The influence of N-Acetylcysteine on locomotor activity, brain oxidative damage, and demyelination in MK-801 model of schizophrenia

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Abstract

The purpose of this study is to investigate the effects of N-acetylcysteine (NAC) on potential cerebral demyelination, oxidative damage and schizophrenia-like behaviors caused by MK-801 which is an N-methyl-D-aspartate glutamate receptor antagonist. For this, 4 groups were formed by dividing 24 male BALB/c mice into groups of six. The control group was given a saline solution (10 ml/kg) intraperitoneally (i.p.). While MK-801 (1 mg/kg-i.p.) was given both alone and with NAC (100 mg/kg-i.p.), the last group was given only NAC (100 mg/kg-i.p.). The injections were made for 14 days. It was observed that, MK-801 caused behavioral problems. When the brain was examined, it was determined that it caused a reduction in the weight of the brain, glial cell infiltration, vacuolization in neurons and shrinking in the cell nuclei in the hippocampus. A reduction in myelin basic protein (MBP) secretion was also observed. In the mice given NAC as a protector, it was observed that behavioral problems covered, antioxidant levels increased, and other impairments were repaired. It was concluded that NAC may have a neuroprotective effect.

Introduction

Schizophrenia is a chronic, severe mental disease that is characterized by disrupted social behaviors and abnormal brain function (Lewis and Lieberman 2000; Kruk-Slomka et al. 2016). Schizophrenia, which affects approximately 1% of the world's population, is 60% genetic, and associated with environmental factors such as pre-birth and post-birth stress factors and drug abuse by 40% (Greenstein and Greenstein 2000; Afifi and Bergman 2005). When the brains of schizophrenic patients are examined, it is observed that there are various pathological changes such as narrowing in some regions of the brain and growth in the ventricles (Carey 2002; Savas et al. 2002). These changes are generally seen in the limbic system (Buchsbaum et al. 2007). In microscopic examinations, disorders in synapse transmission in the cortex and reductions in the sizes of nerve cells, dendrite spine density and length were detected. Additionally, dysfunction in oligodendrocytes and shrinkage in the myelin layer in connection to this are encountered (Xiu et al. 2014). The myelin basic protein (MBP), which is the second most frequently found protein in the central nervous system, constitutes 30% of the total protein and 10% of the dry weight of the myelin layer. Problems experienced in the white matter may lead to transmission dysfunctions in axons, and afterwards, result in several problems from psychiatric diseases and weakness in the motor system (Boggs 2006; Matute and Ransom 2012). Researchers have reported that there is also a noticeable reduction in volume in the hippocampus of schizophrenia patients (Nelson et al. 1998; Savas et al. 2002). Similarly, Elfaki et al. (2013) reported that there is a statistically significant volumetric difference in the white matter of the brain in schizophrenia patients, and the white matter volumes of schizophrenia patients are lower in comparison to those of healthy individuals.

The glutamatergic N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis is proposed by many researchers to help understand the etiology and pathophysiology of schizophrenia (Kruk-Slomka et al. 2016). This hypothesis is based on the idea that a reduction in neurotransmission from the cortical regions to the brain stem causes schizophrenia-related dopaminergic irregularity (Javitt 2007). The fact

that an NMDA receptor antagonist like MK-801 causes schizophrenia-like behaviors in rodents is one of the reliable findings supporting this hypothesis. Previous studies have successfully shown that applying an NMDA receptor antagonist such as MK-801 revealed schizophrenia-like behavioral and neurobiological changes in rodents (Yu et al. 2011; Xiu et al. 2014; 2015; Kruk-Slomka et al. 2016). Yu et al. (2011) reported that MK-801 revealed various schizophrenia symptoms in mice such as biting, forced climbing, falling sideways, failure to respond to light and sounds and reduction in willingness to live with other animals and search for food. Besides these, in addition to psychotic behaviors, MK-801 applications may lead to vacuolization and neurodegeneration in some regions of the brain (Zhang et al. 1996; Horvàth et al. 1997; Kovacic and Somanathan 2010; Xiu et al. 2014; 2015). Moreover, previous studies have reported that MK-801 caused reduction in the white matter volume of the brain, reduction in the length of myelinated nerve fibers, disintegration in the myelin layer, demyelination, shrinkage in the diameters of nerve fibers and decrease the MBP production in mice (Xiu et al. 2014; 2015).

It was proposed that oxidative stress contributes to the pathophysiology of schizophrenia. This is because brain tissue is more sensitive to the toxic effects of reactive oxygen species than other organs of the body as it has high oxygen consumption and low antioxidant production rates. In addition to this, brain cell membranes are more easily affected by oxidative stress as they densely contain polyunsaturated fatty acids (Salim 2014). Short-term or long-term high-dose applications of MK-801 lead to damage in the brain tissue (Sharp et al. 1991; Xiu et al. 2014; 2015). Studies have provided findings that MK-801 leads to oxidative stress by increasing reactive oxygen species in the brain (Ozyurt et al. 2007a; 2007b; Ozyurt et al. 2014). As glutamate released into the environment cannot bind to the NMDA receptor that is subjected to hypofunction by MK-801, excessive amounts of glutamate start to accumulate in the intercellular space, and the glutamatergic system balance that is disrupted based on this leads to formation of oxidative stress and injuries in nerves (Ozyurt et al. 2007a; Genius et al. 2013). Previous studies have determined that MK-801 increases oxidant substance levels in rats (Ozyurt et al. 2007a; 2007b; Ozyurt et al. 2014). There is evidence in studies conducted on schizophrenia patients that reactive oxygen species are formed as a result of the damage it induces on central nervous system cell membranes. It was reported that there are residues related to lipid peroxidation in the blood samples of schizophrenia patients (Halliwell 1992; Virit et al. 2009).

