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ORIGINAL RESEARCH

Neuroprotection Induced by *N*-acetylcysteine and Selenium Against Traumatic Brain Injury-Induced Apoptosis and Calcium Entry in Hippocampus of Rat

Mustafa Nazıroğlu · Nilgün Şenol · Vahid Ghazizadeh · Vehbi Yürüker

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Abstract Neurodegeneration associated with acute central nervous system injuries and diseases such as spinal cord injury and traumatic brain injury (TBI) are reported to be mediated by the regulation of apoptosis and oxidative stress through Ca^{2+} influx. The thiol redox system antioxidants, such as *N*-acetylcysteine (NAC) and selenium (Se), display neuroprotective activities mediated at least in part by their antioxidant and anti-inflammatory properties. However, there are no reports on hippocampal apoptosis, cytosolic reactive oxygen species (ROS), or Ca^{2+} values in rats with an induced TBI. Therefore, we tested the effects of Se and NAC administration on apoptosis, oxidative stress, and Ca^{2+} influx through TRPV1 channel activations in the hippocampus of TBI-induced rats. The 32 rats were divided into four groups: control, TBI, TBI + NAC, and TBI + Se groups. Intraperitoneal administrations of NAC and Se were performed at 1, 24, 48, and 72 h after TBI induction. After 3 days, the hippocampal neurons were freshly isolated from the rats. In cytosolic-free Ca^{2+} analyses, the neurons were stimulated with the TRPV1 channel agonist capsaicin, a pungent compound found in hot chili peppers. Cytosolic-free Ca^{2+}, apoptosis, cytosolic ROS levels, and caspase-3 and -9 activities were higher in the TBI group than control. The values in the hippocampus were decreased by Se and NAC administrations. In conclusion, we observed that NAC and Se have protective effects on oxidative stress, apoptosis, and Ca^{2+} entry via TRPV1 channel activation in the hippocampus of this TBI model, but the effect of NAC appears to be much greater than that of Se. They are both interesting candidates for studying the amelioration of TBIs.

Keywords Apoptosis · Hippocampus · *N*-acetylcysteine · Oxidative stress · Traumatic brain injury · TRPV1 channels

Abbreviations

$[Ca^{2+}]_i$	Intracellular Ca^{2+}
CAP	Capsaicin
DMSO	Dimethyl sulfoxide
ROS	Reactive oxygen species
PBS	Phosphate-buffered saline
Se	Selenium
TBI	Traumatic brain injury
TRP	Transient receptor potential
TRPV1	Transient receptor potential vanilloid 1
NAC	*N*-acetylcysteine

Introduction

Oxidative stress occurs in the body during physiological functions such as phagocytosis and mitochondrial functions. If not controlled by antioxidants it can induce tissue damage (Nazıroğlu 2007; Daiber et al. 2013). It has been demonstrated that local and systemic inflammation responses, as well as neurodegenerative disease, are also associated with reactive oxygen species (ROS). ROS are scavenged by enzymatic and nonenzymatic antioxidants (Nazıroğlu 2007). Selenium is well established as an essential trace mineral, which plays critical roles in many

M. Nazıroğlu (✉) · V. Ghazizadeh
Neuroscience Research Center, University of SuleymanDemirel, TR-32260 Isparta, Turkey
e-mail: mustafanaziroglu@sdu.edu.tr

N. Şenol · V. Yürüker
Department of Neurosurgery, Faculty of Medicine, University of Suleyman Demirel, Isparta, Turkey

biological processes (Nazıroğlu and Yürekli 2013). In fact, selenium is known primarily for its antioxidant activity as a component of glutathione peroxidase (GSH-Px) and, in therapeutic aspects, for its anti-inflammatory and antiviral properties (Dalla Puppa et al. 2007). Selenium is also required for the catalytic activity of mammalian thioredoxin reductase, another significant antioxidant enzyme (Yeo and Kang 2007). *N*-acetylcysteine (NAC) is a thiol-containing (sulphydryl donor) antioxidant, which contributes to regeneration of glutathione and also acts through a direct reaction with free radicals (Özgül and Nazıroğlu 2012; Nazıroğlu et al. 2013a). In addition, positive clinical responses obtained during therapy with NAC and selenium in neurodegenerative diseases have provided substantial evidence for the important role of ROS in pathological processes of traumatic brain injury (TBI) (Jeo and Kang 2007; Chen et al. 2008). TBI is a leading cause of morbidity and mortality and represents a major public health burden (Woodcock and Morganti-Kossmann 2013). After TBI, a complex cascade of pathophysiological processes rapidly damages nervous tissue. This increased vulnerability due to TBI may depend on a change in the nature and timing of the activation of a number of neuroprotective and neurodegenerative molecular signals in the injured brain (Raghupathi 2004). The initial damage spreads to surrounding tissue by different mechanisms, including oxidative stress (Jeo and Kang 2007) and activation of cation channels (Nazıroğlu et al. 2013b). These changes can force injured tissue beyond a point of no return and precipitate an irreversible neurodegenerative process. A better knowledge of the molecular signals activated in a state of increased vulnerability due to trauma can aid in devising future treatment strategies, and enable the prediction of neurological outcomes after TBI.

