Neurobehavioral assessment of the impact of vitamins C and E following acute exposure to sodium azide-induced neurotoxicity

Taiwo A. Abayomi1*, Olorunfemi S. Tokunbo2, Benjamin T. Adebisi1, Mayowa U. Fadare1, Ismail T. Gbadamosi3, Justina O. Akinwale1, Elizabeth A. Sawyer1 and Tolulope T. Arogundade5

1Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University, PMB 4494, Osogbo, Osun State, Nigeria
2Neuroscience Unit, Department of Anatomy, Faculty of Basic Medical Sciences, Adeleke University, Ede, Osun State, Nigeria
3Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

ABSTRACT

The effect of acute exposure to NaN₃ on different neurobehavioral tasks that relates to learning and memory, fear and anxiety as well as motor and coordination activities were assessed in rats and the impact of vitamins C and E before and after the exposure were investigated in this study. Thirty six (36) male wistar rats weighing between 170 - 190g were randomly divided into six groups (n=6): groups A (Control), B (NaN₃), C (NaN₃ + Vit. E), D (NaN₃ + Vit C), E (Vit E + NaN₃) and F (Vit C + NaN₃). Acute neurotoxicity was induced in experimental groups by oral administration of 15mg/kg sodium azide for 5 days while Vitamins C and E were administered for 21 days before and after neurotoxicity was induced. Different neurobehavioral studies were assessed. Group B showed significantly reduced body weight compared to other groups. Open field test revealed decreased exploratory activity and significant impairment in habituation (emotionality) in group B compared to the control and other treated groups. Learning and memory measured using novel object recognition (NOR) test showed reduced episodic memory retention in group B while the other treatment groups showed appreciable memory retention. Moreover, the rotarod test showed severe motor deficit in group B when compared to other groups. Our results suggest that acute exposure to NaN₃ has neurodegenerative effects and that supplementation of Vit C and E before or after exposure to NaN₃ can either be neuroprotective or ameliorative.


Received January 1, 2019; Accepted January 16, 2019; Published February 25, 2019.

Copyright: © 2019 Abayomi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. JETPH is a journal publication of BRSF.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: taiwo.abayomi@uniosun.edu.ng

Keywords: sodium azide neurotoxicity, neurobehavioral studies, brain regions, vitamin C, vitamin E

1. INTRODUCTION

Sodium azide is a useful probe reagent and a preservative use in the hospitals and laboratories, and is used in agriculture for pest control of soil-borne pathogens and as a mutagen for crop selection of plants [1]. However, it is an environmental pollutant in certain occupational settings. It is reported to impair brain cytochrome oxidase activity and causes neurobehavioral abnormalities including learning and memory deficits similar to those found in certain neurodegenerative diseases [2, 3]. Studies carried out to elucidate the compound’s mechanism of cellular toxicity implicated it as neuronal mitochondrial toxin [4].

Vitamins C and E has been implicated in the neuroprotection of various parts of the brain as relates to learning and memory as well as motor coordination following oxidative stress and this have since been established in both human and animal models [5, 6]. Vitamin E has many biological functions, the antioxidant function being the best known (Other functions include enzymatic activities, gene expression, and neurological functions [7]. Vitamin C also functions as an antioxidant and plays a role in immune function and it has demonstrated antiviral and antibacterial effects in vitro; plays a role in microsomal hydroxylation reactions that catalyze cholesterol catabolism and detoxification of xenobiotic chemicals; and is involved in the metabolism of neurotransmitters
In performing their roles as antioxidants, Vitamin C, provides protection against oxidative stress-induced cellular damage by scavenging of reactive oxygen species, while vitamin E by neutralization of lipid hydroperoxyl radicals, and by protecting proteins from alkylation by electrophilic lipid peroxidation products [7]. Therefore, this study was designed to elucidate the neurobehavorial changes associated with acute sodium azide neurotoxicity as well as evaluate the neuroprotective and ameliorative role of vitamins C and E following sodium azide induced neurotoxicity in adult wistar rats.