N-acetylcysteine (NAC) is a substance with strong antioxidant and anti-inflammatory properties (Scalley and Conner 1978; Dean et al. 2011). It has been used as an antioxidant in paracetamol poisonings for more than three decades. As years have passed, and the effect mechanisms of NAC have been revealed, there has been an increase in the number of trials conducted on it. NAC, which is a mucolytic, is being used in various pulmonary and renal diseases today (Dean et al. 2011). As it easily passes through the blood-brain barrier, and successful results were obtained after its trial in Alzheimer patients as an alternative treatment method, its usage areas in psychiatry are increasingly broader today (Adair et al. 2001; Farr et al. 2003; Dean et al. 2004; Dean et al. 2011).

Reactive oxygen species that are formed during oxidative stress lead to the start of a process that may result in death by causing oxidation of the DNA, proteins and lipids in the cell. As an antioxidant, N-

acetylcysteine neutralizes free radicals before they damage the cells. It does this by increasing the cysteine-glutathione ratios. It allows renewal of antioxidants like glutathione that have been degraded by free radicals in the cells (Tardiolo et al. 2018). This is because NAC is the preliminary substance of glutathione production (Turkmen et al. 2019). This way, it increases the endogenous antioxidant defense mechanism and destroys free radicals. It was reported that such activities are also seen in the onset of neurodegenerative diseases (Tardiolo et al. 2018). In schizophrenia, against disruptions in the signal pathways, NAC strengthens NMDA receptor responses against glutamate (Himi et al. 2003; Janáky et al. 2007). NAC may be beneficial in treatment of schizophrenia by targeting to fix both oxidative stress and glutamatergic dysfunction (Carlsson 2006). NAC was tried in the clinic on schizophrenia patients, and it was reported that it had beneficial effects on the negative symptoms of schizophrenia and some other symptoms (Berk et al. 2008). While a noticeable rate of improvement was recorded in symptoms, it was reported that plasma glutathione levels increased after NAC applications (Lavoie et al. 2008). Other studies have also reported that NAC application showed beneficial outcomes in schizophrenia symptoms (Bulut et al. 2009). In toxicological studies conducted on the nervous system, it has been shown that NAC has positive, improving and protective effects against demyelination (Mirzakhani et al. 2016; Hichor et al. 2018; Zaki et al. 2018). Besides, so far, no study has been encountered to examine the effects of NAC on neurotoxicity associated with schizophrenia.

In the light of the information above, schizophrenia studies have shown that neuronal survival is an important parameter. This is why the use of neuroprotective agents in schizophrenia model studies is promising for further studies. In many experimental studies, it has been shown numerous times that NAC has neuroprotective effects against various neurotoxic agents. Furthermore, there is no study examining the effects of NAC on schizophrenia-associated cellular and molecular disorders. The purpose of this study is to investigate the neuroprotective effects of NAC on degenerative changes that occur in myelin sheaths and oxidative stress levels in brain tissue and protective effects on behavioral change assessed with the open field (OFT) and elevated plus maze (EPM) tests in a mouse schizophrenia model induced by chronic MK-801 application.

Material And Method

Drugs, chemicals and kits

NAC (600 mg/20 tablet) was purchased from Basel Drug Co. (Istanbul, Turkey). MK-801 (CAS Number 77086-22-7; molecular weight 221.30 g/mol; purity ≥ 99%, a selective NMDA receptor antagonist) and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Total oxidant capacity (TOS) and Total antioxidant capacity (TAS) kits were purchased from Rel Assay Diagnostics (Gaziantep, Turkey). All chemicals that were used in this study were of analytical grade.

Animals

Adult (8-10-week-old) BALB/c mice (n=24) weighing 30-35 g were acquired from the Experimental Animals Research Center of Afyon Kocatepe University. The animals were housed in a light-controlled

room (12 hours of light/dark, light starting at 8:00 am) at 22±1°C and 45±15% humidity with standard commercial pellet feed and free access to tap water. The permission necessary for conducting experiments on the mice was received from the Animal Experiments Local Ethics Board of Afyon Kocatepe University (AKUHADYEK-189-17), and ethical directives were adhered to throughout the experiment.

Groups and Drug Applications

After a week of adaptation, the mice were divided into four equal groups (n=6 in each group): Control, MK-801, NAC+MK-801 and NAC. To the control group, a saline solution was administered at a volume of 10 ml/kg every day in the afternoon. MK-801 (a selective NMDA receptor antagonist) was administered at a dose of 1 mg/kg every day in the afternoon, while NAC was administered at a dose of 100 mg/kg every day in the morning. The drug applications lasted for 14 consecutive days. The dose of NAC was determined according to the description of Fukami et al. (2004), and the dose of MK-801 was determined according to the method of Xiu et al. (2014; 2015). All drugs (Sigma, USA) were dissolved in saline, the solutions were freshly prepared every day, and they were applied intraperitoneally (i.p.). On the fifteenth day, the animals were subjected to the open field test (OFT) and elevated plus-maze test (EPM).

Open Field Test

Locomotor activity was calculated in a 60 x 60 x 24 cm stainless galvanized sheet metal open field test (OFT) area that was separated into 36 equal squares. The squares that were nearest to the wall were labeled "peripheral," while the rest were labeled "central." The mouse was positioned in the center of the arena for OFT, and a video camera manually monitored the number of squares crossed by the four paws (locomotor activity) for 5 minutes (Taksande et al., 2009). After each animal was subjected to the exam, the arena was washed with 70% alcohol to remove the odor.

The Elevated Plus-Maze Test

The EPM test measured exploratory locomotor activity as well as anxiety-like behaviors. A platform made of stainless galvanized sheet metal with two open arms (30 cm x 5 cm) and two enclosed arms of the same size with 15-cm-high walls was used for the EPM test. Both of the armies converged in the middle of the arena (5 cm x 5 cm). Each animal was placed in the middle of the plus maze, facing the enclosed arm, and monitored by a video camera for 5 minutes. The maze apparatus was washed with 70% alcohol after each trial. It was recorded how much time the animals spent in enclosed arms. If the animal's four legs and body moved to that zone, it was determined whether it was in the open arm, enclosed arm, or centre. Mice prefer safe enclosed arms to open arms that cause anxiety. (Akillioglu et al. 2012).