Calcium ion (Ca^{2+}) is an important second messenger that has been shown to be responsible for a number of signal transduction pathways including neuronal excitability, metabolism, cell proliferation, and cell death (Nazıroğlu 2007; Espino et al. 2011). It is well known that Ca^{2+} is involved in the induction of TBI. One family of calcium channels is transient receptor potential (TRP) cation channels, and they were first described in *Drosophila*, in which photoreceptors carrying *trp* gene mutations exhibit a transient voltage response to continuous light (Nazıroğlu 2011). The mammalian TRPs are subdivided into seven major different sub-families including the vanilloid (TRPV) and melastatin (TRPM). Increased ROS production and inflammation can activate redox-sensitive membrane channels in TBI (Zhao et al. 2008). Hence, we are particularly interested in a sub-family of TRPV members, known as TRPV1, because of its potential role in cell death resulting from oxidative stress and inflammation (Graphical Abstract) (Nazıroğlu et al. 2012). As TRPV1 channels are permeable to Ca^{2+} and have previously been implicated in other oxidative stress-induced neurodegenerative disorders (Nazıroğlu 2011; Nazıroğlu et al. 2012), activation of these channels is a potentially important mechanism that may contribute to the pathogenesis of TBI. To our knowledge, there is no report of selenium and NAC treatments on oxidative stress, apoptosis, or Ca^{2+} channels in hippocampal neurons after TBI.

In the present study, we have evaluated, for the first time, the possibility that the exposure to TBI could induce TRPV1 cation channel activation, oxidative stress, and apoptosis in hippocampal neurons of TBI-induced rats, and NAC and selenium administrations may modulate the changes in the neurons.

Materials and Methods

Chemicals

Ethylene glycol-bis(2-aminoethyl-ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA), dimethyl sulfoxide (DMSO), capsaicin (CAP), and Roswell Park Memorial Institute (RPMI) 1640 medium were obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA), *N*-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (ACDEVD- AMC), nonidet-P-40 substitute (NP40), 2-(*N*-morpholino)ethanesulfonic acid hydrate (MES hydrate), PEG, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-[(3-chomalidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), NAC, selenium (sodium selenite), and dithiothreitol (DTT) were obtained from Sigma-Aldrich Chemical Co. (Istanbul, Turkey). Dihydrorhodamine-123 (Rh 123) and *N*-acetyl-Leu-Glu-His-Asp-7-amino-4-methylcoumarin (AC-LEHD-AMC) were purchased from Bachem (Bubendorf, Switzerland). All organic solvents were purchased from Santa Cruz (Istanbul, Turkey). Fura-2/AM was purchased from Promega (Eugene, OR, USA). The reagents were equilibrated at room temperature for half an hour before an analysis was initiated or reagent containers were refilled.

Animals and Induction of TBI

All experimental procedures were approved by the Medical Faculty Experimentation Ethics Committee of Süleyman Demirel University (Protocol Number; 2013-12/02). 36 male adult Sprague–Dawley rats (aged 6 months and weighing 330 ± 20) were used in the current study. The rats were maintained at 21 ± 2 °C under a 12/12 h light/dark cycle with rodent chow and water ad libitum. Animals were injured using a 1-cm inner diameter ×10-cm-long glass tube, through which a 300 g weight (0.5-cm diameter) was dropped onto the

brain at the craniotomy site, causing a contusional head trauma (Marmarou et al. 1994).

Study Groups

The animals were randomly divided into four groups as follows:

Group I: Control group ($n = 8$): Placebo was supplemented to the first group.
Group II: TBI group ($n = 8$): TBI was performed on each animal through 300 g weight causing a contusional head trauma (Marmarou et al. 1994).
Group III: TBI plus NAC group ($n = 8$): NAC (150 mg/kg body weight) was orally (via gastric gavage) given to the animals at 1, 24, 48, and 72 h after brain trauma (Oksay et al. 2013; Senol et al. 2014).
Group IV: TBI plus selenium group ($n = 8$): Selenium (1.5 mg/kg body weight) was intraperitoneally administrated to the animals at 1, 24, 48, and 72 h after brain trauma (Nazıroğlu et al. 2008; Senol et al. 2014).

