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Research report

Creatine reduces oxidative stress markers but does not protect against seizure susceptibility after severe traumatic brain injury

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ABSTRACT

Achievements made over the last years have highlighted the important role of creatine in health and disease. However, its effects on hyperexcitable circuit and oxidative damage induced by traumatic brain injury (TBI) are not well understood. In the present study we revealed that severe TBI elicited by fluid percussion brain injury induced oxidative damage characterized by protein carbonylation, thiobarbituric acid reactive species (TBARS) increase and Na^+,K^+-ATPase activity inhibition 4 and 8 days after neuronal injury. Statistical analysis showed that after TBI creatine supplementation (300 mg/kg, p.o.) decreased the levels of protein carbonyl and TBARS but did not protect against TBI-induced Na^+,K^+-ATPase activity inhibition. Electroencephalography (EEG) analysis revealed that the injection of a subconvulsant dose of PTZ (35 mg/kg, i.p.), 4 but not 8 days after neuronal injury, decreased latency for the first clonic seizures and increased the time of spent generalized tonic–clonic seizures compared with the sham group. In addition, creatine supplementation had no effect on convulsive parameters induced by a subconvulsant dose of PTZ. Current experiments provide evidence that lipid and protein oxidation represents a separate pathway in the early post-traumatic seizures susceptibility. Furthermore, the lack of consistent anticonvulsant effect exerted by creatine in this early phase suggests that its apparent antioxidant effect does not protect against excitatory input generation induced by TBI.

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1. Introduction

Traumatic brain injury (TBI) is a heterogeneous disorder characterized by clinical multifaceted and complex consequences [36]. TBI-induced damage can be classified into primary damage induced by direct mechanical force to the head occurring at the time of injury and secondary damage characterized by complications starting during the first minutes, hours, and days after the injury [36]. In line with this view, it has been demonstrated that numerous abnormalities after injury induce alterations in brain excitability and spontaneous seizures or epilepsy [29,36]. Clinically, post-traumatic epilepsy (PTE) refers to the condition where recurrent spontaneous seizures occur more than one week after TBI [10,37]. Considering that patients with early seizure may have a higher risk of late epilepsy [17], there is a great need for biomarkers providing quantitative measures of the process of post-traumatic epileptogenesis.

In this context, recent studies have suggested that oxidative stress, imbalance between oxidant and antioxidant, is a significant component of the secondary injury cascade accompanying TBI, and the ultimate effects of this process can be seen, among other techniques, by measuring lipid peroxidation and protein carbonylation [20,35,44]. Although evidence supporting the idea that any cellular constituent may be a target for free radical damage, the inhibition of some selected targets such as Na^+,K^+-ATPase may play an important role in the TBI-induced hyperexcitability [21]. In fact, Na^+,K^+-ATPase is a membrane bound enzyme known to play a pivotal role in cellular ionic gradient maintenance and particularly sensitive to reactive species [30]. However, little information is available regarding the relationship between lipid peroxidation and protein carbonylation and Na^+,K^+-ATPase enzyme in seizure development at early phases after severe TBI.

Creatine (N-[aminoiminomethyl]-N-methyl glycine) is a guanidine compound endogenously produced from glycine, methionine, and arginine in the liver, kidney, pancreas and brain [2]. Dietary

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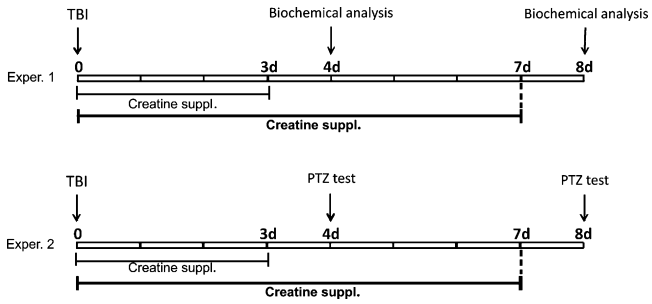

Fig. 1. Study design. Rats receiving intragastric gavage of creatine (300 mg/kg) or vehicle (CMC), 30 min after TBI and once a day until third or seventh day after TBI. In the fourth and eighth days after TBI, biochemical analyses (experiment 1) or PTZ (35 mg/kg, i.p.) test (experiment 2) were performed.

supplementation of creatine monohydrate is used by many athletes to enhance performance. In addition to the pool of creatine in the muscle, high levels of creatine are found in the brain [31]. Although many of molecular mechanisms are not well understood, creatine supplementation has been proposed and/or proven partially in a variety of animal/cellular models of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's [1]. In addition, it has been shown that creatine pre-treatment reduces TBI-related tissue damage in cortical controlled impact model [42]. Mechanisms underlying the creatine-induced neuroprotection may involve the maintenance of mitochondrial integrity and consequent decrease in production of reactive species, or may be due to increased stores of cellular phosphocreatine [43]. However, the role of creatine in the process of post-traumatic seizure susceptibility has not been evaluated to date. Therefore, the purpose of this study was to investigate the involvement of lipid peroxidation and protein carbonylation and Na^+,K^+-ATPase activity in the process of post-traumatic seizure susceptibility and the role of early creatine administration in these deleterious effects.

