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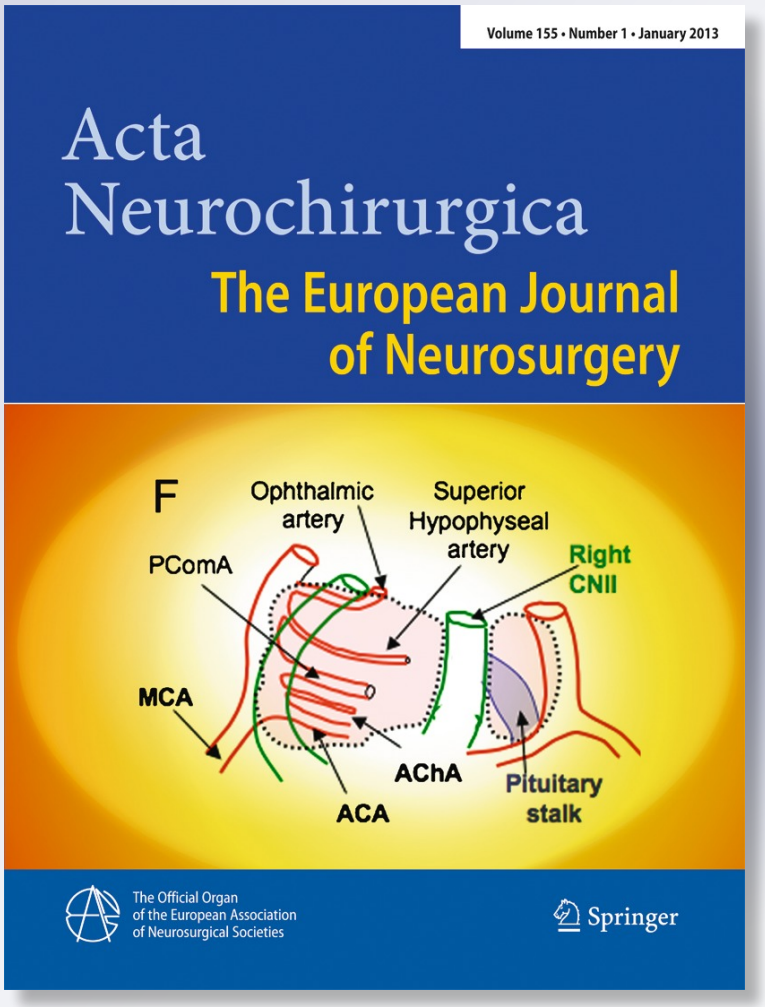
Acta Neurochirurgica
The European Journal of Neurosurgery

ISSN 0001-6268
Volume 155
Number 1

Acta Neurochir (2013) 155:141-149
DOI 10.1007/s00701-012-1501-3



Springer

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EXPERIMENTAL RESEARCH

The protective effect of 2-mercaptoethane sulfonate (MESNA) against traumatic brain injury in rats

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Received: 15 June 2012 / Accepted: 13 September 2012 / Published online: 29 September 2012

Abstract
Background The agent, 2-mercaptoethane sulfonate (MESNA), is a synthetic small molecule, widely used as a systemic protective agent against chemotherapy toxicity, but is primarily used to reduce hemorrhagic cystitis induced by cyclophosphamide. Because MESNA has potential antioxidant and cytoprotective effects, so we hypothesized that MESNA may protect the brain against traumatic injury.
Method Thirty-two rats were randomized into four groups of eight animals each; Group 1 (sham), Group 2 (trauma), Group 3 (150 mg/kg MESNA), Group 4 (30 mg/kg methylprednisolone). Only skin incision was performed in the sham group. In all the other groups, the traumatic brain injury model was created by an object weighing 450 g falling freely from a height of 70 cm through a copper tube on to the metal disc over the skull. The drugs were administered immediately after the injury. The animals were killed 24 h later. Brain tissues were extracted for analysis, where levels of tissue malondialdehyde, caspase-3, glutathione peroxidase, superoxide dismutase, nitric oxide, nitric oxide synthetase and xanthine oxidase were analyzed. Also, histopathological evaluation of the tissues was performed.
Results After head trauma, tissue malondialdehyde levels increased; these levels were significantly decreased by MESNA administration. Caspase-3 levels were increased after trauma, but no effect of MESNA was determined in caspase-3 activity. Following trauma, both glutathione peroxidase and superoxide dismutase levels were decreased; MESNA increased the activity of both these antioxidant enzymes. Also, after trauma, nitric oxide, nitric oxide synthetase and xanthine oxidase levels were increased; administration of MESNA significantly decreased the levels of nitric oxide, nitric oxide synthetase and xanthine oxidase, promising an antioxidant activity. Histopathological analysis showed that MESNA protected the brain tissues well from injury.