2. MATERIALS AND METHODS

2.1 Animal procurement and care

All protocols and treatment procedures were done according to the Institutional Animal Care and Use Committee (IACUC) guidelines and as approved by the Faculty of Basic Medical Sciences Ethics Review Committee, Osun State University, Nigeria. Thirty-six (36) male Wistar rats, weighing 170 - 190 g were procured and housed in the Animal house of the College of Health Sciences, Osun State University, Osogbo. The animals were acclimatized for two weeks under standard laboratory conditions of temperature 27-30°C in a 12:12 light and dark cycle. The animals were fed with rat pellet (Topleeds Ltd. Ibadan, Oyo State, Nigeria) with access to drinking water ad libitum.

2.2 Animal grouping

Rats were divided into 6 groups (A, B, C, D, E and F) (n=6).
Group A served as the control and was administered with only distilled water; Group B was administered with 15mg/Kg of NaN₃ for 5 days; Group C was administered 15mg/Kg of NaN₃ (for 5 days) followed by 100mg/Kg of Vitamin C for 21 days; Group D was administered 15mg/Kg of NaN₃ (for 5 days) followed by 100mg/ Kg of Vitamin E for 21 days; Group E was administered 100mg/ Kg of Vitamin C for 21 days followed by 15mg/Kg of NaN₃ for 5 days; Group F was administered 100mg/ Kg of Vitamin E for 21 days followed by 15mg/Kg of NaN₃ for 5 days; Administration was done orally using an oral gavage daily at 0800 hours.

2.3 Treatment solutions

Crystalline salts of NaN₃ was obtained from Sigma and was dissolved in distilled water (15 mg/ml) and adjusted to pH 7.4 with 0.1 M phosphate-buffer saline (PBS). Vitamin C salt was also obtained from Sigma and was dissolved in distilled water (100mg/ml). Vitamin E was procured from Ladoke Akintola University of Technology Teaching Hospital pharmacy department, Osogbo. 100mg/ml was pipetted using a syringe. These solutions were freshly prepared each morning of administration and kept at 4° C before use [9].

2.4 Neurobehavioral paradigms

2.4.1 Open field test

The locomotor activity and freezing, a form of non-associative learning, were measured in the open field test [10, 11]. Rats were gently placed into a corner of the arena and allowed to explore the apparatus for 3 minutes. During the three minutes of exploration, the time spent freezing (no movement) was quantified. Exploratory measures as well as non-exploratory behaviours were recorded by the observer [12]. Exploratory motor activity (EMA) measures included horizontal locomotion (the number of squares crossed) as well as vertical activity (rearing). Non-exploratory measurements comprised only the vegetative behaviours (numbers of urination episodes and defecation boli). After the 3 minutes test session, the rat was returned to its home cage and the open field was cleaned using 70% ethyl alcohol (to avoid odour cues) and permitted to dry between tests. To assess the process of habituation to the novelty of arena, rats were exposed to the apparatus for a 3 minutes test session, on three consecutive days.

2.4.2 Novel Object Recognition (NOR) test

Object Recognition (OR) was performed in 40 x 60cm wooden box, with a frontal glass wall, the inside of which was painted with clear colours. The recognition objects were made of plastic. Before starting the trainings, all animals had two free exploration sessions for contextual habituation with no objects inside the box. Animals were allowed to explore two different objects centrally placed for a duration of 5 min (sample phases). The total time spent exploring the two objects was recorded with the assistance of two stop watches: “object exploration” is defined as directing the nose and vibrissae to the object at a distance of less than 2 cm, as if “smelling” it with caution; bumping, turning around or sitting upon the object were not considered exploratory behaviours. At the end of the training trial, the animals were removed from the box and returned to its home cage. After an interval (the retention delay) of 15 min, the animal was reintroduced into the box for the test session (choice phase), now with a different set of objects - one familiar (identical to, but not the same one previously explored object), and the other, a new/unexplored object - both placed in the same position as the sample stimuli. The total time spent exploring the two objects was recorded.