2. Methods

2.1. Animal and reagents

Adult male Wistar rats (250–300 g), maintained at 12 h light/dark photoperiod with free access to water and food were used. All experimental protocols (including statistical evaluation) were designed to use a minimum number of animals. Animal utilization reported in this study has been conducted in accordance with national and international legislation (guidelines of the Brazilian College of Animal Experimentation (COBEA) and Use of Laboratory Animals – PHS Policy) as well as with the approval of the Ethics Committee for Animal Research of the Federal University of Santa Maria (process number 114/2010). All reagents were purchased from Sigma (St. Louis, MO, USA) except the thiobarbituric acid, which was obtained from Merk (Darmstadt, Germany). Solutions were prepared in type I ultra pure water. Pentylenetetrazol (PTZ) was prepared in 0.9% sterile saline solution.

2.2. Study design

The study design is summarized in Fig. 1 and consisted of two experiments. Experiments were conducted as follows:

Experiment 1: In order to determine the role of creatine on TBI-induced oxidative damage (characterized by measuring the thiobarbituric acid reactive species (TBARS), total protein carbonyl content) and the possible TBI-induced effects to the Na^+,K^+-ATPase activity, injured and sham animals received intragastric gavage of either creatine (300 mg/kg body weight) suspended in 0.5% carboxymethylcellulose (CMC) or vehicle (CMC) [26,38], 30 min after TBI, once a day until third day after TBI. Another subset of animals received creatine (300 mg/kg body weight) or vehicle (CMC) once a day until seven days after TBI. Twenty-four hours after the last creatine administration, animals were killed by decapitation and their brain was exposed by removing the parietal bone. The brain was quickly removed and the injured hemisphere corresponding to the impact site of injury was rapidly dissected on an inverted ice-cold Petri. After, tissue sample was homogenized in cold 10 mM Tris–HCl buffer (pH 7.4) and then divided in aliquots for subsequent neurochemical analyses.

Experiment 2: The role of creatine in the process of post-traumatic seizure susceptibility after TBI was investigated. For this purpose, injured and sham animals received the same treatment as the animals in experiment 1.Twenty-four hours after the last creatine administration, sham and TBI animals received an injection of saline (0.9% NaCl, 1 ml/kg, i.p.) or subconvulsant dose of PTZ (35 mg/kg, i.p.).

2.3. Traumatic brain injury (TBI) and placement of electrodes for EEG recordings

TBI was induced by fluid percussion brain injury as originally described by McIntosh et al. [28], using the stereotaxic position described by D'Ambrosio et al. [7]. Animals were anesthetized with a single intraperitoneal (i.p.) injection, 3 ml/kg Equitesin (1% phenobarbital, 2% magnesium sulfate, 4% chloral hydrate, 42% propylene glycol, 11% ethanol). Under stereotaxic guidance, a craniectomy 3 mm in diameter was drilled on the right convexity parietal cortex, 2 mm posterior to bregma and 3 mm lateral to the midline, taking care to keep the duramater intact. A plastic injury cap was placed over the craniotomy with dental acrylic cement and was filled with chloranfenicol. During the surgery for the placement of the plastic injury cap, all animals were implanted with electrodes for EEG recordings. Two screw electrodes were placed over the parietal cortex, one rostral to the craniectomy and other contralateral to the craniectomy along with a ground lead positioned over the nasal sinus. All electrodes were connected to a multipin socket fixed to the skull with dental acrylic cement. Subsequently, the animal was removed from the stereotaxic device and returned to its homecage. After 24 h, animals were anesthetized with isoflurane, had the injury cap attached to the fluid percussion device and placed in the heat pad maintained at $37 \pm 0.2\,^{\circ}C$. TBI was produced by a fluid-percussion device developed in our laboratory. A brief (10–15 ms) transient pressure fluid pulse (3.53 ± 0.17 atm) impact was applied against the exposed dura. Pressure pulses were measured extracranially by a transducer (Fluid Control Automação Hidráulica, Belo Horizonte, MG, Brazil) and recorded on a storage oscilloscope (Gould Ltd., Essex, UK). Sham-operated animals underwent an identical procedure with the exception of the fluid percussion injury.

