk of toxicities for millions of infected individuals residing in resource-limited and highly affected regions (WHO 2006). Treatment of HIV includes Duovir-N, a highly active antiretroviral therapy (HAART), available as a fixed dosed combination therapy used for the treatment of human immunodeficiency virus, and comprising lamivudine, zidovudine and nevirapine (NVP) (WHO 2006; WHO 2010).

Duovir-N tablets prevent or slow down the ability of HIV to replicate and spread, which keeps the viral

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load down to a low level, allowing the numbers of CD4+ cells to increase so that the immune system can recover, and reducing the risk of disease progression (WHO 2009). This triple drug therapy with Duovir-N makes it easier to take the medications regularly, which helps improve compliance and prevents resistance of the HIV to individual drugs (Mills et al. 2006; WHO 2010). This combination therapy is highly effective and people with HIV on antiretroviral treatment could live for the rest of their lives without developing the acquired immunodeficiency syndrome (AIDS) (Broder 2009).

Despite these improvements, prolonged benefits of antiretroviral drugs are compromised by numerous side effects, adverse clinical events and toxicities. All antiretroviral drugs can have both short-term and long-term adverse effects, and the risk of specific side effects varies from drug to drug, and from patient's idiosyncrasy. Some of the clinical events include AIDS-related insulin resistance, lipodystropy syndrome, gastrointestinal symptoms and hyperglycaemia (Montessori et al. 2004; Ristig et al. 2005). There is a report that even low concentrations of antiretroviral (ARV) drugs that penetrate the blood brain barrier have detrimental effects on the central nervous system (Heaton et al. 2004).

Cognitive impairment occurs in a substantial proportion of HIV-infected patient (15–50%) on highly active antiretroviral therapy (Xu and Ikezu 2009; Schouten et al. 2011). Neurologic complications such as myelopathy, neuropathy, neuropathic pain, and cognitive decline also occur in patients on HIV treatment (Treisman and Kaplin 2002; Tozzi, et al. 2010). Other toxicities induced by anti-HIV drugs include hepatotoxicity, allergies, hyperglycaemia, lactic acidosis, lipodystrophy, and gastrointestinal disorder (Carr et al. 2001). HAART toxicity is likely going to be a public health issue in Africa very soon due to the increasing number of people exposed to this HIV drugs (UNAIDS Report 2013) as a result of social conflicts and crime leading to rape and subsequent post exposure prophylaxis. Prophylaxis is also practiced by health workers when they have occupational exposure to HIV; they are expected to commence treatment within 72 hours after exposure and to continue with the medication for as long as 30 days. HIV positive pregnant women are also given this medication to prevent mother to child transmission of HIV thereby exposing the mother and the unborn child to this medication.

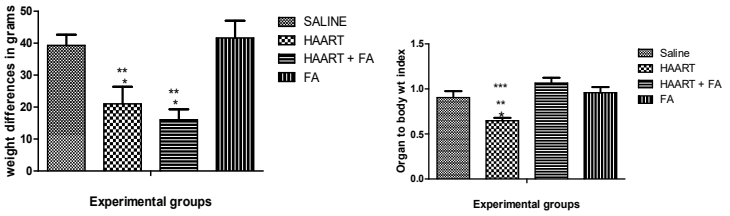
Regarding Nevirapine (NVP), central side effects such as neuropsychiatric complications and headaches have sometimes been associated with its use (Wise et al. 2002). These effects are induced by the action of NVP in the central nervous system (CNS); in fact, NVP has been found in the cerebrospinal fluid in a concentrations of 15 to 40% of plasma levels (van Praag et al. 2002; von Giesen et al. 2002). Further studies demonstrated that the degree of NVP brain uptake was higher compared to other antiviral HIV drug, such as abacavir, amprenavir, and ritonavir (Anthonypillai et al. 2004). It is worth mentioning that the non-nucleoside reverse transcriptase inhibitors (NNRTIs) have good CNS penetration (Wynn et al. 2002; Gibbs et al. 2006). It has also been reported that about 40% of patients treated with efavirenz develop toxicities related to the CNS, with symptoms such as dizziness, insomnia, and depersonalization (Rihs et al. 2006). Olivero et al. (1997) reported that transplacental exposure of mice to zidovudine at 25 mg/day by gavage during the last third of gestation resulted in shorter chromosomal telomeres in the liver and brain of most newborn mice. Combivir, a combination of zidovudine and lamivudine has been implicated in post HAART psychosis and zidovudine was named the likely component that caused the patient's psychosis (Foster et al. 2003).