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Conclusions Although further studies considering different dose regimens and time intervals are required, MESNA was shown to be at least as effective as methylprednisolone in the traumatic brain injury model.

Keywords Antioxidation · Antiapoptosis · 2-mercaptoethane sulfonate · Methylprednisolone · Neuroprotection · Traumatic brain injury

Introduction

Traumatic brain injury (TBI) is still a major worldwide health problem. Traumatic brain injury immediately causes direct mechanical damage to the brain, which is called primary injury, and then induces biochemical pathways that lead to delayed neural cell loss, which is called secondary injury [41]. Lots of studies have been designed to prevent secondary injury after TBI. The aim of these studies was to prevent delayed biochemical injury, suppress oxidative stress and enhance functional outcome after TBI. Despite these efforts, an effective treatment for preventing secondary injury has not yet been developed.

Reactive oxygen species (ROS) play an important role in the pathogenesis of secondary injury after TBI [14, 16]. Trauma-induced production of ROS causes injury via lipid peroxidation, as well as oxidative damage of proteins and DNA. Oxidative stress begins immediately after TBI, and initiates the events resulting in neuronal dysfunction and death [4]. To prevent this oxidative damage, organisms have endogenous enzymatic antioxidant defense systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase. On the other hand, secondary insults often involve apoptotic cell death, which causes complex cascading pathways, resulting in the activation of proteases such as caspase-3 [20, 40].

The agent, 2-mercaptoethane sulfonate (MESNA), is a synthetic small molecule, which has the potential to scavenge ROS by virtue of the sulfhydryl group [13]. It is widely used as a systemic protective agent against chemotherapy toxicity, but is primarily used to reduce hemorrhagic cystitis induced by cyclophosphamide [5]. 2-Mercaptoethane sulfonate has also been found to inhibit the development of bladder tumors in rats [23], and to prevent renal oxidative damage in rats treated with ferric nitrilotriacetate [38]. Thus, the effectiveness of MESNA as an antioxidant drug has been demonstrated in various in vivo and in vitro models [12, 32].

Based on these studies, the aim of this study was to evaluate whether MESNA administration could protect brain tissue from oxidative stress and apoptosis in rats after experimental TBI. We also compared MESNA with methylprednisolone (MP), which has been researched widely in traumatic brain and spinal cord injury.

Materials and methods

Experimental groups

Animal care and all experiments were in concordance with European Communities Council Directive of November 24, 1986 (86/609/EEC) on the protection of animals for experimental use. All experimental procedures used in this investigation were reviewed and approved by the ethical committee of Ministry of Health Refik Saydam Hıfzıssıha Institution. Thirty-two adult male Wistar Albino rats weighing 250–350 g were used. The rats were randomly assigned to four groups, with eight rats per group.

The groups were as follows:

Group 1: Sham (n=8); skin incision only. Rats underwent skin incision and non-traumatic brain samples were obtained 24 h after surgery.

Group 2: Trauma (n=8); rats underwent TBI as described below. After craniectomy, brain samples were removed 24 h post-injury.

Group 3: MESNA (n=8); as for group 2, but rats received a single intraperitoneal dose of 150 mg/kg MESNA (Uromitexan, Eczacıbaşı Baxter, Turkey) immediately following TBI. The dosage of the MESNA used in this study was obtained from the past studies [8].

Group 4: Methylprednisolone (MP) (n=8); as for group 2, but rats received a single intraperitoneal dose of 30 mg/kg MP (Prednol, Mustafa Nevzat, Turkey) immediately following TBI.

Anesthesia and trauma procedure

All rats were kept under environmentally controlled conditions at 22–25 °C, with appropriate humidity and a 12-h light cycle and granted free access to food and water.

The animals were anesthetized by an intraperitoneal injection of 10 mg/kg xylazine (Rompun, Bayer, Turkey) and 50 mg/kg ketamine (Ketalar, Parke Davis, Turkey), and allowed to breathe spontaneously. A rectal probe was inserted and the animals were positioned on a heating pad maintaining the body temperature at 37 °C. A moderate brain-injury model, described by Marmarou et al. [22], and modified by Ucar et al. [37], was applied for head trauma. The rats were placed in a prone position on the table. A midline incision was made on the head, and the coronal and lambdoid sutures were identified. A metallic disc of 10 mm diameter and 3 mm thickness was fixed to the cranium using

bone wax between the two sutures in the midline. Trauma was applied at the point where the disc was placed in the midline. A lead object weighing 450 g was allowed to fall freely from a height of 70 cm through a copper tube on to the metal disc over the skulls of the rats. The animals were supported on a 10-cm foam bed that provided the deceleration after impact. After injury, the metallic disc was removed and the skin sutured before termination of anesthesia.

All the animals were anesthetized with the above mentioned agents at the 24th h after trauma and their brains were extracted immediately without any damage. Samples of neural tissues were obtained by excising the left frontoparietal lobes from the boundary of the interhemispheric fissure and were subjected to biochemical analyses. The remaining parts of the brains were maintained in formaldehyde solution for histopathological analysis.