Sacrifice and Examination of Brain Tissue

All animals were sacrificed on the 16th day by cervical dislocation. The skulls of the animals were opened, and their brains were removed. The brains were weighed and divided into two pieces from the middle. One half of the brains were allocated for stereological-pathological examination and

measurements, while the other half were allocated for TAS-TOS measurements and real-time PCR analyses.

The brain hemispheres allocated for stereological examination were consecutively sliced in compliance with the systematic uniform random sampling method (Gundersen et al., 1988) at thicknesses of 40 microns with a microtome from the polus rostralis towards the caudal. While thick cross-sections were taken for pathological measurements, 5-micron samples were taken from the cross-sections in-between until reaching a thickness of 40 microns, and pathological assessments were made on these cross-sections. On the thick cross-sections, the diameters of the nuclei of cells in the hippocampus CA1 (cornu ammonis 1) region were measured with the help of the M-Shot software (x100). The measurements were made on the cells in the central zone on the thick cross-sections with a safe height interval. The safe height interval was determined as the 10-micron zone in the central zone of the cross-section after the cross-section thickness was measured with the help of a microcator. The pathological results of the light microscopy test were scored as follows: Observed changes were graded as 0 (no changes), 1 (mild changes), 2 (moderate changes), or 3 (severe changes) in a blinded manner.

Preparation of brain tissue homogenate

The brain tissues were separately weighed after washing with a 0.9% NaCl solution and transferred into thick-walled glass tubes. Afterwards, to dilute the brain tissues, cold phosphate buffer (pH 7.4, 50 mM) was added onto them by ten times their weight, and they were homogenized in a flask full of ice for 10 s at the first speed setting (IKA Ultra Turrax-T18, Germany). The homogenization process was ended after observing that the tissues were homogenously disintegrated inside the tube at 10-s intervals. The obtained homogenates were then centrifuged at 2795 g for 10 min (Nuve NF 1000R, Ankara, Turkey). All procedures were carried out at 4°C. The brain samples were kept at -80°C before the oxidative stress parameters were assessed.

TAS-TOS measurement in brain tissue homogenate

The TOS of the tissues was measured based on the method described by Erel (2005) by using a total oxidant level kit (Rel Assay Diagnostics, Gaziantep, Turkey). The oxidants in the sample convert ferrous ion chelator complexes into ferric ions. Ferric ions form a colored complex with the chromogenic solution. The absorbance of this complex was measured at 530 nm with an ELISA reader set at 25°C to determine the TOS levels, and it was directly proportional to the oxidant amount in the sample. The results are presented as μmol H_2O_2 equiv/L.

The TAS of the tissues was measured based on the method described by Erel (2004) by using a TAS Rel Assay brand kit (Rel Assay Diagnostics, Gaziantep, Turkey). The measurement is made based on the discoloration of antioxidant molecules. According to the instructions of the kit, 500 μL of reagent 1 (measurement buffer) and 30 μL supernatant were combined, and the absorbance was measured at 660 nm by an ELISA kit set at 25°C to determine the TAS levels. After this, 75 μL of reagent 2 (colored ABTS solution) was added to the mixture, and the product was incubated for 10 min. The TAS levels were

determined by reading the absorbance at 660 nm after incubation. Trolox was used as a calibrator, and the results are presented as mmol Trolox equiv/L.

Real-Time PCR

Total RNA was extracted from the brain tissue according to the instructions of the manufacturing firm using a kit with an RNA isolation capacity (GeneAll Hybrid R, RiboEx-Seoul/Korea). For this aim, a 100 mg of tissue was homogenized by dividing into small pieces with a lancet, and it was vortexed by adding 1 mL RiboEx onto it. The kit that was used for the cDNA synthesis was a HyperScript™ First strand Synthesis, GeneAll Hybrid R (Seoul/Korea). For preparing a master mix, the RNA sample, primer, dNTP mix and RNase-free distilled water were used. After the cDNA master mix was prepared, it was incubated for 5 min at 65°C. After incubation, the mixture was put onto ice. After this, onto the cDNA master mix, the 10X RTase Reaction Buffer, 0.1 M DTT, Reverse Transcriptase and RNase Inhibitor components were added, and a homogenous mixture was ensured. After preparing the master mix for cDNA synthesis, the next step was the reverse transcription reaction. The reverse transcription reaction was carried out in two steps. By leaving for 60 min at 55°C in the first step and for 5 min at 85°C in the second step, the cDNA synthesis was completed. The cDNA samples were frozen at -80°C. In the real-time PCR, for MBP amplification, the forward primer TGGAGAGATTCACCGAGGAGAGGC and reverse primer TGAAGCTCGTCGGACTCTGAGGGC primer sequences and a kit (RealAmpTM SYBR qPCR Master mix) were used. As the master mix components used for the real-time qPCR, 2X MasterMix (with SYBR-Green), ROX Dye, Forward Primer (10 µM), Reverse Primer (10 µM), cDNA Template and RNase-free distilled water were used. The real-time qPCR reaction was carried out in an Applied Biosystems™ 7500 Fast Real-Time PCR device. The PCR program was started with 1 cycle for 300 s at 95°C as the initial denaturation. This was followed by 40 cycles at 95°C for 15 s and 55-68°C for 60 s, and the program was ended with a melting curve analysis.

Statistical Analysis

The data obtained in the study were analyzed by using the SPSS 21.0 for Windows package software. In the statistical analysis, firstly a normality test was applied to see whether or not there was a normal distribution among the groups. The parametric test of analysis of variance (ANOVA) was used in the comparison of the data confirmed to be normally distributed based on the groups. Duncan's test was applied in the pairwise comparisons of the groups. The level of statistical significance for the difference between groups was taken as 0.05.