We did not add to the study groups NAC and Se administrated animals without TBI because antioxidant and Ca^{2+} entry modulator roles of the antioxidant have been recently investigated (Weber, 2012; Nazıroğlu et al. 2013a).

Preparation of Hippocampal Samples

The animals were killed by ether asphyxiation and cervical dislocation in accordance with SDU Experimental Animal legislation. Primary cultures of rat hippocampal neurons were prepared as previously described (González et al. 2007). Hippocampal tissue was dissected and placed in cold Hanks' solution prior to mechanical dissociation by trituration and incubated for 30 min with trypsin and mixed every 10 min. It was centrifuged (at 500 g for 5 min), and the supernatant was discarded and replaced by Hanks' solution for twice and then used in assays. Cells were plated at a density of $<1 \times 10^6$ cells/ml on either of the 35-mm culture dishes.

Measurement of Intracellular-Free Calcium Concentration ($[Ca^{2+}]_i$)

The hippocampus cells were loaded with fura-2 by incubation with 4 μM fura-2/AM for 45 min at room temperature according to a procedure published elsewhere (Uğuz and Nazıroğlu 2012; Uğuz et al. 2012). Once loaded, the cells were washed and used within the next 2–4 h. All groups were exposed to CAP for stimulation of ($[Ca^{2+}]_i$) influx. Fluorescence was recorded from 2 ml aliquots of magnetically stirred cellular suspension at 37 °C by using a spectrofluorometer (Carry Eclipsys, Varian Inc, Sydney, NSW, Australia) with excitation wavelengths of 340 and 380 nm and emission at 505 nm. Changes in $[Ca^{2+}]_i$ were monitored using the fura-2 340/380 nm fluorescence ratio and were calibrated according to the method of Grynkiewicz et al. (1985).

Apoptosis Level and Caspase Activity Assays

The APOPercentage assay kit was used according to the instructions provided by Biocolor Ltd. (Belfast, Northern Ireland) and described elsewhere (Uğuz and Nazıroğlu 2012; Nazıroğlu et al. 2013c).

The determination of caspase-3 and -9 activities was based on a method as previously reported (Espino et al. 2011; Nazıroğlu et al. 2013a). Stimulated or resting cells were washed once with PBS. After centrifugation, cells were resuspended in PBS at a concentration of 10^5 cells/ml. 15μl of the cell suspension was added to a microplate and mixed with the appropriate peptide substrate dissolved in a standard reaction buffer that was composed of 100 mM HEPES, pH 7.25, 10 % sucrose, 0.1 % CHAPS, 5 mM DTT, 0.001 % NP40 and 0.04 ml of caspase-3 substrate (AC-DEVD-AMC) or 0.1 M MES hydrate, pH 6.5, 10 % PEG, 0.1 % CHAPS, 5 mm DTT, 0.001 % NP40, and 0.1 mM of caspase-9 substrate (AC-LEHD-AMC). Substrate cleavage was measured with the microplate reader (Infinite pro200; Tecan Austria GmbH, Groedig, Austria) with excitation wavelength of 360 nm and emission at 460 nm. The data were calculated as fluorescence units/mg protein and presented as fold-increase over the pretreatment level (experimental/control).

Intracellular ROS Measurement

Rhodamine 123 (Rh 123) is a nonfluorescent, noncharged dye that easily penetrates cell membranes. Once inside the cell, DHR 123 becomes fluorescent upon oxidation to Rh 123, fluorescence being proportional to ROS generation. Rh 123 was found to be a nontoxic and about threefold more sensitive indicator of granulocyte respiratory burst activity than 2′,7′-dichlorofluorescin diacetate (DCFH-DA) (Rothe et al. 1988). The fluorescence intensity of Rh 123 was measured in an automatic microplate reader (Infinite pro200). Excitation was set at 488 nm and emission at 543 nm (Espino et al. 2011; Nazıroğlu et al. 2013a). Treatments were carried out in triplicate. The data are presented as fold-increase over the pretreatment level (experimental/control).

Statistical Analyses

All results were expressed as mean ± SD. Significant values in the four groups were assessed with an unpaired Mann–Whitney *U* test. Data were analyzed using the SPSS statistical program (version 17.0 software, SPSS Inc.