Mobility and motor coordination are key factors in physical well being and these are controlled by the cerebellum. Motor performance deficits for older adults appear to be due to dysfunction of the central and peripheral nervous systems, as well as the neuromuscular system (Seidler et al. 2010). These deficits have a negative impact on the ability of older adults to perform functional activities of daily living. Despite the above growing body of evidence for CNS side effects of HAART in HIV patients, there is still dearth of information on the neurobehavioural effects and toxicity of this combination widely used in Nigeria on rodent models. This necessitated this research to investigate the potential effects of this drug and the possible ameliorative effect of folic acid, a free radical scavenger with reported antioxidant activity (Joshi et al. 2001; Sankrityayan and Majumdar 2015; Beltagy et al. 2016) on the histology of the cerebellum, locomotor and anxiety related behaviour in Wistar rats using open field maze.

MATERIALS AND METHODS

Animals

Forty male Wistar rats weighting 161 - 259 g at the time of acquisition and acclimatization were used in this study. They were kept at the animal house of the University of Uyo. The animals were allowed 12 hours light and 12 hours dark cycles at 27^{o}C - 30^{o}C room temperature. They were fed standard rat pelletized diet (Grand Cereals Ltd, Nigeria) and water *ad libitum*. The research was approved by the Ethics committee of the University of Uyo, Nigeria. The guidelines of the Institutional Animal Care and Use Committee (IACUC) were strictly followed throughout in handling the animals.



(a) b)

Fig. 1: Effect of Douvir-N and folic acid on body weight and organ to body weight ratio at *p≤0.05