Biochemical procedures

Tissue Malondialdehyde (MDA) analysis

Malondialdehyde is formed from the breakdown of polyunsaturated fatty acids, and serves as an important and reliable index for determining the extent of peroxidation reactions [21]. Tissue MDA levels were determined by a method based on the reaction with thiobarbituric acid (TBA). Briefly, the samples were mixed with two volumes of cold saline solution containing 0.001 % butylated hydroxytoluene (BHT) (200 μl of 0.01 % BHT solution in methanol) and 0.07 % sodium dodecyl sulfate (SDS) (20 μl of 7 % SDS). Then 1 ml of samples was added to 500 μl of 0.01 NH2SO4 and 500 μl of the TBA reagent (0.67 % TBA in 50 % acetic acid) to precipitate protein. The samples were heated in boiling water for 60 min. After cooling, an equal volume (2 ml) of *n*-butanol was added to each test tube and mixed. The mixture was centrifuged at 4,000 rpm for 10 min at room temperature. The absorbance of the organic layer in a 1-ml cell was read at 535 nm (Molecular Devices Corporation, Sunnyvale, CA, USA). MDA concentrations were expressed as nmoles per milligram tissue wet weight.

Tissue caspase-3 analysis

Tissues were homogenized in physiological saline (1 g in 5 ml) and centrifuged at 4,000 g for 20 min. The upper layer of clear supernatant was removed and used in the analyses. Before the analysis, the supernatant samples were adjusted so that they contained equal protein concentrations. The protein concentrations of the supernatant samples were measured using the Lowry method. The Lowry method depends on the reactivity of the nitrogen in peptides with copper ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteau phosphomolybdic-phosphotungstic acid to heteropolymolybdenum blue by the copper catalyzed oxidation of aromatic amino acids. Absorbance measurements were made at 700 nm using a spectrophotometer. The protein concentration of the sample was determined using a protein calibrator. The caspase-3 activity of the tissue samples were measured using the Caspase-3 Colorimetric Detection Kit (907-013; Assay Designs, Ann Arbor, MI, USA). The kit involves the conversion of a specific chromogenic substrate for caspase-3 (acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide), followed by colorimetric detection of the product (*p*-nitroaniline) at 405 nm. The absolute value for caspase-3 activity can be determined by comparison with a signal given by the *p*-nitroaniline calibrator. Activity measurements were quantified by comparing the optical densities obtained with standards with the *p*-nitroaniline calibrator. One unit of caspase-3 activity was defined as the amount of enzyme needed to convert 1 pmol of substrate per min at 30 °C. The results were expressed as U/mg protein.

Tissue glutathione peroxidase (GPx) analysis

Glutathione peroxidase activity was measured by following changes in NADPH absorbance at 340 nm [26]. In the activity calculations (IU, international unit), extinction coefficients NADPH were used for GSH-Px. The results were expressed as IU/mg protein.

Tissue superoxide dismutase (SOD) analysis

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al. [35]. The principle of the method is based on the inhibition of nitrobluetetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50 % inhibition in the NBT reduction rate. SOD activity was expressed as U/mg protein.

Tissue nitric oxide (NO) and nitric oxide synthetase (NOS) analysis

The level of NO was estimated by the method based on the diazotization of sulfanilic acid by NO at acid pH and subsequent coupling to *N*-1-napthyl-ethylene diamine (Griess reaction) as described before [9]. Since nitrate anion does not give a diazotization reaction with sulfanilic acid, the samples were treated with cadmium (a reducing agent) to reduce nitrate anions into nitrite anions before the NO estimation. The total NOS activity method is based on the

Griess reaction. The results were expressed as μmol/mg protein and IU/mg protein respectively.

Tissue xanthine oxidase (XO) analysis

Serum XO activity was measured by the method of Prajda and Weber [28], where activity is measured by determination of uric acid from xanthine. Serum samples were incubated for 30 min at 37 °C in 3 ml of the phosphate buffer (pH 7.5, 50 nM) containing xanthine (4 mM). The reaction was stopped by addition of 0.1 ml 100 % (w/v) TCA and the mixture was then centrifuged at 4,000*g* for 20 min. Urate was determined in the supernatant by measuring absorbance at 292 nm against blank and expressed as mIU/mg protein. The calibration curve was constructed by using 10–50 mU/ml concentrations of standard XO solutions (Sigma X-1875). One unit of activity was defined as 1 μmol of uric acid formed per minute at 37 °C, pH 7.5.

Histopathological procedures

The brain parenchymal tissues of all rats in all the groups were embedded in paraffin and fixed with 10 % buffered formalin for 24 h. Using a microtome, 5-μm-thick serial sections were taken from the paraffin blocks, and stained with hematoxylin-eosin (H&E) for routine histopathological observations. Sections of all tissue samples were observed under a light microscope by a neuropathologist who was blinded to the study design.