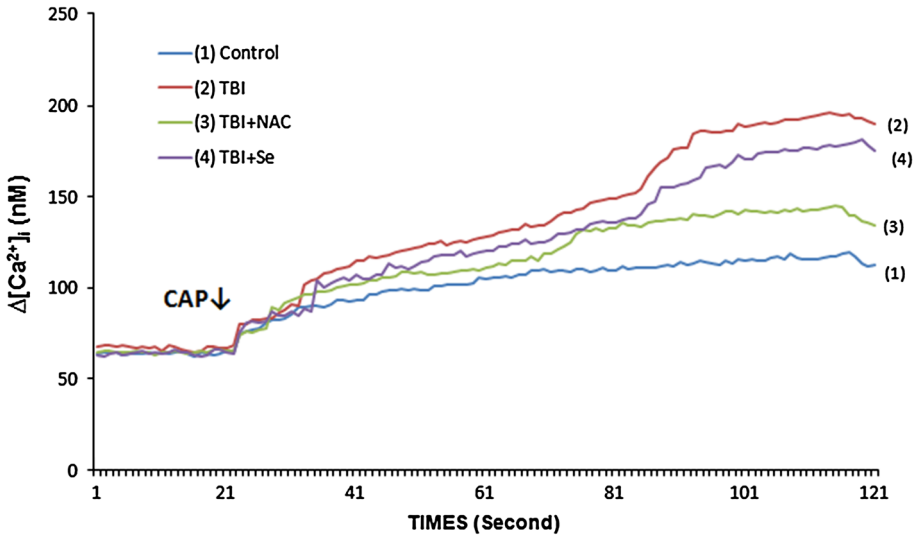

Fig. 1 Effects of *N*-acetyl cysteine (NAC) and selenium (Se) on cytosolic free Ca^{2+} concentrations of hippocampal neurons in TBI-induced rat. ($n = 8$ and mean ± SD). *CAP* capsaicin

Chicago, Illinois, USA). p values of less than 0.05 were regarded as significant.

Results

Effects of NAC and Selenium on Cytosolic-Free Calcium ($[Ca^{2+}]_i$) concentration

The results of $[Ca^{2+}]_i$ concentrations in the control, TBI, TBI + NAC, and TBI + Se are shown in Figs. 1 and 2. The $[Ca^{2+}]_i$ concentration was significantly ($p < 0.001$) higher in the TBI group than in control. The hippocampal neurons in the control, TBI, TBI + NAC, and TBI + Se groups were stimulated by CAP which is a TRPV1 channel agonist and pungent compound in hot chili peppers. After the stimulation, the $[Ca^{2+}]_i$ concentrations were significantly ($p < 0.001$) higher in the TBI groups than in control. Hence, TBI induced Ca^{2+} entry through TRPV1 channel activation in the neurons. After the CAP stimulation, the $[Ca^{2+}]_i$ concentration was significantly lower in TBI + NAC ($p < 0.01$) and TBI + Se ($p < 0.05$) groups than in the TBI group. The results indicated that NAC and selenium modulated TBI-induced Ca^{2+} entry through TRPV1 channel activation in the neurons. The $[Ca^{2+}]_i$ concentration was also significantly ($p < 0.05$) lower in the TBI + NAC group than in the TBI + Se group, and it seems that the modulator role of NAC on TBI-induced $[Ca^{2+}]_i$ entry via TRPV1 channels in the hippocampus is more significant than that of selenium.

Effects of NAC and Selenium on Apoptosis and Caspase Values

We investigated the effects of TBI exposure and the modulator role of NAC and selenium administrations on the rate of programmed cell death, apoptosis, and caspase values in the hippocampal neurons. The results of apoptosis, caspase-3, and -9 values in the control, TBI, TBI + NAC and TBI + Se groups are shown in Figs. 3, 4, and 5, respectively. The apoptosis ($p < 0.001$), caspase-3 ($p < 0.05$), and caspase-9 ($p < 0.05$) values in the TBI group were significantly higher than in the control group. However, the apoptosis ($p < 0.001$), caspase-3 ($p < 0.001$) and caspase-9 ($p < 0.05$) values were significantly lower in the TBI + NAC and TBI + Se groups than in the control and TBI groups. The values were also significantly ($p < 0.05$) lower in the TBI + NAC group than in the TBI + Se group, and it seems that the modulator role of NAC on programmed cell death in the hippocampus is more significant than that of selenium.

Effects of NAC and Selenium on Cytosolic ROS Production Value

The ROS values in the control, TBI, TBI + NAC, and TBI + Se groups are shown in Fig. 6. The ROS and mitochondrial membrane depolarization values were increased by the TBI induction. The ROS ($p < 0.01$) and mitochondrial membrane depolarization values ($p < 0.001$) in the TBI group were significantly higher than those in the control group. However, the values were significantly ($p < 0.001$) lower in the TBI + NAC and TBI + Se groups than in the control and TBI groups. The values were also significantly ($p < 0.05$) lower in the TBI + NAC group than in the TBI + Se group.