2.4. PTZ test, behavioral evaluation and EEG recordings

To determine whether the seizure threshold was reduced after TBI and detect any enhance seizure susceptibility after TBI, we used a single subconvulsant dose of 35 mg/kg (i.p.) body weight of PTZ, appropriate and tested for Wistar rats [25]. On the day of the experiments, each animal was transferred to a Plexiglas cage (25 cm × 25 cm × 40 cm) and acclimatized for 20 min before EEG recording. The rat was then connected to the lead socket in a swivel inside a Faraday's cage, and the EEG recorded using a digital encephalographer (Neuromap EQSA260, Neurotec Ltd, Itajubá, MG, Brazil). EEG signals were amplified, filtered (0.1–70.0 Hz, bandpass), digitalized (sampling rate 256 Hz) and stored in a PC for off-line analysis. Routinely, a 10 min baseline recording was obtained to establish an adequate control period. After baseline recording, PTZ (35 mg/kg, i.p.) was administered. Animals were observed for the appearance of clonic and generalized tonic–clonic convulsive episodes for 20 min according to Ferraro et al. [8], who describes clonic convulsions as episodes characterized by typical partial clonic activity affecting face, head, vibrissae, and forelimbs. Such clonic events typically last 1–2 s and can occur either individually or in multiple discrete episodes before generalization. Generalized convulsive episodes were considered as generalized whole-body clonus involving all four limbs and tail, rearing, wild running and jumping, followed by sudden loss of upright posture and autonomic signs, such as hypersalivation and defecation, respectively. During the 20-min observation period, latencies for the first clonic and first generalized tonic–clonic convulsions were measured. EEG recordings were visually analyzed for seizure activity, which were defined by the occurrence of the following alterations in the recording leads [27]: isolated sharp waves ($\geq 1.5 \times$ baseline); multiple sharp waves ($\geq 2 \times$ baseline) in brief spindle episodes (≥ 1 s ≥ 5 s); multiple sharp waves ($\geq 2 \times$ baseline) in long spindle episodes (≥ 5 s); spikes ($\geq 2 \times$ baseline) plus slow waves; multispikes ($\geq 2 \times$ baseline, ≥ 3 spikes/complex) plus slow waves; major seizure (repetitive spikes plus slow waves obliterating background rhythm, ≥ 5 s). Rhythmic scratching of the electrode headset by the animal rarely caused artifacts. These recordings were easily identified and discarded.

2.5. Biochemical analyses

2.5.1. Measurement of thiobarbituric acid reactive species (TBARS)

TBARS content was estimated by the method of Ohkawa et al. [33]. Briefly, homogenates were diluted 1:2 (v/v) in type I ultrapure water and incubated in medium containing 0.2 ml brain homogenate, 0.1 ml of 8.1% sodium dodecyl sulfate (SDS), 0.4 ml buffered acetic acid (500 mM, pH 3.4), and 0.75 ml of 0.81% thiobarbituric acid (TBA). The mixture was made up to 2 ml with type I ultrapure water and heated at 95 °C for 90 min in water bath using a glass ball as condenser. Absorbance of each sample was measured in the supernatant at 532 nm in a Hitachi U-2001 spectrophotometer (Hitachi Instruments Incorporation, Schaumburg, IL, USA).

2.5.2. Measurement of protein carbonyl content

Total protein carbonyl content was determined by the method described by Yan et al. [46], adapted for brain tissue [34]. Briefly, homogenates to

