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RECOGNITION MEMORY IMPAIRMENT AND THE ROLE OF DMSO, ALA AND VITAMIN C DURING TRAUMATIC BRAIN INJURY IN ALBINO RATS

TANIMA HAFIZASI BOZUKLUĞU VE ALBİNO SIÇANLARINDAKİ TRAVMATİK BEYİN YARALANMALARI ESNASINDA DMSO, ALA VE C VİTAMİNİN ROLÜ

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Abstract

Expression of cognitive and functional disorders is a common clinical development of traumatic brain injury (TBI) that is essentially determined by the site and severity of the insult. The present study sought to examine the effects of closed-head TBI on memory in albino rats, in order to further examine the potential efficacy of an acute antioxidants treatment with Dimethyl Sulfoxide (DMSO), Vitamin C and Alpha µ- lipoic acid (ALA). The rat model of closed-head injury by weight drop method was applied on anesthetized rats. The treatment protocol included single oral administration of DMSO, Vitamin C and ALA in three different doses (22.5, 45 and 67.5 mg/kg) 1hr post-TBI and continued for two weeks. The Novel Object Recognition Test as well as the Modified Neurological severity score (mNSS) were employed to assess post-TBI memory and neurological function respectively. Our results revealed a recognition memory deficit that was significant 7 days after TBI up to 14 days post-TBI. Most importantly, DMSO, Vitamin C and ALA were able to attenuate the memory impairment by TBI. The mNSS of the treated groups decreased significantly than the non-treated group in the first and second week. Conclusively, the use of antioxidants can help in the management of TBI by reducing oxidative stress and improving cognitive function.

Keywords: traumatic brain injury, impairment, memory, neurological severity score

Özet

Bilişsel ve işlevsel bozukluklar, esasen hasarın meydana geldiği bölge ve şiddetine bağlı olarak belirlenen travmatik beyin yaralanmasının (TBI) yaygın görülen bir klinik gelişmesi olarak ifade edilebilir. Mevcut çalışma, Dimetil Sülfoksit (DMSO), C vitamini ve Alfa lipoik asit (ALA) içerikli bir akut antioksidan tedavisinin potansiyel etkinliğini daha derinden inceleyebilmek adına kapalı kafa travmatik beyin yaralanmasının albino sıçanlarının hafızaları üzerindeki etkilerini araştırmayı amaçlamaktadır. Ağırlık düşürme yöntemiyle kapalı kafa yaralanmasının sıçan modeli uyuşturulmuş sıçanlar üzerinde uygulandı. Tedavi protokolü, travmatik beyin yaralanmasından bir saat sonra DMSO, C vitamini ve ALA'nın üç farklı doz halinde (22.5, 45 ve 67.5 mg/kg) ve tek seferde ağızdan alınmasını öngörmüştür. Travmatik beyin yaralanması sonrası sırasıyla mevcut hafızayı ve nörolojik işlevi değerlendirmek üzere Değiştirilmiş Nörolojik Şiddet Skorunun (mNSS) yanı sıra Yeni Nesne Tanıma Testi uygulanmıştır. Elde edilen sonuçlar, travmatik beyin yaralanmasını takip eden yedi ila on dört gün boyunca önemli ölçüde tanıma hafızası eksikliğini ortaya çıkarmıştır. Daha da önemlisi; DMSO, C vitamini ve ALA, travmatik beyin yaralanmasından kaynaklı hafıza bozukluğunu azaltma eğilimi göstermiştir. Müdahalede bulunulan deney guruplarına ait Değiştirilmiş Nörolojik Şiddet Skoru'nda (mNSS) müdahale edilmeye guruptakilere nazaran birinci ve ikinci haftada önemli ölçüde düşüş gözlenmiştir. Sonuç olarak antioksidan kullanımı, oksidatif stresi azaltarak ve bilişsel işlevi geliştirerek travmatik beyin yaralanması tedavisine katkıda bulunabilir.

Anahtar Kelimeler: *travmatik beyin yaralanması, bozukluk, hafıza, nörolojik şiddet skoru*

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1. Introduction

Traumatic brain injury (TBI) is a major health and socioeconomic challenge throughout the world (Peeters et al.,2015). It is widespread all over the world and anybody can be a victim. Because outcome resulting from TBI are usually not instantly discernable, the society is mostly ignorant of the effect of TBI (Koskinen & Alaranta 2008). The major effect of TBI is the significant disabilities its victims are surviving with after recovery. Such deficits can include impaired thinking or memory, movement, sensation (e.g., vision or hearing), or emotional functioning. These problems not only affect individuals but can have long-term impact on families and communities. In the United States for example, TBI is linked with the death of about 51,000 people each year and causes long-term disability that affects an approximate 70,000 to 90,000 persons yearly (Thurman & Guerrero, 1999).