Discussion

We found that hippocampal apoptosis, caspase-3, caspase-9, cytosolic ROS, and $[Ca^{2+}]_i$ values were increased by induction of TBI. Hence, TBI inductions to the rats are

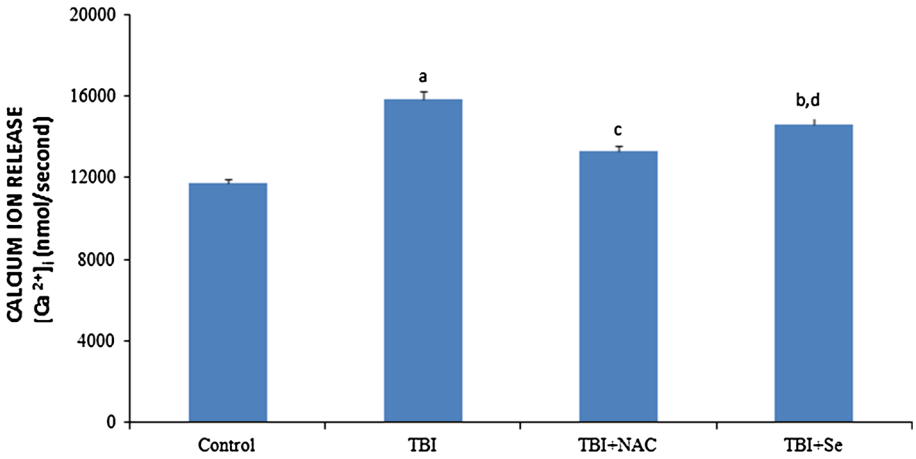

Fig. 2 Effects of *N*-acetyl cysteine (NAC) and selenium (Se) on cytosolic free Ca^{2+} concentrations of hippocampal neurons in TBI-induced rat. ($n = 8$ and mean ± SD). Fura-2-loaded rat hippocampal neurons were stimulated with capsaicin (CAP and 0.1 mM) in the presence of normal extracellular calcium ($[Ca^{2+}]_o = 1.2$ mm for 120 s. The traces shown are representative of eight separate experiments. (mean ± SD). $^{a}p < 0.001$ and $^{b}p < 0.05$ versus control. $^{c}p < 0.01$ versus TBI. $^{d}p < 0.05$ versus TBI + NAC

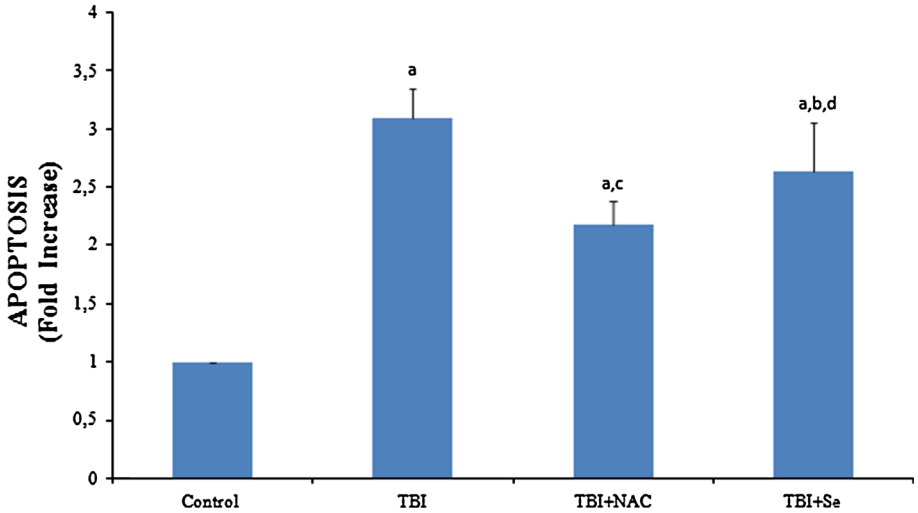

Fig. 3 Effects of *N*-acetyl cysteine (NAC) and selenium (Se) on apoptosis levels in hippocampus of control and TBI-induced rat. ($n = 8$ and mean ± SD). The values are expressed as fold increase over the pretreatment level (experimental/control). $^{a}p < 0.001$ versus control. $^{b}p < 0.05$ and $^{c}p < 0.001$ versus TBI group. $^{d}p < 0.01$ versus TBI + NAC