A semiquantitative scoring system, ranging between 0 and 3, was used for grading both histopathological changes (vascular congestion, intraparenchymal hemorrhage, inflammation, neuronal loss and gliosis) and the neuronal degenerative signs (nuclear pyknosis, nuclear hyperchromasia, cystoplasmic eosinophilia and axonal edema) in the brain tissues of all samples. Nine different parameters assessed histopathologically were scored as follows: 0, absent; 1, mild; 2, moderate; 3, common. Pathological score for each brain was calculated by averaging the scores of these nine different parameters.

Statistical analysis

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS, Chicago, IL, USA). Whether the distributions of continuous variables were normally or not were determined by Shapiro Wilk test. The Levene test was used for the evaluation of homogeneity of variances. Data were shown as mean ± standard deviation or median (IQR), where applicable. While, the mean differences among groups were analyzed by one-way ANOVA, otherwise, Kruskal Wallis test was applied for comparisons of the median values. When the *P* value from one-way ANOVA or Kruskal Wallis test statistics were statistically significant, post hoc Tukey HSD or Conover's non-parametric multiple comparison tests were used to determine which group differed from the others. A *P* value of less than 0.05 was considered statistically significant.

Results

Tissue malondialdehyde (MDA) analysis

When mean tissue MDA levels were compared between the sham and the trauma groups, there was a statistically significant difference observed ($P<0.001$); as a result we concluded that after TBI, due to elevated lipid peroxidation, tissue MDA levels were increasing. When we compared the MESNA and the MP groups with the trauma group, there was a statistically significant difference observed ($P<0.001$ and $P<0.05$ respectively). These data showed that both MESNA and MP prevented an increase in MDA levels in the brain (Fig. 1a).

Tissue caspase-3 analysis

There was a statistically significant difference between the sham and the trauma groups with regard to mean caspase-3 activity ($P<0.001$). However, these data showed that TBI clearly elevated caspase-3 activity in the damaged tissue. When the MESNA group was compared with the trauma group, there was no statistically significant difference determined ($P=0.658$). On the other hand, the MP group showed a statistically significant decrease in caspase-3 activity compared with the trauma group ($P<0.05$) (Fig. 1b). This data concluded that MP prevented an increase in caspase-3 activity and effectively inhibited apoptotic cell death, but no such anti-apoptotic activity was determined with MESNA.

Tissue glutathione peroxidase (GPx) analysis

When mean tissue GPx levels were compared between the sham and the trauma groups, there was statistically significant difference observed ($P<0.001$); so we concluded that after TBI, due to highly elevated oxidative stress, tissue GPx levels were decreasing. When we compared the MESNA group with the trauma group, a statistically significant difference was observed ($P<0.001$). As in the MESNA group, the comparison between the MP and the trauma groups showed a statistically significant difference ($P<0.05$) (Fig. 1c). These data showed that both MESNA and MP prevented oxidative stress of the brain by elevating GPx activity.