TBI is a complex disease process based on its pathophysiology (Masel & DeWitt 2010) that causes structural damage and functional deficits due to both primary and secondary injury mechanisms leading to neuronal cell death (Davis, 2000). Cell death can be acute or chronic (Raghupathi, 2004). Both acute cell death and delayed apoptosis contribute to functional deficits after TBI. However, even mild TBI with no remarkable cell death can lead to cognitive deficits, which are possibly related with diffuse axonal injury (DAI) (Niogi et al., 2008). Among the factors leading to functional deficit are biochemical cascades which occur in response to primary and secondary injury. These mechanisms generate oxidative stress.

Oxidative stress (OS) is one of the characteristics of TBI that can initiate the pathophysiology ensuing disruption and protracted neuronal function. OS an imbalance between oxidant and antioxidant agents can result in neural dysfunction and death. After TBI, a group of oxidant such as ROS and RNS are produced resulting in oxidative damage of macromolecules in the brain, while antioxidant defense enzymes decrease. This imbalance is directly related to the pathogenesis of TBI (Rodríguez-Rodríguez et al.,2014).Therefore, the development of antioxidant strategies is of key interest in ongoing efforts to optimize brain injury treatment. Vitamin C (ascorbic acid) is a water-soluble antioxidant that is found throughout the body as the ascorbate anion (Harrison et al., 2014). It inhibits peroxidation of membrane phospholipids and acts as a scavenger of free radicals (Straber and Stevens 1997). The high concentration of vitamin C (10-fold higher than its plasma levels) and its asymmetric distribution in different regions of the brain indicates its vital role in the brain (Harrison et al., 2014).

Dimethyl sulfoxide is a dipolar, aprotic and highly hygroscopic solvent with a broad spectrum of biological activities that suggest efficacy as neuroprotectant (Lu and Mattson, 2001). ALA, or just lipoic acid (LA), is a unique and potent antioxidant that can deliver antioxidant activity in both fat- and water-soluble mediums (Hirai et al., 2001). Loss of mitochondrial function entails a reduction of the energy-transducing systems partly due to oxidative/nitrative damage.

At present there is no single pharmacological agent capable of fully restoring cognitive and motor –sensory function post TBI. Nevertheless, the possibility of an effective treatment is based upon the fact that even though some of the neural injury is due to the primary mechanical events (i.e. shearing of nerve cells and blood vessels), the majority of posttraumatic neuronal damage and degeneration is due to a pathochemical and pathophysiological cascade of secondary events occurring during the first minutes, hours and days following the injury which exacerbate the damaging effects of the primary injury. The aim of this study is to assess the effect of antioxidants (DMSO, ALA and Vitamin C) on recognition memory of rats induced with TBI.

2. Materials and Methods

2.1. Source of Experimental Chemicals

Ascorbic acid, (vitamin C), and □- lipoic acid, were obtained from Sigma® Chemicals Limited Paderborn, Germany. Dimethylsulfoxide was obtained from Cayman® Chemical Company, Ann Arbor, USA. Ketamine hydrochloride was obtained from Rotexmedica®, Trittau, Germany.

2.2. Experimental Animals

Fifty five apparently healthy albino rats of winstar strain weighing between 180-200g were purchased from the Animal House of the Biological Sciences, Usmanu Danfodiyo University, Sokoto Nigeria. The rats were allowed to acclimatize to the research laboratory. The experimental animals were subjected to a 12 hours light/12 hour dark schedule. The rats were fed with growers mash of vital® feed and clean water was given ad-libitum.

2.3. Experimental design

The experimental animals were randomly divided into eleven groups of five rats each. TBI was induced in groups 1 to 9 and treated with three different doses of Vitamin C, DMSO and ALA for two weeks. Group 10 was not treated (traumatized not treated) while group 11 was used as negative control (non-traumatized non-treated).

2.4. Induction of TBI

Head injury was induced in the entire experimental animals except in the negative control group by weight drop method using an acceleration impact devise of Marmaru (1994) as modified by Heath and Vink (1995). The experimental rats were properly restrained and anaesthesized using a dissociative anaesthetic agent Ketamine at a dose rate of 80mg/kg body weight.