2.4.3 Elevated plus maze test

The elevated plus maze has been described as
a simple method for assessing anxiety responses of rodents. The rats were taken out of the cage and placed at the junction of the open and closed arms, facing the open arm opposite to where the experimenter is, in a consistent manner. Precaution was taken to start data collection as soon as the animal was placed in the maze so that the behaviour of each animal was consistently recorded for 5 min. The number of arm entries and time spent in each open/closed arm was recorded on the data sheets with a timer. Animals were first pre-exposed to the elevated plus maze (training session) for 5 min. The number of times the rats entered the open arm or closed arm and the time spent in the arms was recorded. They were returned back to their cage after the training session. After an interval of 1 hour, the procedure was repeated (test session).

2.4.4 Rotarod test

The rotarod performance test is a performance test based on a rotating rod with forced motor activity being applied, usually by a rodent. The test was used to measure the motor activity of the experimental rats. Apparatus was set to accelerate from 4 to 40rpm in 300s, and animals from same cage were placed in separate lanes on rod initially rotating at 4rpm. Testing began when acceleration was started and ended when animal fell off the rod. Apparatus was cleaned with 70% ethanol between test.

2.5 Statistical analysis

All quantitative data were analyzed using GraphPad Prism® (version 6) software. Neurobehavioral assessments outcomes were plotted in ANOVA followed with Tukey’s multiple comparisons test. Significance was set at *p<0.05, **p<0.01 and ***p<0.001. The results were represented in bar charts with error bars to show the mean and standard error of mean (Mean±SEM) respectively.

3. RESULTS AND DISCUSSION

Figure 1. Effects of vitamins c and e on body weight changes prior and after sodium azide induced neurotoxicity.

Lower body weight gain was significantly seen in groups of animals treated with NaN₃ only compared to the control animals (p<0.05) (Fig.1). Animals treated at various stages with Vitamin E and C had higher body weight gain when compared with those treated with NaN₃.

Figure 2. Open field test.
Fig. 2 shows the level of exploratory activity (Horizontal locomotion) and number of times freezing occurred in different treatment groups during the Open field test. Animals in group B treated with NaN₃ only, displayed significantly reduced horizontal locomotion (crossing) when compared to the control group and other treated groups (p< 0.05). There was no significant difference in the behaviour of rats treated with vitamins C and E when compared with the control group. Also, animals in group B spent more time freezing than their counterparts in the control group and other groups pre and post treated with vitamins C and E.

Fig. 3. Novel Object Recognition.

Fig. 3 shows the level of working memory of rats in each group as revealed by the novel object recognition test. Rats in group B spent significantly less time exploring the novel object when compared with the control group and other treatment groups other (p<0.05). This signifies severe working memory deficits and probable damage to the prefrontal cortex in group B animals.

Fig. 4. Elevated Plus Maze.

Fig. 4 shows the time spent in the closed arm by animals in each group. NaN₃ animals spent significantly more time in the closed arm when compared to the control group and other treated groups (p< 0.05). This is an indication of fear and anxiety.

Fig. 5 shows that animals treated with NaN₃ spent significantly lesser time on the rotarod compared with the control group. Those pre-treated and post-treated with vitamin C and vitamin E performed better in the test.

Decreased motor function and memory are two main behavioral parameters observed in neurodegenerative diseases [6]. In an effort to elucidate the impact of vitamins C and E in relation to the neurobehavioral patterns of rats following sodium azide induced neurotoxicity, we investigated different neurobehavioral paradigms to test for episodic memory, exploratory activity, fear and anxiety as well as motor coordination and balance in adult male wistar rats.

In this study, rats administered with 15 mg of
**Figure 5. Rotarod Test.**

 NaN₃ only, (group B) showed progressively less activity and general weakness as well as reduced appetite and body weights when compared to the control and other treatment groups. Leptin, a cytokine primarily secreted by white adipose tissue is implicated in the inhibition of food intake [13] and increasing energy metabolism [14]. From this study, it is possible that NaN₃ could cause an increased breakdown of white adipose tissue and subsequently increase leptin secretion since the concentration of circulating leptin is strongly correlated with the adipose tissue present in the body; though the mechanism by which NaN₃ may effect this increase is not yet understood. Hence acute NaN₃ toxicity is likely to be associated with increased leptin level which may be responsible for the reduced body weight observed unlike fasting [15] and cold exposure [16] which is associated with reduced leptin concentration. However, ananimals pre-treated and post treated with vitamins C and E showed significant body weight gain compared to NaN₃ group which implies that supplementation with vitamins C and E prior or after NaN₃ toxicity is associated with preserving and restoring normalcy to the rate of breakdown of white adipose tissue, hence leptin level is regulated and appetite and body weight is restored.