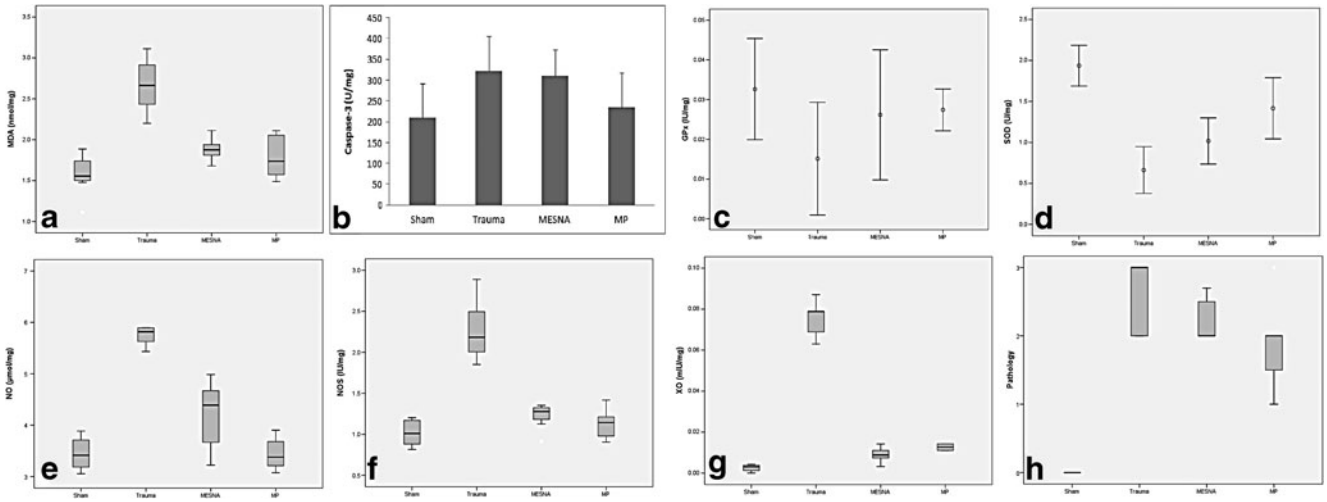

Fig. 1 Biochemical results. **a** Comparison of median MDA levels in patients among groups. The *horizontal lines* in the middle of each box indicates the median, while the *top and bottom borders* of the box mark the 25th and 75th percentiles, respectively. The *whiskers* above and below the box mark the maximum and minimum MDA levels. **b** Caspase-3 levels. **c** Comparison of mean GPx levels in patients among groups. The *circles* in the middle of each line indicate the mean, while the *top and bottom borders* of the line marks are ± SD GPx. **d** Comparison of mean SOD levels in patients among groups. The *circles* in the middle of each line indicate the mean, while the *top and bottom borders* of the line marks are ± SD SOD. **e** Comparison of median NO levels in patients among groups. The *horizontal lines* in the middle of each box indicate the median, while the *top and bottom borders* of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum NO levels. **f** Comparison of median NOS levels in patients among groups. The *horizontal lines* in the middle of each box indicates the median, while the *top and bottom borders* of the box mark the 25th and 75th percentiles, respectively. The *whiskers* above and below the box mark the maximum and minimum NOS levels. **g** Comparison of median XO levels in patients among groups. The *horizontal lines* in the middle of each box indicates the median, while the *top and bottom borders* of the box mark the 25th and 75th percentiles, respectively. The *whiskers* above and below the box mark the maximum and minimum XO levels. **h** Comparison of median pathology scores in patients among groups. The *horizontal lines* in the middle of each box indicates the median, while the *top and bottom borders* of the box mark the 25th and 75th percentiles, respectively. The *whiskers* above and below the box mark the maximum and minimum pathology scores

Tissue superoxide dismutase (SOD) analysis

As in GPx levels, SOD levels were significantly decreased when the sham group was compared with the trauma group ($P<0.001$). When the trauma group was compared with the both MESNA and MP groups, the differences were statistically significant ($P<0.001$ and $P<0.05$ respectively) (Fig. 1d). Both MESNA and MP caused elevated SOD activity and protected the brain from oxidative stress.

Tissue nitric oxide (NO) and nitric oxide synthetase (NOS) analysis

Trauma caused significant elevation in the NO levels when compared with the sham group ($P<0.001$). Both MESNA and MP treatment showed statistically significant reduction in the NO levels ($P<0.001$ and $P<0.05$ respectively) (Fig. 1e).

After trauma, total NOS levels were increased significantly when compared to the sham group ($P<0.001$). Both MESNA and MP inhibited NOS activity significantly when compared with the trauma group ($P<0.001$ and $P<0.05$ respectively) (Fig. 1f).

Tissue xanthine oxidase (XO) analysis

Xanthine oxidase activity found to be higher in the trauma group when compared with the sham group ($P<0.001$). Both MESNA and MP decreased the XO activity following TBI ($P<0.001$ and $P<0.05$ respectively) (Fig. 1g).

Histopathological assessment

The parenchymal features of the white and gray matter, the neuronal morphology and the vascular structures were assessed to be normal in the sham group (Fig. 2a). Following trauma, marked edema in the white and gray matter, in addition to vascular congestion were observed with neuronal injury findings, including hyperchromasia in neuronal nuclei, nuclear pyknosis, cytoplasmic eosinophilic degeneration, and axonal edema. Also, focal neuronal loss and gliolitic zones were seen in the trauma group (Fig. 2b).

When pathological scores compared, the trauma group had statistically higher scores than the sham group ($P<0.001$). In the MESNA group, pathological score is lower (1.92±0.46) than the trauma group (2.62±0.51); this difference was statistically significant ($P<0.05$). In the MP group, pathology score was 1.87±0.64 and this score is statistically significantly