They were intubated and ventilated on room air with a Harvard Rodent ventilator. The skull was exposed by midline incision and a stainless steel disc measuring 10mm in diameter and 3mm in depth was cemented centrally along the control suture between the lambda and the bregma with a polyacrylamide adhesive.

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The experimental animals were secured in the prone position on a 10cm deep foam bed. Injury was induced by dropping an eighty gram (80g) brass weight from a distance of 1m. The stainless steel disc was immediately removed from the skull and the animal was allowed to recover in the cage.

2.5. Neurological Assessment

Animals were examined with a modified neurological severity score (mNSS). This evaluation was performed by the modified method of Leonov et al., (1990).The total score of 18 points consisted of three components: consciousness and respiration, cranial nerve function and sensor motor function and coordination. 18 different tasks were used to evaluate these functions. One point is given for failure to perform a task and 0 for success. Scores ranged from zero in healthy uninjured animals to a maximum of 18 indicating severe neurological dysfunction with failure in all tasks. The mNSS immediately after trauma reflects the initial severity of injury.

Immediately after initial evaluation of mNSS the rats were assigned to one of the treatment groups, evenly distributed to achieve homogenous groups.

2.6. Novel object recognition test

The NOR task was used to evaluate recognition memory in rats as described by Rachmany et al (2013). This task is based on the innate tendency of rodents to explore new objects within their environment. The use of this natural tendency allows one to determine whether a rat can discriminate between a familiar and a novel object. Rats were individually habituated to an open field arena with an object (a). After 5minutes, the rat is removed and a novel object (b) is introduced before putting back the rat into the arena. Exploratory behavior was analyzed over a 5 min period. Exploration of an object was defined as rearing on the object, sniffing it at a distance of less than 2 cm and/or touching it with the nose. Successful recognition was represented by preferential exploration of the novel object over the familiar object. The time spent by each rat exploring the novel object over the familiar object was recorded and used to generate a preference index. A discrimination preference index was calculated as following: (time spent near the new object minus time spent near the old object) / (time spent near the new object plus time spent near the old object). After each session, the objects and arena were thoroughly cleaned with 70% ethanol to prevent odor recognition.

2.7. Statistical analysis

All results were expressed as Mean ± Standard Deviation (M ± SD) and analyzed using SPSS version 22. One-way analysis of variance (ANOVA) followed by Tukey post-Hoc test was used to compare the means of treated groups with traumatized non treated and non -traumatized non treated groups. Comparison between different doses of the treatments was also conducted. Differences are considered statistically significant at $p < 0.05$.

3. Results

The result of this study indicated that all the traumatically head injured rats recorded poor mNSS in the first week (VC 22.5mg-8.8±0.84, 45mg-9.4±0.89, 67.5mg-9.6±0.55, ALA 22.5mg-8±1.00, 45mg-8.6±0.89, 67.5mg-8.8±0.84, DMSO 22.5mg-8.6±1.14, 45mg-8.8±1.30, and 67.5mg-9±0.71) which significantly ($p = 0.0001$) improved in the second week (VC 22.5mg-1.6±0.54, 45mg-1.6±0.54, 67.7mg-1.2±0.44, ALA22.5mg-3.6±0.54, 45mg-3.4±0., 67.5mg-543.2±1.00, DMSO 22.5mg-1.8±0.44, 45mg-1.4±0.54 and 67.5mg-1.8±0.83) while the scores of the TNT group showed no significant improvement between the first (9.2±1.09) and second week (7.2±1.09).

The result revealed that TBI caused memory deficit seven days Post injury as observed in the recognition index of the traumatized non treated group (36.2±2.28). However treatment with DMSO, ALA and vitamin C prevented the occurrence of the deficit as indicated by the NORT in these groups (figure2).

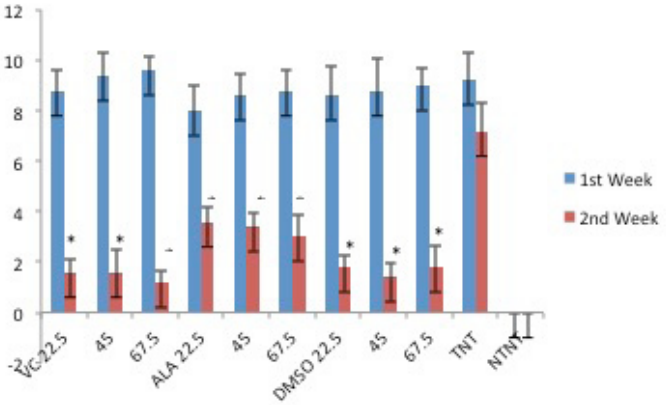

Figure 1: Modified Neurological Severity Score of the various groups
Bars with asterisk are statistically significant at p value of 0.0001
Key; DMSO – dimethyl sulfoxide, ALA – alpha lipoic acid, VC – vitamin C, TNT – traumatized non treated NTNT-Non Traumatized Non treated