Results

Effects of NAC on schizophrenia-like behaviors induced by MK-801 applications

Half an hour after the injection of MK-801 to the mice, the mice displayed falling sideways, trying to climb forcedly, locomotor hyperactivity, incoordination disorder, and loss of willingness to seek food. In the group given NAC against MK-801, while the symptoms like falling sideways, trying to climb forcedly, and

incoordination disorder decreased much, it was observed that the locomotor hyperactivity continued. In the open field test, the distance traveled (squares counted) (Fig. 1A) and the time spent in the central zone (seconds) (Fig. 1B) were examined to detect locomotor problems. Besides, time spent in the enclosed arms (sec) (Fig. 2) was examined to detect anxiety problems in the elevated plus maze test. In these tests conducted the next day after the last drug application, there was no statistically significant difference between the groups in terms of behavioral problems ($p>0.05$).

Effects of MK-801 and NAC on brain weight

After sacrificing, the brains of the mice were taken out of their skulls and weighed. It was observed that the mean brain weight of the group that was given only MK-801 decreased in comparison to the other groups. However, this decrease was not statistically significant (Table 1; $p>0.05$).

Effects of NAC on oxidative stress parameters

When the groups were compared in terms of their total oxidant statuses, it was observed that the TOS levels increased only in the MK-801 group in comparison to the other groups, but this increase was not statistically significant (Fig. 3A; $p>0.05$).

When the groups were compared in terms of their total antioxidant statuses, it was observed that the TAS levels decreased in the MK-801 group significantly in comparison to the NAC group ($p<0.05$), while this decrease was not statistically significant in comparison to the control and NAC + MK-801 groups (Fig. 3B; $p>0.05$).

Histopathological Findings

The histopathological changes in the brains of the animals in the experiment groups are shown in Fig. 4. When the cross-sections stained with hematoxylin-eosin were examined under a light microscope, it was observed that the brains of the control group mice showed a familiar general histological structure (Fig. 4A). In the brain cortices of the mice that were given only MK-801, vacuole formations were noticeable (Fig. 4C). The vacuole formation in the brains of the mice given NAC for protective purposes was lower in comparison to the MK-801 group (Figure 4D; Table 2-3). In addition to this structural disorder noticed in the brain cortex, there was also intense glial cell infiltration in the brains of the mice given MK-801 (Figure 4C; Table 2-3), while the infiltration in the group given NAC for protective purposes was close to that in the control group (Figure 4A, D; Table 2-3).

Hematoxyline-eosin staining of neurons in the hippocampal CA1 region of mice indicated extensive damage with a large number of dark-stained shrunken neurons and shrunken cell nuclei in the MK-801 group (Fig. 5C; Table 4). In contrast, the NAC+MK-801 group represented significantly reduced degenerative neurons. The neurons in this group were in the normal posture with well-outlined nuclei (Figure 5D; Table 4). When the nucleus diameters of the cells in the hippocampus CA1 region were examined, it was revealed that the neuron nucleus diameters of the mice given MK-801 (Fig. 5C; Table 4)

were smaller in comparison to the other groups. In the mice in the NAC + MK-801 group (Figure 5D; Table 4), the values were very close to those in the control group.

MBP Expression Findings

Figure 6 presents the data on the mRNA expression of MBP in the structure of the myelin sheath of the groups. It was observed that the MBP mRNA expression levels in the NAC + MK-801 group increased in comparison to the MK-801 group, and the NAC application increased the MBP mRNA expression (Fig. 6.; $p<0.01$).

Discussion

This study investigated the protective effects of NAC against degeneration and schizophrenia symptoms that arise in the brain after MK-801 application in mice. While it was found that MK-801 application in the mice led to falling sideways, trying to climb forcedly, locomotor hyperactivity, difficulty in walking, incoordination disorder and loss of willingness to seek food, it was observed that it also led to vacuole formation, glial cell infiltration in the brain, shrinkage in the hippocampus neuron nuclei and reduction in the MBP release. After administering NAC for protective purposes against MK-801, although the locomotor hyperactivity continued in the mice, they started to walk more regularly, had reduced behaviors like falling sideways and trying to climb forcedly, had lower glial cell infiltration rates, and shrinkage in their hippocampus cell nuclei was prevented.

The cause of difficulty in motor activities in schizophrenia started to be researched in animal models, and it was seen that this situation especially arose as a result of disruptions in cell function in oligodendrocytes in the white matter and myelination problems (Walther and Strik 2012). Degeneration of the myelin layer may suggest not only schizophrenia but also multiple sclerosis (MS), which is another disease related to problems in myelin sheath production (Karussis 2014). The myelin sheath is subjected to degeneration also in this disease, and as a result, problems in motor activities such as difficulty in walking are encountered. The layer in the brain that is rich in myelin sheaths is the white matter region. Degeneration of the myelin layer found in the white matter results in weakness in the motor system in patients after a certain time. It was observed in previous studies that MK-801 leads to volumetric shrinkage in the white matter of the brain, and it was found that this shrinkage was caused by degeneration in the neurons and myelin sheaths found in this region (Xiu et al. 2014; 2015).