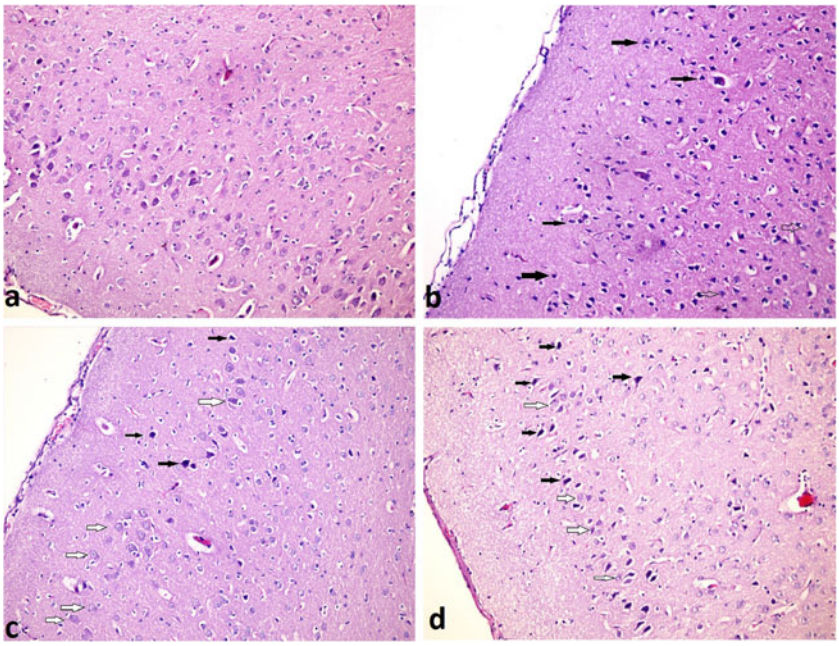

Fig. 2 **a** Photomicrograph of the sham group showing regular brain parenchyma (H&E, ×20 obj.). **b** Photomicrograph of the trauma group showing marked edema, vascular congestion and neuronal injury findings (H&E, ×20 obj.). The *filled arrows* indicate the injured neurons; notice only a few normal-appearing neurons (*hollow arrows*). **c** Photomicrograph of the MESNA group showing less edema and fewer degenerated neurons (*filled arrow*). Note the normal-appearing neurons (*hollow arrow*) (H&E, ×20 obj.). The brain tissues were well protected from injury. **d** Photomicrograph of the MP group showing fewer degenerated neurons (*filled arrow*), and more normal-appearing neurons (*hollow arrow*) (H&E, ×20 obj.)

lower than the trauma group ($P<0.05$) (Fig. 1h). Histopathologically, both MESNA and MP protected the brain from TBI (Fig. 2c, d).

The biochemical results and pathology scores are summarized in Table 1.

Discussion

Head trauma causes brain injury by leading to primary and secondary damage in the neural tissue. The most important secondary factors, which leads to further neuronal death, are

Table 1 Biochemical and histopathological alterations among the groups

Variables	Sham	Trauma	MESNA	MP	*P* value
MDA (nmol/mg)	1.55 (0.26)[a, b, c]	2.66 (0.50)[a, d, e]	1.87 (1.50)[b, d]	1.74 (0.51)[c, e]	<0.001
Caspase-3 (U/mg)	210.4±80.02[a, b, c]	321.8±83.67[a, d]	309.6±64.41[b]	233.4±85.4[c, e]	0.658
GPx (IU/mg)	0.033±0.006[a, b]	0.015±0.007[a, d, e]	0.026±0.008[b, d]	0.027±0.003[e]	<0.001
SOD (U/mg)	1.94±0.12[a, b, c]	0.66±0.14[a, d, e]	1.02±0.14[b, d, f]	1.42±0.19[c, e, f]	<0.001
NO (μmol/mg)	3.41 (0.62)[a, b]	5.82 (0.28)[a, d, e]	4.39 (1.17)[b, d, f]	3.38 (0.65)[e, f]	<0.001
NOS (IU/mg)	1.01 (0.32)[a, b]	2.18 (0.69)[a, d, e]	1.28 (0.18)[b, d]	1.14 (0.27)[e]	<0.001
XO (mIU/mg)	0.003 (0.003)[a, b, c]	0.079 (0.010)[a, d, e]	0.009 (0.004)[b, d]	0.013 (0.003)[c, e]	<0.001
Pathology	0.0 (0.00)[a, b, c]	3.0 (1.00)[a, d, e]	2.0 (0.25)[b, d]	2.0 (0.75)[c, e]	<0.001

The values are expressed as mean ± SD or median (IQR), where applicable

MESNA 2-mercaptoethane sulfonate, *MP* methylprednisolone, *MDA* malondialdehyde, *GPx* glutathione peroxidase, *SOD* superoxide dismutase, *NO* nitric oxide, *NOS* nitric oxide synthetase, *XO* xanthine oxidase

[a] Sham vs Trauma ($P<0.001$)

[b] Sham vs MESNA ($P<0.05$)

[c] Sham vs MP ($P<0.05$)

[d] Trauma vs MESNA ($P<0.001$)

[e] Trauma vs MP ($P<0.05$)

[f] MESNA vs MP ($P<0.05$)

lipid peroxidation [3, 4, 19, 24], apoptosis [20, 27], and the development of the ROS [16, 34].