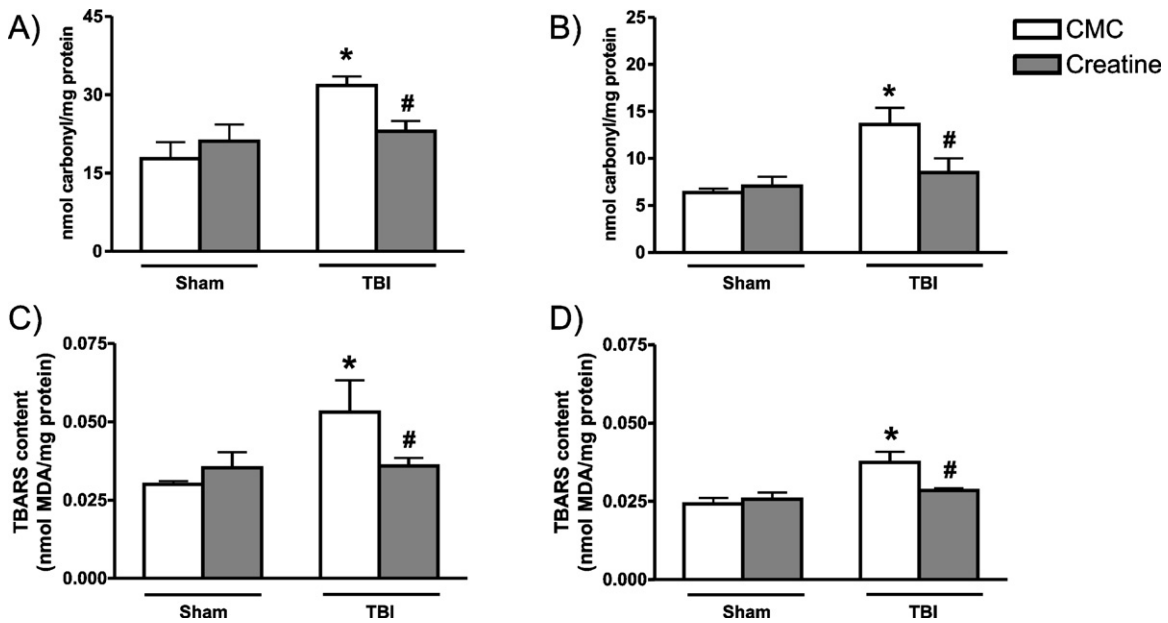

Fig. 2. Effect of creatine supplementation (300 mg/kg, p.o.) for 3 (A and C) and 7 days (B and D) after TBI on the total protein carbonylation (A and B) and TBARS (C and D) levels measured 4 (A and C) and 8 days (B and D) after TBI. Data are mean + S.E.M. for any $n = 8–10$ in each group. '*' indicates a significant difference ($p < 0.05$) compared with the sham group. '#' indicates a significant difference compared with the TBI group.

759–800 μg/ml protein in each sample and 1 ml aliquots were mixed to 0.2 ml 2,4-dinitrophenylhydrazine (DNPH, 10 mM) or 0.2 ml HCl (2 M). After incubation at room temperature for 1 h in dark environment, 0.6 ml denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 1.8 ml heptane (99.5%) and 1.8 ml ethanol (99.8%) were added sequentially and mixed under vortex agitation for 40 s and centrifuged for 15 min. Afterwards, protein isolated from the interface was twice washed with 1 ml ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 ml denaturing buffer. Each DNPH sample was read at 370 nm in a Hitachi U-2001 spectrophotometer against the corresponding HCl sample (blank) and total carbonylation calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1}, as described by Levine et al. [19].

2.5.3. Na^+,K^+-ATPase activity measurement

Na^+,K^+-ATPase activity was performed according to Wyse et al. [45]. Briefly, the reaction medium consisted of 30 mM Tris–HCl buffer (pH 7.4), 0.1 mM EDTA, 50 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, and 50 μg protein in the presence or absence of ouabain (1 mM) to 350 μl final volume. The reaction was started by adding adenosine triphosphate (ATP) to 5 mM final concentration. After 30 min at 37 °C, reaction was stopped by adding 70 μl trichloroacetic acid (TCA, 50%). Saturating substrate concentrations were used, and the reaction was linear with protein and time. Appropriate controls were included in the assays for ATP's non-enzymatic hydrolysis. The amount of inorganic phosphate released was quantified by the colorimetric method described by Fiske and Subbarow [9] and the Na^+,K^+-ATPase activity was calculated by subtracting the ouabain-sensitive activity from the overall activity (in the absence of ouabain).

2.5.4. Protein determination

Protein content was colorimetrically determined by the method of Bradford [5] using bovine serum albumin (1 mg/ml) as standard.

2.5.5. Statistical analyses

Data from ex-vivo total carbonyl, TBARS and Na^+,K^+-ATPase activity determinations were analyzed by two-way ANOVA (analysis of variance). The first myoclonic jerk latency and generalized convulsion latency were analyzed by Scheirer–Ray–Hare test for two-way ANOVA for non-parametrical data. A probability of $p < 0.05$ was considered significant. All data are expressed as mean ± S.E.M. A Student–Newman–Keuls and Dunn tests were run to perform the *post hoc* analysis.

3. Results

Fig. 2 shows the effect of creatine supplementation on protein carbonylation (2A and 2B) and TBARS (2C and 2D) in the ipsilateral cortex 4 and 8 days after TBI. Statistical analysis revealed that TBI induced protein carbonylation [$F(1,19) = 4.527$; $p < 0.05$; Fig. 2A] and TBARS [$F(1,24) = 4.540$; $p < 0.05$; Fig. 2C] increase and that creatine supplementation protected against such increase 4 days after neural injury (significant treatment, creatine or vehicle, by pretreatment, TBI or sham, interaction: TBARS [$F(3,24) = 5.840$; $p < 0.05$] and protein carbonylation [$F(3,19) = 7.820$; $p < 0.05$]. Statistical analysis also revealed that TBI induced protein carbonylation [$F(1,21) = 4.898$; $p < 0.05$; Fig. 2B] and TBARS content [$F(1,21) = 4.933$; $p < 0.05$; Fig. 2D] increase and that creatine supplementation protected against this increase 8 days after neural