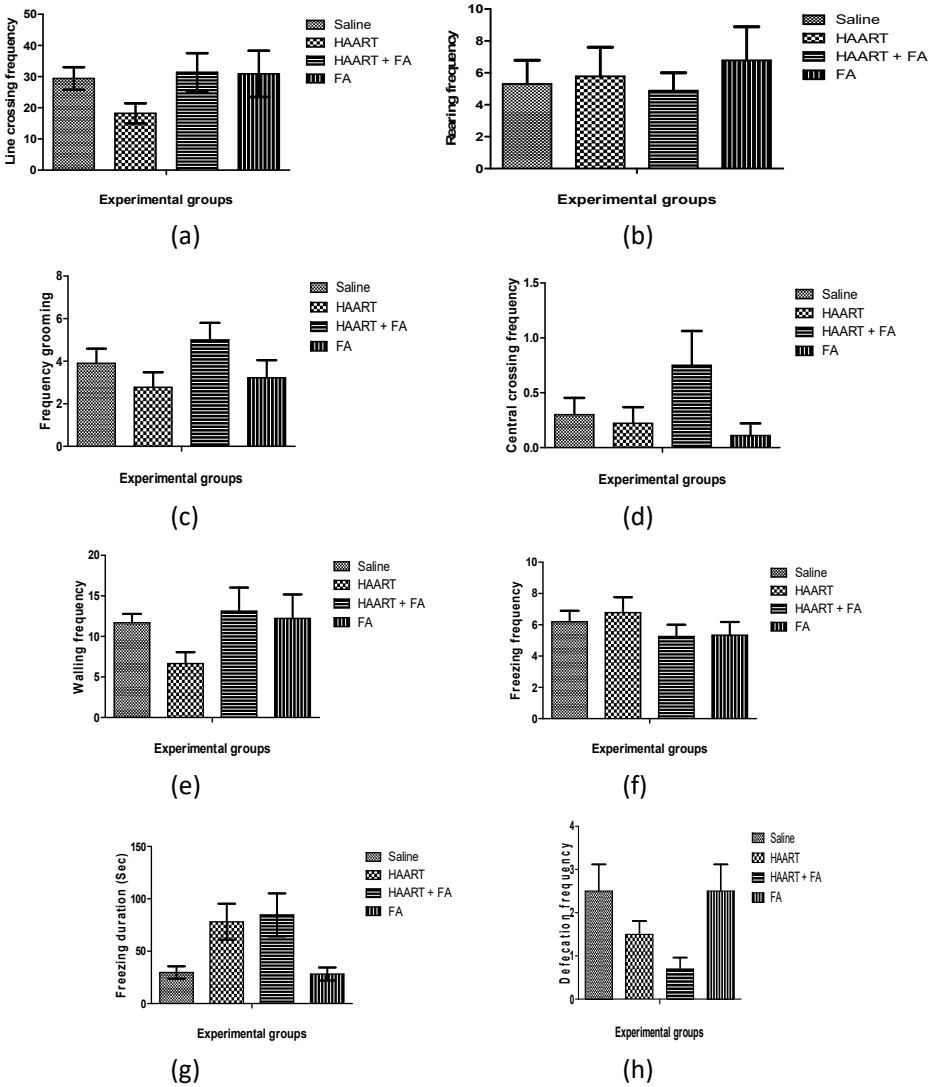
Experimental Design

The animals were randomly divided into four groups of 10 rats each. Group 1 was the control and groups 2, 3 and 4 were the test groups which were administered with 9.28 mg/kg of Duovir-N only, 9.28 mg/kg of Duovir-N and 0.07 mg/kg of folic acid, 0.07 mg/kg folic acid, respectively. The drugs were administered orally, twice daily for 30 days, after which the neurobehavioural test in the open field maze was performed.

Open Field Test and Animal Sacrifice

Animals were individually placed in an open field apparatus made of perspex plastic with dimensions (40 × 60 × 50 cm) and the floor was divided into 25 equal squares by lines. The numbers of squares crossed with all paws (frequent line crossing) were counted in a 5 min session and the following were recorded: (1) frequency of line crossing, (2) freezing and freezing period, (3) rearing frequency (vertical postures of the rat with its hind paws on the floor and forepaws on the wall), (4) central crossing and central crossing duration, (5) grooming, (6) defecation and (7) urination (Brown et al. 1999).

After the open field test, the animals were put on overnight fast before they were sacrificed under chloroform anaesthesia and the brains harvested. Each cerebellum was excised, weighed, then processed through dehydration in graded alcohol, clearing in xylene, infiltrated and embedded in paraffin, and stained using haematoxylin and eosin method.



(a) (b) (c) (d) (e) (f) (g) (h)

Fig. 2: Neurobehavioral test of open field behaviour after 30 days of exposure to Dourvir-N and Folic acid.at *p≤0.05

Statistical Analysis

The software package GraphPad Prism (version 6) was used for analysis and graphical representation of data. All data are presented as mean ± standard error of mean (SEM). The data were analyzed by two-way analyses of variance. Data with $P < 0.05$ was considered statistically significant.

RESULTS

Effect of Douvir-N and Folic Acid on Body Weight

Body weight of animals exposed to HAART

(Dourvir-N) were significantly ($p < 0.05$) reduced compared with control and the group that received folic acid only. There was also a significant reduction in the weight of animals administered with Dourvir-N and folic acid compared to the control group (Figure 1a).