The TBI model used in our study was described by Marmarou et al. [22] and modified by Ucar et al. [37]. In the model of Marmarou et al. [22], an object of 450 g falls freely from a height of 2 m, causing 44 % mortality. To lower the mortality rate, we used the moderate head trauma model described by Ucar et al. [37], in which an object of 450 g falls freely from an height of 70 cm. 2-Mercaptoethane sulfonate is a well-known systemic protective agent against chemotherapy toxicity [31]. When MESNA enters the blood stream, a large percentage of the drug is oxidized to MESNA-disulfide, whereas a fraction remains in the reduced form. This oxidation is similar in nature to the actions of glutathione, which forms disulfide bonds to detoxify H_2O_2. The oxidation of this sulfhydryl group contributes to the antioxidant properties of the MESNA [2, 12]. On the other hand, MESNA was found to be an anti-apoptotic agent [8, 42]. Because of these past studies we hypothesized and demonstrated the neuroprotective effects of MESNA against TBI in rats for the first time in our study.

It is known that lipid peroxidation products increase soon after injury [3]. However, lipid peroxidation is recognized as one of the main pathophysiological mechanisms involved in secondary damage [7]. Malondialdehyde, a well-known secondary product of lipid peroxidation in myelin, glial and neural membranes, is formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of lipid peroxidation [29]. Our study showed that, after TBI, levels of MDA had dramatically increased in the trauma group compared with the sham group; after the administration of MESNA and MP, this increase in the MDA levels decreased significantly. These results showed that both MESNA and MP prevented lipid peroxidation in the traumatic brain.

Following TBI, secondary insults often involve apoptotic cell death in regions caudal to the impact site [18]. Caspase-3 is an interleukin-converting enzyme, and has been suggested to be the principal effector in the mammalian apoptotic and inflammatory pathways [17]. Caspase-3 is a reliable marker in reflecting the apoptotic activity [18]. In this study, after trauma caspase-3 activity increased significantly, as an indicator of increased apoptosis. MP protected the brain from apoptosis by lowering caspase-3 activity. Unfortunately,