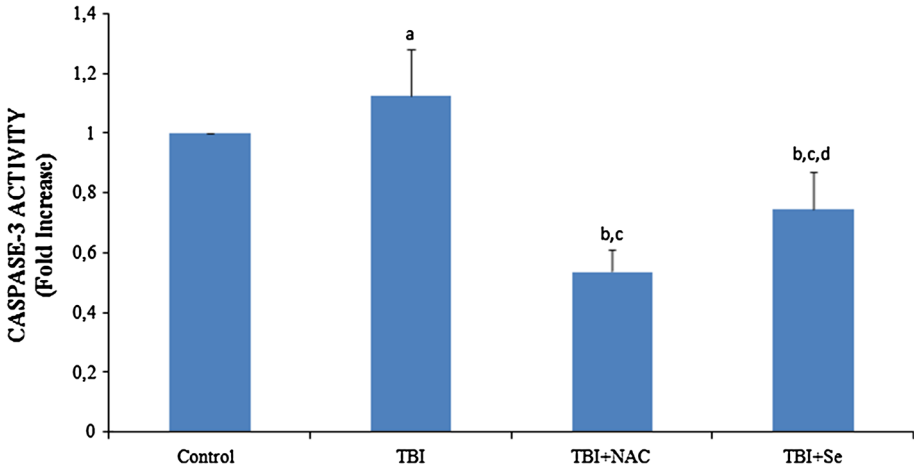

Fig. 4 Effects of *N*-acetyl cysteine (NAC) and selenium (Se) on hippocampus caspase-3 activity control and TBI-induced rat. ($n = 8$ and mean ± SD). The values are presented as mean ± SD of 8 separate experiments and expressed as fold increase over the pretreatment level (experimental/control). $^{a}p < 0.05$ and $^{b}p < 0.001$ versus control. $^{c}p < 0.001$ versus TBI group. $^{d}p < 0.05$ versus TBI + NAC group

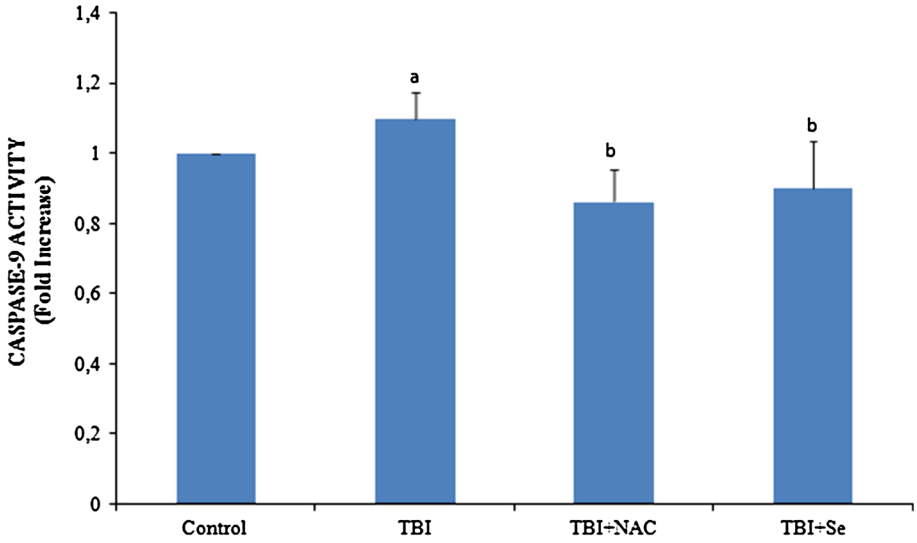

Fig. 5 Effects of *N*-acetyl cysteine (NAC) and selenium (Se) on hippocampus caspase-9 activity in control and TBI-induced rat. The values are presented as mean ± SD of 8 separate experiments and expressed as fold increase over the pretreatment level (experimental/control). [a]$p < 0.05$ versus control. [b]$p < 0.05$ versus TBI group