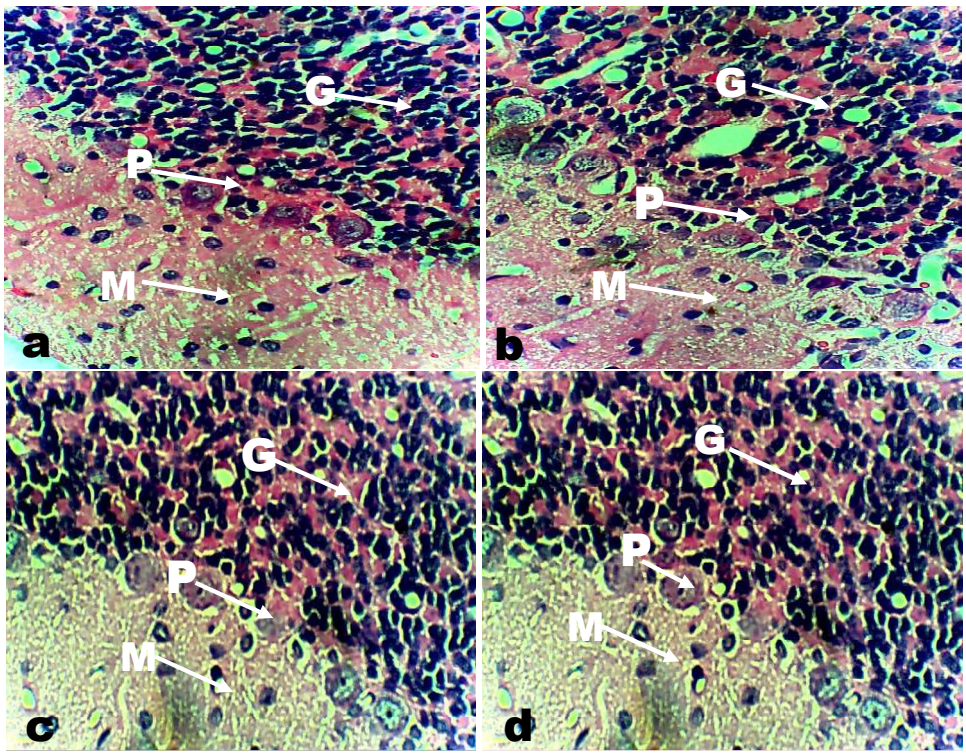


Fig. 3: Photomicrographs of the histology of the cerebellum of rats treated with Duovir-N and/or folic acid and the control.
a. The cerebellar section of control group. b. The cerebellar section of group administered 9.28 mg/kg of Duovir-N. c. The cerebellar section of the group administered with 9.28 mg/kg of Duovir-N and 0.07mmg/kg of folic acid. d. The cerebellar section of the group administered with 0.07mg/kg of folic acid. Molecular (M) layer, granular (G) layer, and Purkinje (P) cells (arrows). H & E ×400

Effect of Douvir-N and Folic Acid on Organ to Body Weight Ratio

Following exposure to HAART, there was significant reduction in brain to body weight ratio in the HAART group compared to the control and folic acid groups (Figure 1b).

Effect of Douvir-N and Folic Acid on Neurobehavioral Test of Open Field

Following exposure to the open field, there was no significant difference in locomotor, rearing and grooming activities following administration of Dourvir- N and folic acid compared to the control (Figures 2a, 2b and 2c). In addition, central line crossing, walling activity, freezing frequency, freezing duration and frequency of urination were not significantly different between the treatment group and the control (Figure 2d, e, f, g).