In recent years, various studies have revealed that NAC has a protective effect against neurotoxicity and myelin degeneration (Mirzakhani et al. 2016; Zaki et al. 2018). In the study by Zaki et al. (2018), where NAC was applied for protective purposes against cisplatin toxicity, it was reported that the myelin sheath on the sciatic nerve was affected by cisplatin toxicity, this led to folding, dissolution and degeneration in the myelin sheath, whereas NAC had a protective effect in the NAC-given rats, and in these rats, the structure of the myelin sheath was close to that in the control group. In another study, where Mirzakhani et al. (2016) investigated the protective activity of NAC, it was stated that NAC applied on rats whose sciatic nerves were damaged by compression increased the recovery in the sciatic nerves and reduced the

negative changes in the myelin sheath. Abd-Ellah et al. (2018) investigated the protective activity of the NAC and folic acid combination against aspartame neurotoxicity and reported that NAC had a protective effect against degeneration in the myelin layer. The researchers stated that NAC could be used for protective purposes against neurotoxicity. Haber et al. (2018) applied NAC combined with minocycline plus for protection against trauma-related damage induced on the brain and determined that NAC protected the myelin layer and stimulated remyelination. In a study by Hichor et al. (2018) examining the protective role of NAC on mice against sciatic nerve damage, the researchers detected damage in the myelin layer on the sciatic nerve, and it was observed that NAC application noticeably reduced this damage. NAC was also successful in bringing the myelin protein values within normal limits. Furthermore, the movement problems of the animals given NAC disappeared, and their values were normalized.

Oxidative stress refers to disruption of the balance between reactive oxygen species produced by the cell and antioxidant mechanisms that counter these. As the brain consumes excessive amounts of oxygen and is rich in lipids, it is highly sensitive to oxidative stress (Salim 2014). Today, there are still conflicting results regarding the antioxidant status of schizophrenia patients. Copoglu et al. (2015) found that TOS levels increased, and TAS levels decreased in individuals with schizophrenia in comparison to the control group. Bahceci et al. (2015), on the other hand, determined that, in comparison to healthy individuals, TOS levels increased, but TAS levels did not change in schizophrenic individuals. The relationship between experimental schizophrenia models and oxidative stress has been a focus among researchers in recent studies. In these studies, it has been emphasized that MK-801-induced neurotoxicity decreased, and locomotor problems disappeared by using various antioxidants such as CAPE (caffeic acid phenethyl ester), omega 3 fatty acid and melatonin (Ozyurt et al. 2007a; 2007b; Ozyurt et al. 2014). In this study, although it was shown that MK-801 applications increased the TOS levels and reduced the TAS levels, it was demonstrated that these differences were not significant, and the combined applications of NAC with MK-801 did not create a significant change in the TOS and TAS levels.

In the study by Jatana et al. (2006) where the protective activity of NAC on the brain was assessed, the authors observed the protective effect of hypothermia and subsequently applied NAC in the hypoxic brain. While a highly substantial loss was observed in the brain volumes of the hypoxic animals when they were examined 2 and 4 weeks later, the brain volume values of the rats that were applied hypothermia + NAC were found very close to those in the sham group that received no intervention. Likewise, when the brains of the rats in the group that was applied hypothermia + NAC were examined, an increase was determined in myelin sheath production and MBP release. Previous studies have reported that MK-801 applied for 14 days reduced MBP mRNA release at the end of this duration (Xiu et al. 2014; 2015). In mouse brains, MK-801 reduces the expression of the MBP gene that is associated with myelin sheath production. In this study, it was similarly observed that the reduced MBP mRNA release was increased by administering NAC.

In this study, when the diameters of the cell nuclei in the hippocampus CA1 region of the brain were calculated, a statistically significant reduction was determined in the diameters of the nuclei of the mice

given MK-801. To the best of our knowledge, this is the first such report on the cell nucleus morphology in this region of the brain. This shrinkage in the neuron nuclei may be an indicator of the presence of neurodegeneration (Bonde et al. 2002). In both previous studies and this study, when the brains of the mice in the groups given MK-801 were examined under light microscopes, vacuole formations and glial cell infiltrations as neurodegeneration indicators were noticed (Olney et al. 1989; Sharp et al. 1991). It is also known that MK-801 applications significantly reduce NMDA receptor release in the hippocampus (Kim et al. 2014). Previous studies have reported that there is a volumetric reduction in the hippocampus in individuals with schizophrenia (Nelson et al. 1998; Savas et al. 2002). As it may be understood based on the results obtained from the MK-801 group in this study, the cause of the volumetric shrinkage in the hippocampus may be related to the reduction in the myelin sheath production and neurodegeneration.

Conclusion

In this study, it was observed that NAC that was used for protective purposes against schizophrenia symptoms induced with MK-801 protected the brain and neurons against degeneration, repaired locomotor activity disorders to a certain extent, increased the antioxidant levels in the brain and could have protective effects on myelin sheath production. Consequently, although NAC has neuroprotective effects, it is needed to conduct more studies on it to discover its exact benefits.

Declarations

Compliance with ethical standards

Ethical Approval The Animal Experiments Local Ethics Board, Afyon Kocatepe University, Afyon, (Registration number: AKUHADYEK-189-17) has approved this study.

Consent to Participate Not applicable

Consent to Publish Not applicable

Conflict of interest The authors declare that they have no conflict of interest.

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Availability of data and materials The datasets used/analyzed in this study are available from the corresponding author on reasonable request.

Authors' contributions: The idea of the research was recommended by M.S.A, who also took part in the design of the study. M.S.A. and R.T. performed the experimental work and wrote/drafted/edited the manuscript and interpreted the results. M.S.A., R.T. and H.H.D. performed the laboratory analyses. All authors were involved in revising the manuscript critically for important intellectual content, and all authors approved the final version to be published.

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Tables

Table 1: Brain weights of mice

Measurements	Control	MK-801	NAC+MK-801	NAC	P value
Brain weight (gr)	0.45±0.02	0.43±0.01	0.44±0.03	0.44±0.02	0.07

Table 2: Effects of repeated MK-801 (1mg/kg), *N*-acetylcysteine (NAC-100 mg/kg), and NAC (100 mg/kg) + MK-801 (1 mg/kg) on histopathological changes in the brain of mice.

Tissue	Histopathological changes	Control	NAC	MK-801	NAC + MK-801
Brain	Vacuolization in neurons	-(5/6) +(1/6)	-(5/6) +(1/6)	-(2/6) +(1/6) ++(3/6)	-(3/6) +(3/6)
	Focal glial cell infiltration in neurons	-(6/6)	-(6/6)	-(1/6) +(1/6) ++(4/6)	-(4/6) +(2/6)

The findings were evaluated in a blinded manner and scored as follows: −, no lesion; +, mild; ++, moderate; +++, severe.