The open field test provides simultaneous measures of both exploratory motor activity (EMA) and habituation. Significant reduction in EMA was observed in the NaN₃ treated animals (p<0.01) when compared to other treatment groups. This reduced exploratory motor activity is corroborated by the increased habituation (time spent freezing) in the open field test as seen in (Fig. 2.) Hence, NaN₃ toxicity is confirmed to have deleterious impact on the brain as it relates to memory [9]. Chronic supplementation with vitamins C and E were observed to restore the damage on EMA exerted by NaN₃ as well as prevent any damage that could be caused following NaN₃ toxicity. Furthermore assessment of the time spent in the closed arm during the elevated plus maze test confirms the increased anxiety level that could be associated with neuronal damage in the amygdale region of the brain following NaN₃ toxicity, Whereas, the anxiety level was observed to be reduced with vitamins C and E intake before and after neurotoxic induction. This goes to confirm that these antioxidants are capable of protecting and restoring the assault induced in the amygdale by neurotoxic substances.

Motor impairment was evident in animals treated with NaN₃ in the rotarod test. From the study it can be inferred that NaN₃ has damaging effect on the cerebellum which is responsible for motor coordination and balance. Following vitamins C and E administration, motor coordination and balance was maintained and restored significantly (p<0.05) as seen in Fig. 5. This confirms the basis that the antioxidant properties in vitamins C and E can help restore and maintain cerebellar function [17,18].

Previous studies have shown that NaN₃ can lead to neurochemical changes associated with impairment in learning and memory [2]. In this present study, the deleterious effects of NaN₃ in different parts of brain in rats (the hippocampus, prefrontal cortex, amygdale and cerebellum) is associated with decreased body weight, memory impairment, increased anxiety as well as motor deficit. This assault is associated with the inhibition of brain cytochrome oxidase [2]. NaN₃ may affect oxygen metabolism and induce free radicals which appears to play a role in most neurodegenerative disorders [19,20]. Vitamin C treatment, through dietary supplementation may provide valuable protection against the neurodegenerative changes associated with cognitive and motor impairments. Vitamin E is essential for maintaining and restoring the morphological and functional integrity of the cells that are critical regulators of motor coordination [18] as well as cognitive function. The antioxidant properties of vitamins C and E, against sodium azide-induced toxicity is apparently responsible for their neuroprotective and ameliorative roles in this study.
4. CONCLUSION

Our study indicated that exposure to NaN₃ resulted in obvious deleterious effects on brain of rats as reflected in impaired learning and memory, increased anxiety and motor deficit. We were also able to show that vitamins C and E were able to protected and maintain the integrity of the neurons in the brain against NaN₃ induced damage. However, vitamin E proved to have more protective effect.

ACKNOWLEDGEMENTS

The duration of this project has been brilliantly enriched and made deeply enjoyable due to the support we got from some of our colleagues. First, many thanks go towards Drs. Oyeyipo I.P. and Obembe O.O. for being extremely generous and granting us unrestricted access to some of the behavioural paradigms in the physiology laboratory of the college of Heath Sciences, Osun State University. Second, we would like to extend our gratitude to the supporting cast of faculty - Drs. Falana B.A, Atere T.A, Adeleke S.O., and Dare B.J. - that have been a valuable source of advice, dialogue, and discussion, and have set great examples as researchers. None of the experiments conducted would have been possible without the invaluable help in the lab from Ms. Awonuga and Mr. Olaniyi. We are grateful that they have met every question, inquiry, and request with patience and kindness.

AUTHOR CONTRIBUTIONS

TAAdesigned the study and performed the statistical analysis. OST wrote the first draft manuscript and also contributed to the research design. ITG contributed to the draft manuscript. BTA, TTA, and MUF took part in the set up and interpretation of the neurobehavioural study. EAS and JOA took part in the bench work that included administration and care of the experimental animals. All authors read and approved the final manuscript.

REFERENCES