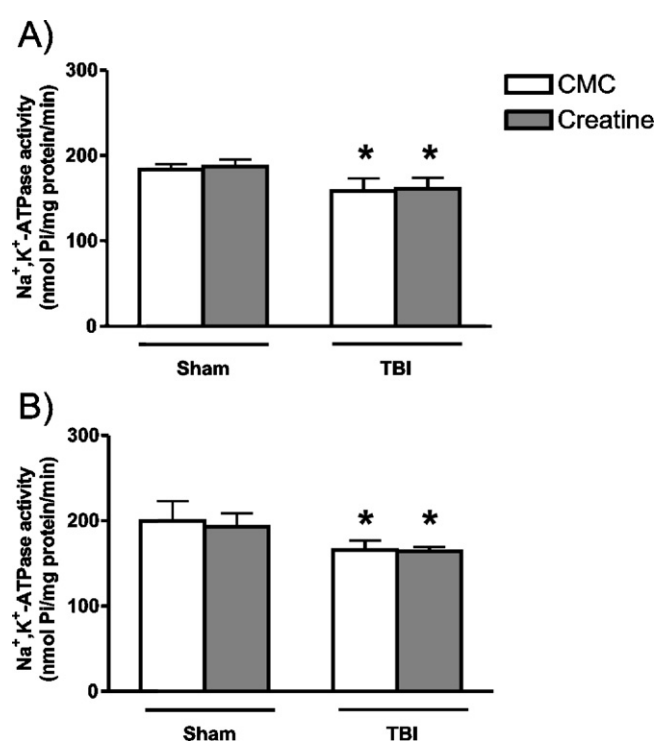

Fig. 3. Effect of creatine supplementation (300 mg/kg, p.o.) for 3 (A) and 7 days (B) after TBI on Na^+,K^+-ATPase activity. Data are mean + S.E.M. for any $n = 6–7$ in each group. * indicates a significant difference ($p < 0.05$) compared with the control group.