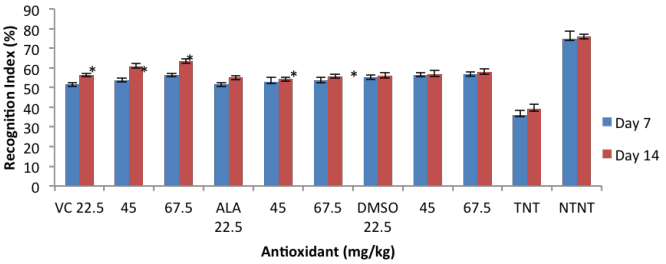

Figure 2: Effects of vitamin C, ALA, and DMSO on Object Recognition Following TBI. Bars with asterisk are statistically significant with p values of 0.0001, 0.0002, 0.0001, 0.0005 and 0.04 respectively
Key; DMSO – dimethyl sulfoxide, ALA – alpha lipoic acid, VC – vitamin C, TNT – traumatized non treated NTNT-Non Traumatized Non treated

The findings of this study revealed that TBI induction by weight drop method causes deficit in memory 7 days post induction. This was demonstrated using NORT carried out

on all the experimental animals. DMSO, vitamin C and ALA had promoting effect on memory in comparison with TNT rats. Previously, Siopi et al, 2012 demonstrated that rat model of CHI causes memory deficit 3 weeks post induction while Tsenter et al, 2008 reported impairment of memory by CHI (at its peak) only 3 days post injury in rat model.

Oral administration of 22.5, 45 and 67.5mg/kg bw of DMSO in this work attenuated the impeding effect of TBI on memory of rats by having significantly ($p<0.05$) higher recognition index than TNT group. In another work, DMSO has shown improvement in memory after observed memory deficit in rats with head injury (de la Torre, 1995).

The observed effect of DMSO on memory in this study can be attributed to one or combination of the following. DMSO restores acetyl cholinesterase activity, which has an essential role in learning and memory processes. Since mitochondrial dysfunction is one of the important factors in cognitive dysfunction following TBI, the Preventive role of DMSO on mitochondrial damage during intracellular calcium overload and other destructive processes following TBI (Sams, 1967) might be one of the mechanisms.

Oxidative stress is considered to be a probable cause of memory deficit due to white matter degeneration (which is associated with memory disorders) and impairment in hippocampal function (Silver et al., 2004), therefore the antioxidant effect of DMSO also contribute to the mechanism.

The result of this study also showed that acute treatment with three doses of vitamin C prevented the impairment of memory by TBI seven days post induction through 14 days. Similarly, Arz, et al., (2004) reported that oral supplementation of vitamin C could attenuate the risk of dementia in aged mice. Shahidi et al. (2008) showed that intraperitoneal (i.p) administration of vitamin C could improve learning and memory in intact rats. Two different studies also showed that i.p. injection of vitamin C could be useful in retention of memory in the scopolamine treated rats and impede amnesia in homocysteine administered rats (Lee, et al., 2001).

This outcome can be due to the ability of vitamin C to modulate neurotransmitter system such as serotogernic and cholinergic which has essential role in cognition (Lee et al., 2001). It might also be due to the scavenging and reducing effect of this vitamin on ROS and free radicals which causes lipid peroxidation and oxidative stress. lipid peroxidation and oxidative stress can interfere with hippocampal function which has a role in memory process (Naber et al., 2000).

Administration of ALA in this work indicated that impairment of cognitive memory by TBI was successfully mitigated. Since mitochondrial dysfunction can lead to memory impairment (Zhanga & Gou qaing, 2001), preventive and restorative effect of ALA on mitochondria during neurodegenerative diseases (Saeed et al., 2008), might probably be the reason for the observed effect. Previous work on ALA supplementation by Moriera et al, (2007) showed amelioration of cognitive decline in AD patients.

4. Conclusion

The result of this research work indicated the potential roles of these antioxidants in mitigating memory dysfunction associated with TBI. It can therefore be concluded that these antioxidants could have a memory increasing effect and /or neuroprotective reole in the mangement of TBI. However, additional work is recommended to further confirm this effect.

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