Table 3: Statistical analysis for histopathological findings in the different study groups.

Groups	Vacuolization in neurons	Focal glial cell infiltration in neurons
Control	0.14 ± 0.37^{b}	0.00 ± 0.00^{b}
NAC	0.22 ± 0.37^{b}	0.14 ± 0.38^{b}
MK-801	1.04 ± 0.95^{a}	1.42 ± 0.78^{a}
NAC + MK-801	0.49 ± 0.48^{ab}	0.36 ± 0.44^{b}
P value	0.029	0.000

Note: 0: no observed changes; 1: mild changes; 2: moderate changes; 3: severe changes.

^{a,b,c} In the same column, values with different letters show statistically significant differences in vacuolization and focal glial cell infiltration in neurons among the different groups ($p < 0.05$). n=6 per group (data expressed as mean ± SD). Abbreviations: NAC, *N*-acetylcysteine

Table 4: Vacuolization in neurons, focal glial cell infiltrations and nucleus diameter measurements (µm) according to results of histopathological assessments

Measurements	Control	NAC	MK-801	NAC+MK-801	*P* value
Vacuolization in neurons	0.14 ± 0.37^{b}	0.22 ± 0.37^{b}	1.04 ± 0.95^{a}	0.49 ± 0.48^{ab}	0.049*
Focal glial cell infiltrations	0.22 ± 0.34^{b}	0.10 ± 0.01^{b}	1.42 ± 0.78^{a}	0.36 ± 0.44^{b}	0.000**
Hippocampus neuron nucleus shrinkage	18.7 ± 0.80^{a}	18.2 ± 1.20^{a}	16.7 ± 0.50^{b}	19.2 ± 1.3^{a}	0.000**

[a,b] In the same column, values with different letters show statistically significant differences in vacuolization and focal glial cell infiltration in neurons among the different groups ($p < 0.05$). n=6 per group (data expressed as mean ± SD). Abbreviations: NAC, *N*-acetylcysteine

Figures

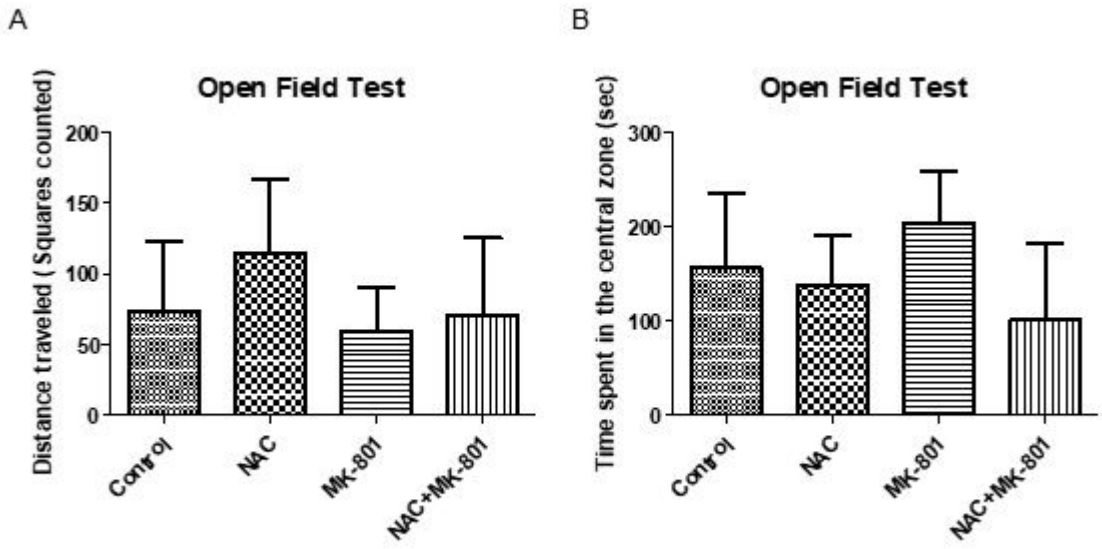

Figure 1

Effects of NAC on schizophrenia-like behaviors induced by MK-801 applications. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days, and placed on an open field device 24 hours later. The distance traveled (A) in 5 min on the open field device did not significant differences between the groups. (B) Time spent in the central zone in 5 min on the open field device did not significant differences between the groups; n=6 per group (data expressed as mean ± SD). Abbreviations: sec, seconds; NAC, N-acetylcysteine..

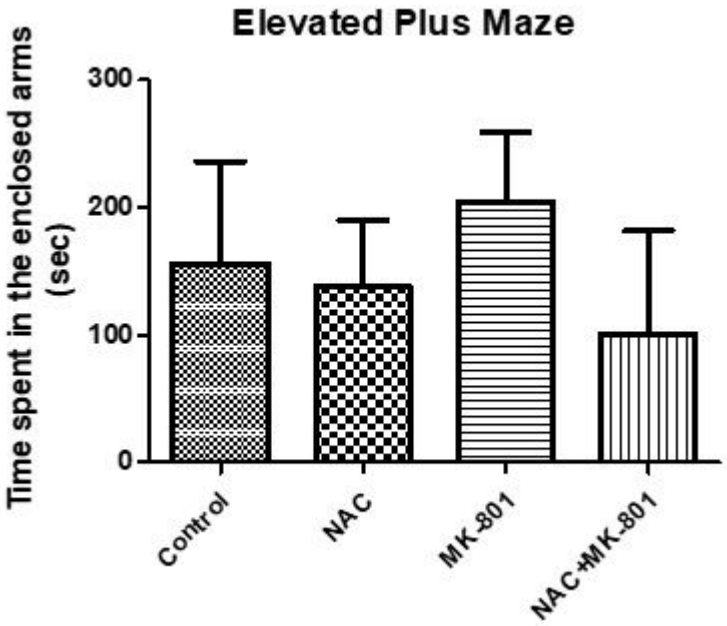

Figure 2

Effects of NAC on schizophrenia-like behaviors induced by MK-801 applications. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days, and placed on an elevated plus maze device 24 hours later. Time spent in the enclosed arms in 5 min on the elevated plus maze device did not significant differences between the groups; n=6 per group (data expressed as mean ± SD). Abbreviations: sec, seconds; NAC, N-acetylcysteine.