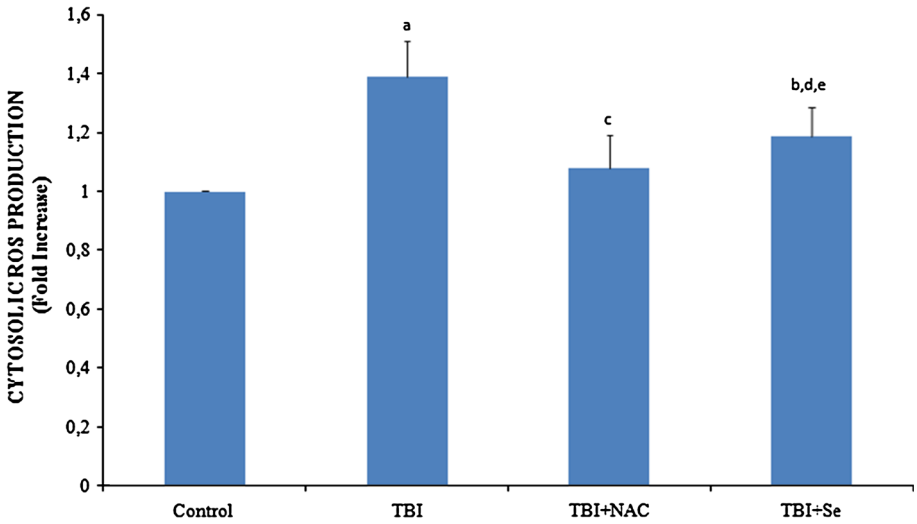

Fig. 6 Effects of *N*-acetyl cysteine (NAC) and selenium (Se) on intracellular ROS level in control and TBI-induced rat. ($n = 8$ and mean ± SD). ($n = 8$ and mean ± SD). The ROS values are expressed as fold increase over the pretreatment level (experimental/control). [a]$p < 0.001$ and [b]$p < 0.05$ versus control. [c]$p < 0.001$ and [d]$p < 0.05$ versus TBI group. [e]$p < 0.05$ versus TBI + NAC group

characterized by increased oxidative stress, Ca^{2+} influx, and apoptosis. However, treatment with selenium and NAC exerts beneficial effects on the hippocampal values in a rat model of TBI. A limited number of in vivo and in vitro studies in tissues except the hippocampus of experimental TBI-animals have been reported regarding the effects of NAC on oxidative stress and caspase activities (Al-Samsam et al. 2000; Yeo and Kang 2007; Yeo et al. 2008; Hoffer et al. 2013). To the best of our knowledge, the current study is the first to compare NAC and selenium administrations with particular reference to their effects on oxidative stress, Ca^{2+} signaling, and the apoptosis redox system in TBI-induced rats.

Oxidative stress induces lipid, protein, and DNA damages. The oxidative damage is mediated by ROS, which can be generated following cell lysis and oxidative burst (as a part of immune response) in TBI (Woodcock and Morganti-Kossmann 2013). Macrophage activation-dependent oxidative stress plays important roles in the removal of myelin debris and the products of neuronal degeneration after trauma in the brain (Sierra et al. 2013). NAC and selenium can alleviate these effects by playing an antioxidant role and by modulating the downstream antioxidant redox system pathways. In the current study, TBI exposure induced a significant increase in the cytosolic ROS production level of hippocampal neurons, although this level was decreased by NAC and selenium treatments. Hence, we provide experimental support for the hypothesis that NAC and selenium may play a pivotal role as an the antioxidant role, in part by significantly inhibiting production of ROS, an important mediator in the etiology of inflammatory disease such as TBI. Our results are confirmed by the results of previous reports of oxidative stress

increments in the brain and hippocampus after TBI (Al-Samsam et al. 2000; Yeo and Kang 2007; Yeo et al. 2008; Karalija et al. 2012; Hoffer et al. 2013).

A large number of studies linked TBI-induced cell damage to excitotoxic mechanisms (Weber 2012). TBI-induced inflammation can result in augmented glutamate release, leading to Ca^{2+} uptake through receptor and calcium channels (Limbrick et al. 2003). High $[Ca^{2+}]_i$ levels in TBI can induce the persistent opening of the mitochondrial permeability transition pore and trigger a host of effects. These include Ca^{2+} release, cessation of oxidative phosphorylation, matrix swelling, and eventually the rupture of the outer membrane with the release of cytochrome *c* and other apoptogenic proteins (Liu et al. 2009; Espino et al. 2011). Thus, the dysregulation of mitochondrial Ca^{2+} homeostasis is now recognized to play a crucial role in triggering mitochondrial dysfunction and subsequent apoptosis. However, NAC and selenium induce transitional mitochondrial permeability by modification of protein thiol groups, which results in cytochrome *c* release and the loss of mitochondrial membrane depolarization (Kim et al. 2002; Hallak et al. 2008). It was also reported that respiratory dysfunction in mitochondria after TBI in mice induced Ca^{2+} entry and oxidative stress although the mitochondrial dysfunction was recovered by increased selenium-dependent GSH-Px activity (Xiong et al. 2004). The modulator role of NAC on TRPV1 channel activity in neuronal cells was also recently reported (Nazıroğlu et al. 2013a). In the current hippocampus ROS and $[Ca^{2+}]_i$ values were increased by TBI exposure although administrations of NAC and selenium decreased the $[Ca^{2+}]_i$ concentration through modulation of TRPV1 channel activity in the neurons. Modulation of TRPV1 in hippocampal cells by means of the treatment with NAC and selenium might be the cause of decreased mitochondrial ROS production, apoptosis, and cell membrane Ca^{2+} influx.