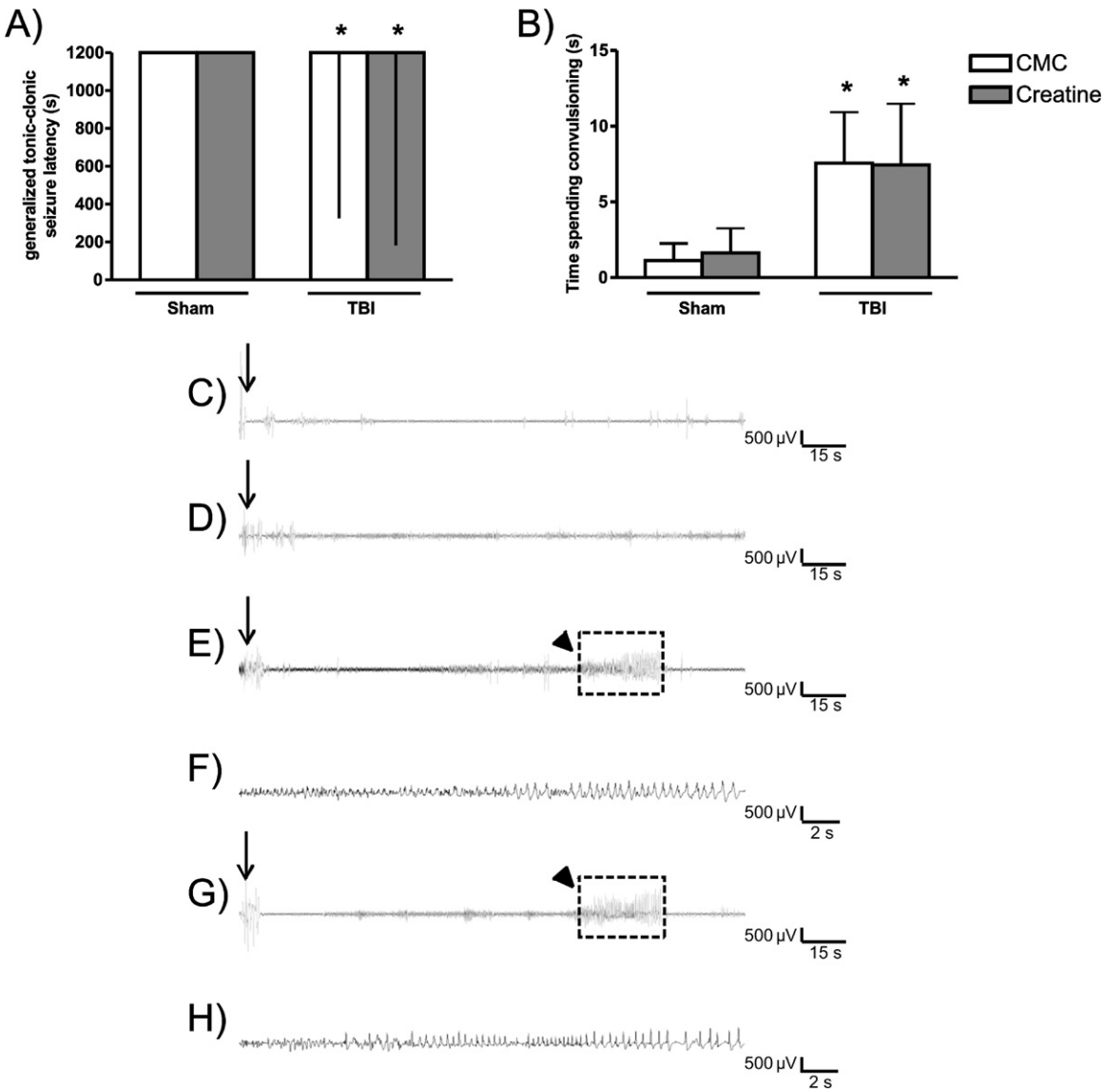

Fig. 4. Effect of creatine supplementation (300 mg/kg, p.o.) on the onset latency for generalized seizure (A) induced by subconvulsant dose of PTZ (35 mg/kg, i.p.) and time spend in convulsion 4 days after TBI (B). In (A) date are median and interquartile ranges for $n = 7–9$ in each group, while in (B) date are mean ± S.E.M. '*' indicates a significant difference ($p < 0.05$) from the sham group. Typical electrographic registers are represented after the PTZ administration (35 mg/kg, i.p.) in sham/CMC (C), sham/creatine (D), TBI/CMC (E) and TBI/creatine (G) groups in this figure as well. In all traces the arrow indicates PTZ administration; the arrowhead indicates the onset of EEG pattern associated with generalized seizure. The expanded waveforms from the EEG recording outline by the traced boxes are shown in (F) and (H) registers.

injury (significant treatment, creatine or vehicle, by pretreatment, TBI or sham, interaction: TBARS [$F(3,21) = 4.05$; $p < 0.05$] and protein carbonylation [$F(3,21) = 6.02$; $p < 0.05$]).

Considering that Na^+,K^+-ATPase is target for free radicals generation [20,21] and that a decrease in its activity involves a neuronal hyperexcitability, we decided to evaluate the role of this enzyme in the TBI-induced toxicity. Statistical analysis revealed a significant decrease of Na^+,K^+-ATPase activity after 4 [$F(1,20) = 5.300$; $p < 0.05$; Fig. 3A] and 8 days [$F(1,18) = 4.442$; $p < 0.05$; Fig. 3B] neural injury and that creatine supplementation did not protect against this decrease when analyzed 4 [$F(3,20) = 0.714$; $p > 0.05$; Fig. 3A] and 8 days after neural injury [$F(3,18) = 1.34$; $p > 0.05$; Fig. 3B].

Figs. 4 and 5 display quantitative time course study for EEG recordings in the ipsilateral cortex from sham and TBI rats when analyzed 4 (Fig. 4) and 8 days (Fig. 5) after TBI respectively. Statistical analysis revealed that the injection of subconvulsant dose of PTZ decreased latency for first generalized tonic–clonic seizures in 4 [$H(1) = 6.515$; $p < 0.02$; Fig. 4A] but not 8 days after TBI [$H(1) = 2.694$; $p > 0.05$; Fig. 5A] and creatine had no effect both in 4 [$H(1) = 0.002$; $p > 0.05$; Fig. 4A] and 8 days after TBI [$H(1) = 3.307$; $p > 0.05$; Fig. 5A]. In animals that had a tonic–clonic seizure with the PTZ test (4 days after TBI) was observed increase in the length of tonic–clonic seizures [$F(1,30) = 4.327$; $p < 0.05$; Fig. 4B] when compared with the sham group. Representative EEG recordings also revealed that PTZ injection (35 mg/kg) caused only minor behavioral and EEG alterations in sham group (Fig. 4C and D). However, this subconvulsant dose caused the appearance of generalized tonic–clonic seizures characterized by multispikes plus slow waves and major seizure activity characterized by 2–3 Hz high-amplitude activity in TBI group (Fig. 4E–H). After the ictal discharge, post-ictal EEG suppression and slow waves were observed correlating with behavioral catalepsy. Statistical analysis revealed that creatine supplementation (300 mg/kg) after severe TBI had no effect on the convulsive parameters induced by PTZ 4 days after neural injury (Fig. 4). In addition, EEG recordings showed that supplementation of this guanidine compound does not alter wave patterns when compared with the sham group 8 days after neural injury (Fig. 5A–E).