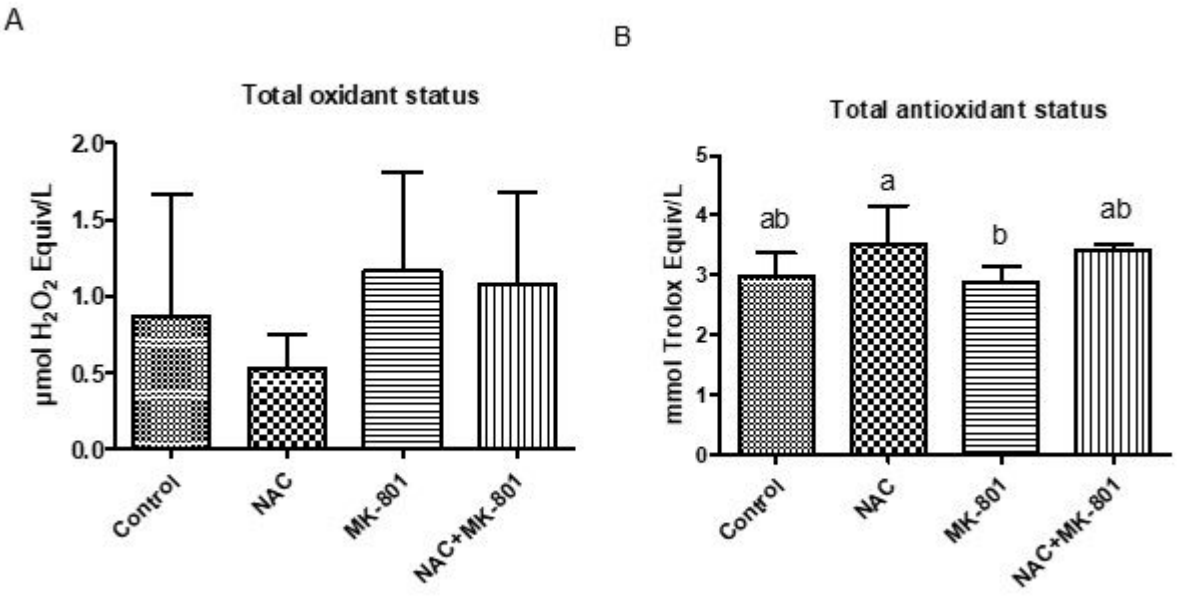

Figure 3

Effects of NAC and MK-801 on levels of TOS and, TAS among the different groups. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days. 48 hours later, mice brains were obtained. TOS and, TAS were measured by a colorimetric method as described in the methodological section. Values bearing different letters on the bars show statistically significant differences on levels of TOS (A) and, TAS (B) among the different groups ($p < 0.05$); n=6 per group (data expressed as mean ± SD). Abbreviations: TOS, total oxidant status; TAS, total antioxidant status; NAC, N-acetylcysteine.

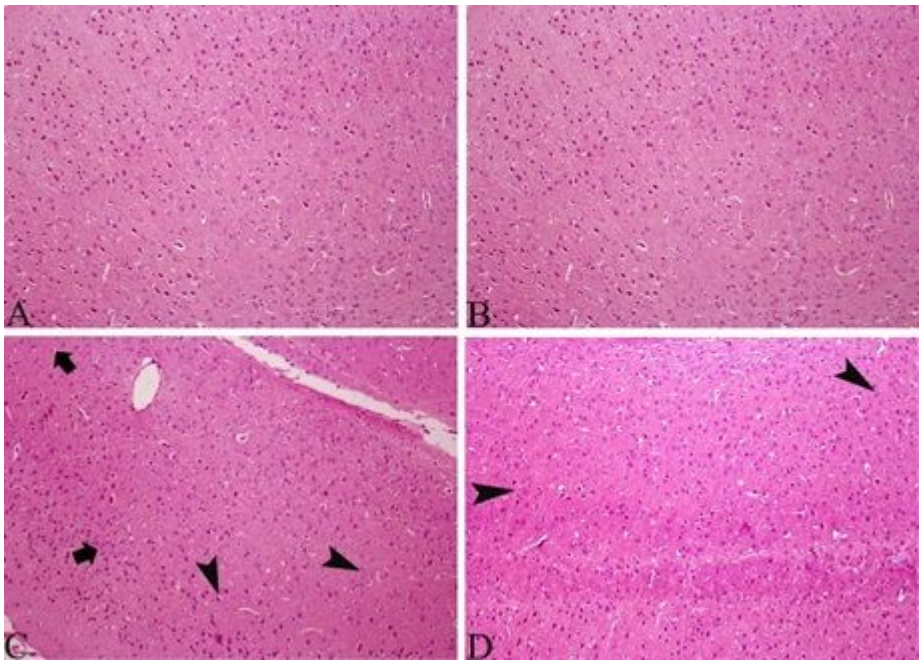

Figure 4

Effects of N-acetylcysteine on histopathological changes induced by MK-801 applications. Representative figures were stained with Hematoxylene and eosine . The original magnification was ×20 and the scale bars represent 100 μm. Arrows indicate vacuolization (C) and arrow head indicate focal glial cell infiltration in neurons (C, D) in the brain. A. Control group; B. NAS group; C. Animals treated with 1 mg/kg/day MK-801 group; D. Animals treated with 100 mg/kg/day NAC and 1 mg/kg/day MK-801 (NAC+MK-801 group)