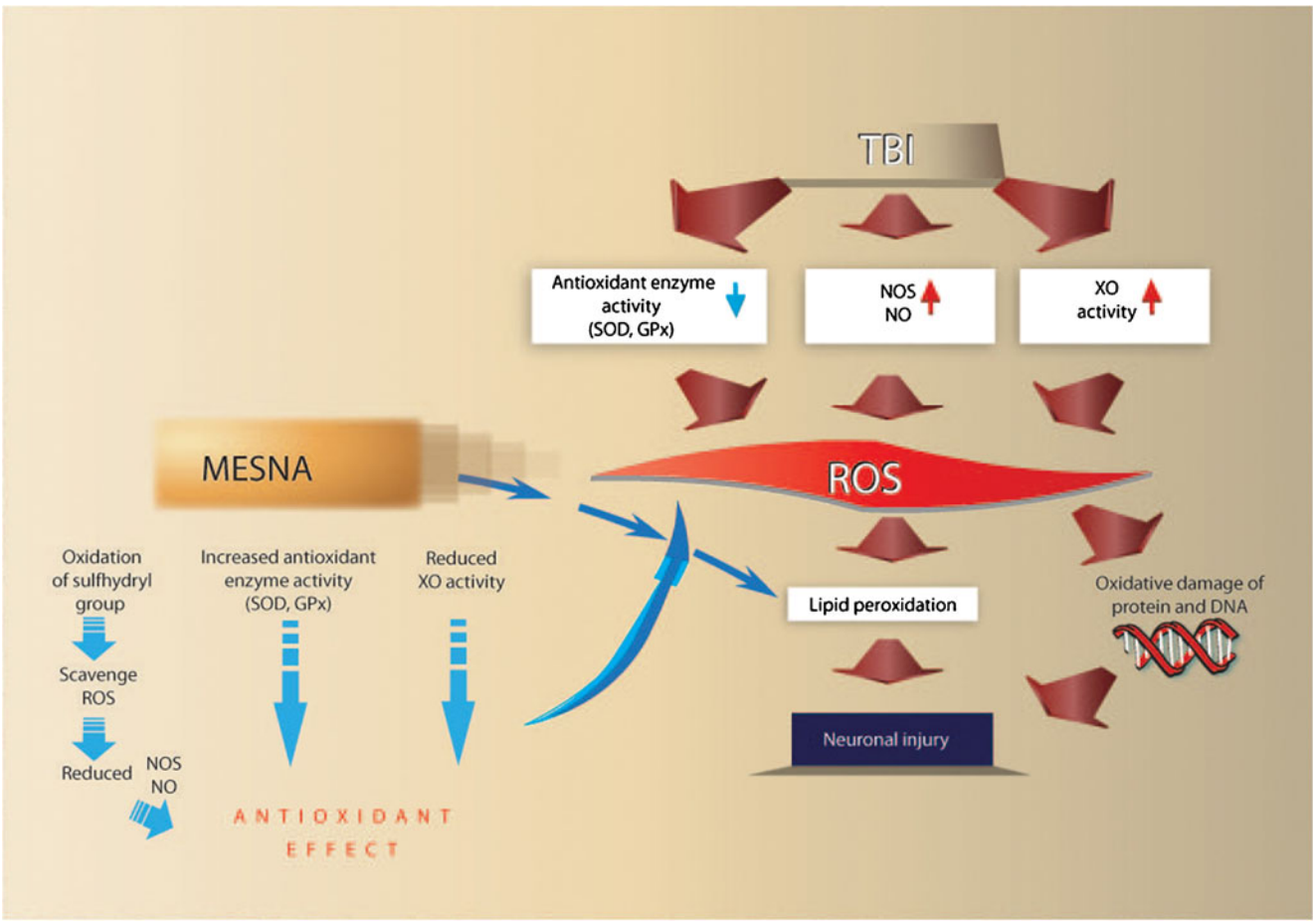

Fig. 3 The pathophysiological mechanisms of MESNA interaction with TBI. *TBI* traumatic brain injury, *SOD* superoxide dismutase, *GPx* glutathione peroxidase, *NOS* nitric oxide synthetase, *NO* nitric oxide, *XO* xanthine oxidase, *ROS* reactive oxygen species

MESNA was not capable of preventing apoptosis after TBI.

Reactive oxygen species play a key role in mediating secondary injury induced by trauma [33, 36]. The brain is particularly vulnerable to oxidative injury because of its high rate of oxygen consumption, intense production of reactive radicals and high levels of transition metals, such as iron, which can catalyze the production of reactive radicals [11, 25]. When tissues are exposed to oxidative stress, they increase the activity and expression of antioxidant enzymes as a compensatory mechanism against free radical-mediated damage. Nevertheless, the increased activity of the antioxidant enzymes may be inadequate to counteract the potential damage in many conditions of oxidative stress [30]. Moreover, antioxidant enzyme activities have been found to be diminished under highly elevated oxidative stress conditions as a result of molecular damage [10, 39]. In our moderate head trauma model, we demonstrated that both SOD and GPx levels decreased significantly after TBI. We hypothesized that this decrease is due to highly elevated oxidative stress. After the administration of the both MESNA and MP, these antioxidant enzymes increased significantly, promising an antioxidant activity of both drugs.

The role of NO in the pathophysiology of TBI has not been fully understood [6]. Endogenous NO is generated from L-arginine by a family of three distinct isoforms of NOS, including endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) [1]. Our study demonstrated that 24 h after TBI NO and total NOS levels increased significantly. Treatment with both MESNA and MP resulted in significant reduction of NO and total NOS levels. This is further evidence for the antioxidant activity of the both drugs.

Reactive oxygen species could be generated by a variety of sources at the cellular level. Xanthine oxidase is an important source for ROS. Xanthine oxidase is shown to be elevated in pathological conditions [15]. After TBI, secondary processes may also promote the generation of ROS and lipid peroxidation via the XO pathway [3]. We demonstrated that, after TBI, XO levels increased significantly. As expected, due to antioxidant activity, both the MESNA and the MP reduced XO levels significantly.

Histopathological evaluation included vascular congestion, intraparenchymal hemorrhage, inflammation, neuronal loss, gliosis, and neuronal degeneration. The sham group had normal brain morphology. In the trauma group, marked edema, vascular congestion, and intraparenchymal hemorrhage were observed. Gliosis and the neuronal degeneration were also significant in the trauma group. Both MESNA and MP groups showed better morphological results compared with the trauma group.

These results suggest that, after moderate TBI, both MESNA and MP have beneficial effects on preserving normal brain morphology, both by reducing lipid peroxidation and antioxidant effects. Figure 3 summarizes the pathophysiological mechanisms of MESNA interaction with TBI.

However, this study has some limitations. The number of rats in each group may be augmented and the dose-dependent results may be investigated. Delayed biochemical and histopathological assessment may also promise better results for further studies. Also, another limitation of this study is the lack of functional outcome measures.

Conclusion

In conclusion, biochemical and histopathological analysis revealed that MESNA exhibits meaningful neuroprotective effects over TBI by reducing lipid peroxidation and increasing antioxidant activity. On the other hand, no antiapoptotic activity of MESNA could be shown. Moreover, MESNA has been shown to be at least as effective as MP in protecting brain tissue over TBI. Based on these promising results, MESNA may have potential future clinical therapeutic application in the TBI practice.

Conflicts of interest None

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Comment

In this experimental study, the authors try to perform biochemical and histopathological analysis about the neuroprotective effects of MESNA in TBI. The study is well conceived and their results sound to be reasonable. The authors concluded that MESNA may play a role in TBI by reducing lipid peroxidation and increasing antioxidant activity. On the other hand, no antiapoptotic activity of MESNA could be shown. The idea to use a well-known systemic protective agent against chemotherapy toxicity in TBI should be stuck at in order to obtain a clinical therapeutic application in TBI practice.

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