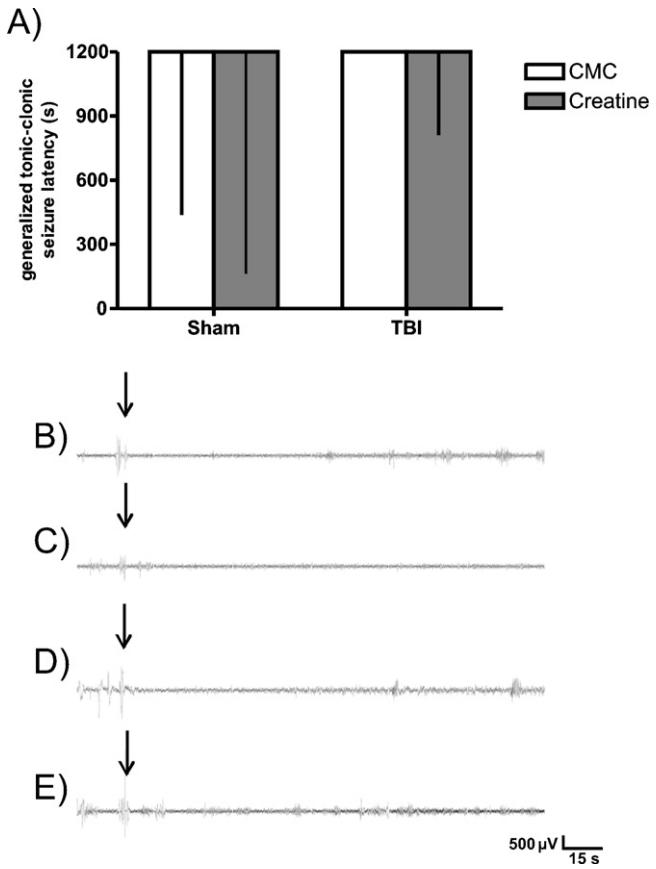

Fig. 5. Effect of creatine supplementation (300 mg/kg, p.o.) on the onset latency for generalized seizure (A) induced by subconvulsant dose of PTZ (35 mg/kg, i.p.). In (A) data are median and interquartile ranges for $n = 7–9$ in each group. There was no significant difference from the respective parameter in vehicle-treated group. Typical electrographic registers are represented after the PTZ administration (35 mg/kg, i.p.) in sham/CMC (B), sham/creatine (C), TBI/CMC (D) and TBI/creatine (E) groups in this figure as well. In all traces the arrow indicates PTZ administration.

4. Discussion

TBI' sequelae, including early post-traumatic seizures, represent a social problem where the importance of brain trauma as a risk factor for the epilepsy development is well established [17]. Nevertheless, clinical trials aiming the epilepsy prevention following TBI have failed [24] due to the multiple epileptogenic processes likely activated simultaneously or sequentially by brain trauma [12]. Thus, significant resources are required to develop a better understanding of the pathophysiologic mechanism as targets for potential therapies.

Results presented in this report have shown that creatine supplementation with 300 mg/kg [26,39] protected against TBI-induced protein carbonylation and TBARS increase 4 and 8 days after the neuronal damage. Other studies have used creatine in a prophylactic manner, exerting effects such as protection against neuronal death, decreased levels of lactate and free fatty acids in a TBI model [40,42]. It was reported that creatine may exert pleiotropic effects by the enzyme creatine kinase [43]. This enzyme is affected by the deleterious effects of oxidative stress in neural tissue, reducing its activity and consequent depleting high-energy phosphate, essential for the cellular function maintenance [13]. However, binding of creatine to mitochondrial creatine kinase can protect its structural conformation, maintaining its activity and consequently, inhibiting the opening of mitochondrial transition pore that would generate free radicals and apoptosis, as well as creatine can exert direct antioxidant effects [43]. However, creatine supplementation did not protect against Na^+,K^+-ATPase inhibition and seizures induced by subconvulsant dose of PTZ. Thus, given the results, we can propose that creatine effects on seizure susceptibility caused by TBI are either very low or inefficient to provide a recovery of normal physiological functions of cells in brain tissue or protect against oxidative damage. It is important to consider that the Na^+,K^+-ATPase activity may be modulated by other mechanisms such as phosphorylation. In this context, studies on the functional effects of PKA/PKC-mediated phosphorylation of Na^+,K^+-ATPase have demonstrated that the activation of protein kinase A (PKA) and protein kinase C (PKC) decreases Na^+,K^+-ATPase [6,32]. In addition, α-subunit phosphorylation at Ser-943 decreases enzyme activity by decreasing enzyme availability at the cell surface [4]. Moreover, targeting products that are very sensitive to oxidative substances such as Na^+,K^+-ATPase may require higher levels of antioxidant defense [11]. However, further studies are necessary to elucidate this point.