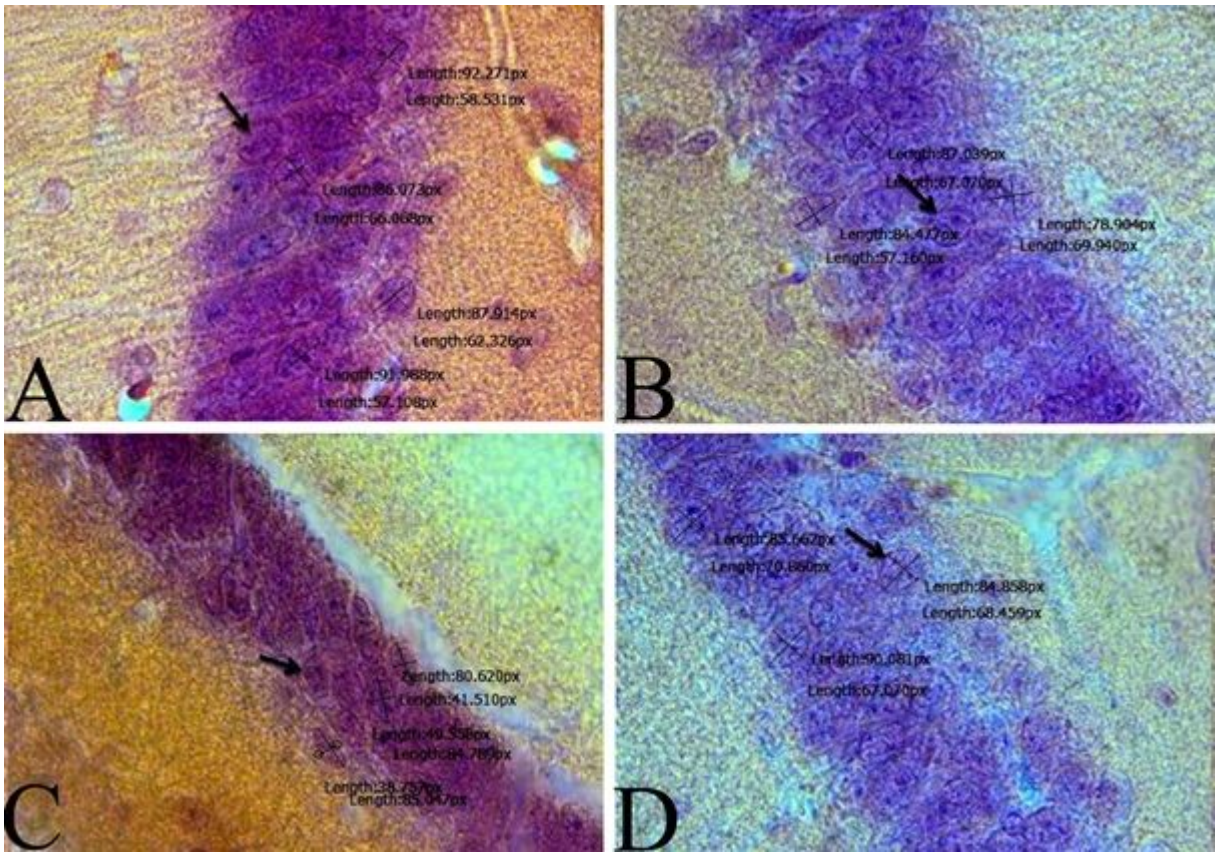

Figure 5

Hippocampal CA1 neuronal cell line. The measurements were performed on the nuclei of the neurons. The original magnification was ×100 and the scale bars represent 40 μm. A. Control group; B. NAS group; C. Animals treated with 1 mg/kg/day MK-801 group; D. Animals treated with 100 mg/kg/day NAC and 1 mg/kg/day MK-801 (NAC+MK-801 group)

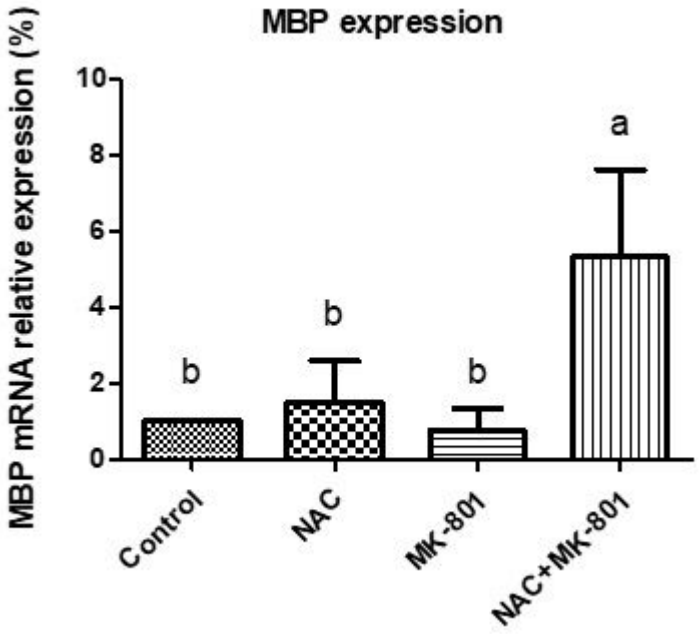

Figure 6

Effects of N-acetylcysteine and MK-801 on the MBP mRNA expression levels among the different groups. Mice were administered with NAC (100 mg/kg, intraperitoneally [ip]) in the morning and with MK-801 (1 mg/kg, intraperitoneally [ip]) in the afternoon for 14 consecutive days. 48 hours later, mice brains were obtained and processed for RT-PCR. Values bearing different letters on the bars show statistically significant differences on the MBP mRNA expression levels among the different groups ($p < 0.05$). n=6 per group (data expressed as mean ± SD). Abbreviations: MBP, myelin basic protein; NAC, N-acetylcysteine.