Effect of Douvir-N and Folic Acid on the Histology of Cerebellum

The histology of the cerebellum of the control group showed normal cells in the three cerebellar cortical layers: molecular, granular and Purkinje cells. The granular cells aggregate and the Purkinje cells were single-lined (Figure 3a). The histology of the cerebellum of animals administered with 9.28 mg/kg of Duovir-N showed similar three cerebellar cortical layers but with a disrupted and shrunken Purkinje cells. The granular cells appear shrunken and scanty (Figure 3b).The histology of the cerebellum of animals administered with 9.28 mg/kg of Duovir-N and 0.07 mg/kg of folic acid showed three cerebellar cortical layers, but the granular layer cells appeared normal compared with Dourvir-N only treated group (Figure 3c). The histology of the cerebellum of animals administered with 0.07 mg/kg of folic acid showed normal three cerebellar cortical layers. The granular cells aggregate, and the Purkinje cells were single-lined as in the control group (Figure 3d).

DISCUSSION

The advent of HAART and advances in antiretroviral therapy continues to reduce morbidity and mortality in AIDS related complications, but organ toxicities are also becoming a major concern to healthcare providers. In our study the reduction in weights observed in the experimental groups may be due to Dourvir-N, unfortunately adjuvant therapy with folic acid was not able to prevent this weight loss as it seemed to worsen it. Antiretroviral drug, adefovi has been shown to be associated with weight loss. Other studies have reported that HAART is associated with incomplete weight recovery in certain subpopulations of HIV-infected subjects (Tang et al. 2002). Previous

reports suggest that a progressive decrease in lean body mass in the HAART era may be related to catabolic cytokines (Roubenoff et al. 2002; Shikuma et al. 2004). This correlated with the result of the brain to body index ratio that was significantly lower in the HAART group, implying that there was shrinkage of the brain size in this group. Organ-body weight ratio is an important indicator of organ toxicity. Azu et al. (2016) reported a reduction in liver size in groups of animals treated with HAART. This finding is particularly important in paediatrics HIV treatment as the brain of the new born is still developing and as such more prone to toxic chemical injuries (Johnson 2008).

The histology showed distortions in the cerebellum of the HAART only group. This included shrunken Purkinje cells, granular cell swelling and loss of granular cells. This might have resulted from mitochondrial damage due to free radicals (Feng et al. 2001). The toxic effect of HAART can lead to motor dysfunction as the cerebellum is the centre for motor control, motor coordination, memory, cognitive processing and emotional control (Chizhikov and Millen 2003; Schmahmann and Caplan 2006; Stoodley and Schmahmann 2010).

These changes were ameliorated in the folic acid HAART combined group compared with the control. This could have been as a result of the antioxidant properties of folic acid. Joshi et al. (2001) and Sankrityayan and Majumdar (2015) reported that folic acid scavenge free radicals very efficiently. A previous study showed that neurovite ameliorated the toxic effects of another ARD, lamivudine in the cerebellum (Peter et al. 2013), which the present result supports.

The adverse effects on the histology did not affect the neurobehaviour of the rats in the open field. The open field maze is used to assess locomotor and exploratory behaviour in a novel environment (Weiss et al. 2000). This neurobehavioral result is likely due to compensatory mechanisms that ensures that homeostasis is maintained in external insults (Fan et al. 2011), but changes can occur if the insult last long enough. Alternatively, it might be due to the behavioural result not reflecting the actual area of insults to the cerebellum as previously reported by Ekong et al. (2014). In our experiment this did not occur after 30 days of HAART administration, which may imply that the drug Dourvir-N has neither anxiolytic nor anxiogenic properties. This is in line with the findings of Romao et al. (2011) who reported that nevirapine, a component of Dourvir-N did not affect locomotor activity in open-field test in mice.

CONCLUSION

In conclusion, the drug Duovir-N affects body weight, and weight and histology of the cerebellum but does not affect neurobehaviour in the open field. Folic acid has the potential of ameliorating the histological distortions of shrunken pyramidal cells and reduction in granular cells possibly through its antioxidant affect on free radicals, thus, protecting the cells from their detrimental effects. Since it affects adult cerebellum, a developing brain will likely be adversely affected and so further studies to investigate the effect of this drug on the developing Wistar rats will help in more understanding of its effects.

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