Reactive oxygen species induce the collapse of mitochondrial membrane potential, and therefore trigger a series of mitochondria-dependent processes including apoptosis (Graphical Abstract) (Espino et al. 2011; Uğuz et al. 2012). Apoptosis is the programmed cell death pathway mainly executed by cysteine proteases known as caspases. The apoptotic cascade of caspases is initiated by the activation of apical (initiator) caspases that include caspase-3, -8, and -9 (Hallak et al. 2008). In response to noxious stimuli and related cellular stress situations, initiator caspases directly or indirectly activate executioner caspases, which in turn orchestrate apoptotic cell death (Zhang et al. 2010; Shang et al. 2011). Although a growing amount of evidence suggests that caspase-mediated apoptosis contributes to neuronal cell death in the TBI, the identification of the caspases that initiate this response remain elusive (Piao et al. 2012). Complex I inhibition results in a decrease in ATP synthesis and an accumulation of oxidative radicals, causing detrimental oxidative stress and cell death (Nazıroğlu 2007). Recent studies have also demonstrated the induction of apoptosis in neurons and cell lines in response to ROS (Celik and Nazıroğlu 2012; Uğuz and Nazıroğlu 2012; Nazıroğlu et al. 2013a). The mechanism of mitochondrial oxidative stress-induced cell death is well studied in hippocampus of the TBI-induced rats. However, it remains to be determined whether and how post-mitotic neurons are affected by mitochondrial oxidative stress-inducing agents. However, NAC and selenium have antioxidant, anti-apoptotic, and anti-inflammatory properties in neuronal cells (Nazıroğlu 2009; Zhang et al. 2010; Özgül and Nazıroğlu 2012; Nazıroğlu et al. 2013a). Similarly, protective roles of NAC (Chen et al. 2008; Englezou et al. 2012) and selenium (Jeo and Kang 2007) in traumatic injury-induced neurons have been reported for ROS production, MAPK/ERK-mediated apoptosis signaling, bax, and caspase-3 and -9 values through blocking the apoptotic cell death. In the current study, hippocampal apoptosis, caspase-3, and -9 values were increased by TBI induction although they were decreased by NAC and selenium treatments. We demonstrate here that NAC and selenium act via antioxidant and anti-inflammatory properties to inhibit apoptotic cell death in the hippocampal cells of rats subjected to trauma.

Apoptotic process is including all caspase activities such as capase-3, -8 and -9 but antioxidants such as NAC and selenium induced protective effects on caspase-3 and -9 activities but not on caspase-8 activities (Zhang et al. 2010; Shang et al. 2011). Apoptosis levels were higher in selenium and NAC groups than those in control although caspase-3 and -9 values were restored to control levels by the treatments and it might have occurred due to effectiveness of the antioxidant on total apoptotic process in the neurons. Cysteine is an oxidative target because of the reactivity of the thiol group that is susceptible to modification by free radicals that may modulate the activity of these proteins, thus making caspase-3 and -9 targets for oxidation-based regulation (Junn and Mouradian 2001; Elphick et al. 2008). In addition, NAC and selenium have modulator roles on thiol groups in neurons (Arakawa and Ito 2007). Due to the thiol group regulator properties of NAC and selenium, the caspase activities (but not apoptosis value) were restored to control values. In addition, previous studies performed to understand the mechanism of TBI-induced apoptosis in various models showed also an involvement of the intrinsic pathway of apoptosis that, which to the activations of caspase-3 and -9 after cytochrome *c* release (Zhang et al. 2010; Shang et al. 2011). In agreement with previous observations, our results also demonstrate that hippocampal cultures from rats engage the intrinsic pathway of apoptosis.

In conclusion, we found that hippocampal apoptosis, caspase-3, caspase-9, ROS, and $[Ca^{2+}]_i$ concentration were increased by induction of TBI, but the values were modulated by NAC and selenium administrations. The effect of NAC appears to be much greater than that of selenium. Hence, we observed striking correlations between the effects of TBI on Ca^{2+} influx through TRPV1 channels, oxidative stress, and apoptosis in the hippocampal neurons of the TBI-induced rats. In addition, NAC and selenium interact with TRPV1 cation channel permeability.

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

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