In this study, in order to determine the presence of seizure susceptibility, we performed the PTZ test in a severe TBI model. PTZ test has been used to show lowered seizure threshold in TBI models at 30 mg/kg body weight [14,18] and at 35 mg/kg body weight in Wistar rats, as a subconvulsant dose for the kindling model [25]. Moreover, susceptibility to PTZ-induced seizures is a standard and widely used experimental model to clinical generalized seizure with well established validity [23].

Our results have showed that the injection of subconvulsant dose of PTZ (35 mg/kg, i.p.) caused seizures in 4 but not in 8 days after a severe TBI episode in rat's parietal cortex. Furthermore, TBI induced Na^+,K^+-ATPase activity inhibition and increased levels of protein carbonylation and TBARS, both 4 and 8 days after the neural injury. These experimental findings suggest that, at least in part, lipid peroxidation, protein carbolylation and seizures induced by subconvulsive dose of PTZ (35 mg/kg; i.p.) represent separate pathways in neurobehavioral disability in this TBI model, especially considering that the lipoperoxidation and protein carbonylation protection exerted by creatine did not alter the susceptibility to seizures during this period. Considering that total protein carbonylation and TBARS content did not show oxidative damage caused specifically in some places, which may contribute little to the total expression of these markers as observed in one study on the antioxidant effect of methylene blue, which decreased protein carbonylation, normalized the activity of Na^+,K^+-ATPase but did not protect against seizures induced by methyl-malonic acid [11].

In fact, it has been shown in previous studies that excitatory currents in layer V pyramidal cells of epileptogenic cortex revealed the presence of enhanced excitatory inputs and hyperexcitable circuits 3 days after the insult [15,29]. These mechanisms occur in parallel with the oxidative damage. Also, the theory of double-hit [16] demonstrates that TBI provides an apparent neuroprotection against cell death at 1 and 6 h in young rats injected with pilocarpine. Still, a study on concussive brain injury in mice showed that after the first trauma episode, there is cellular damage sustained for up to 7 days and these animals when submitted to new trauma in 3, 5 and 7 days, evaluated to cognitive and motor tests, had worsening performance in their activities, except if the trauma was repeated in 7 days [22]. These data corroborate with the finding of this study and it is plausible to conclude that the evolution of pathological process of TBI does not follow a linear development pattern, and at times, still unclear factors that protect against susceptibility to early seizures, in this situation, being perhaps one of the reasons that there is a full consensus whether early seizures (between 24 h and 7 days after injury) are risk factors for developing post-traumatic epilepsy [17].

Markers of oxidative damage seem to have a greater participation on later events. In fact, one work from our group using the same TBI model showed that oxidative marker remains high 3 months

after the insult [20], normalizes at 48 h after TBI as the Na^+,K^+-ATPase activity if animals were submitted to swimming training for 6 weeks previously to TBI [21] and was related to seizure susceptibility in 5 weeks [41]. However, subsequent studies about this issue may be performed.

Limitations of an experimental model should be considered. However, one should take into account that this study did not evaluate on an overall oxidative stress, since we did not measure actual levels of free radicals, so little anti-oxidant systems, such as superoxide dismutase, catalase and glutathione peroxidase. Also, although the markers used are globally accepted to demonstrate the final results of unbalance caused by oxidative stress, such as TBARS and protein carbonylation in the TBI model [3], they may overestimate the actual levels of oxidized lipids and proteins, since TBARS, for example, bind to other substances not necessarily products of lipid peroxidation. Still, biochemical parameters measured were performed in total hemicortex that even though being ipsilateral to the lesion may contain less affected portions by the neuronal damage.

5. Conclusions

The present study showed that TBI causes increase in markers of lipid peroxidation and protein carbonylation at 4 and 8 days after the injury, at which time there is reduced Na^+,K^+-ATPase activity. However, despite the use of creatine has started 30 min after injury being maintained for 3 and 7 days, having reversed the increase in markers of lipid peroxidation and protein carbonylation, it did not reverse the Na^+,K^+-ATPase inhibition. Still, animals tested with subconvulsant dose of PTZ, 4 days after TBI, had lower latency and longer duration for tonic–clonic seizures, effect not reversed by creatine and not observed 8 days after the injury. In summary, we conclude that TBI increased lipid peroxidation and protein carbonylation which were not directly involved with decreased activity of Na^+,K^+-ATPase, and in this critical period these markers were not involved with seizure susceptibility. These results may help to understand the pathophysiology of TBI, showing evidence that TBI may have different periods of susceptibility to early seizure, not necessarily related to oxidative stress markers.

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