Does Decorin Protect Neuronal Tissue via Its Antioxidant and Antiinflammatory Activity from Traumatic Brain Injury? An Experimental Study

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■ BACKGROUND: **The development of secondary brain injury via oxidative stress after traumatic brain injury (TBI) is well known. Decorin (DC) inactivates transforming growth factor β1, complement system, and tumor necrosis factor α, which are related to oxidative stress and apoptosis. Consequently, the aim of the present study was to evaluate the role of DC on TBI.**

■ METHODS: **A total of 24 male rats were used and divided into 4 groups as follows; control, trauma, DC, and methylprednisolone (MP). The trauma, DC, and MP groups were subjected to closed-head contusive weight-drop injuries. Rats received treatment with intraperitoneal saline, DC, or MP, respectively. All the animals were killed at the 24th hour after trauma and brain tissues were extracted. The oxidant/antioxidant parameters (malondialdehyde, glutathione peroxidase, superoxide dismutase, and NO) and caspase 3 in the cerebral tissue were analyzed, and histomorphologic evaluation of the cerebral tissue was performed.**

■ RESULTS: **Levels of malondialdehyde, NO, and activity of caspase 3 were significantly reduced, and in addition glutathione peroxidase and superoxide dismutase levels were increased in the DC and MP groups compared with the trauma group. The pathology scores and the percentage of degenerated neurons were statistically lower in the DC and MP groups than in the trauma group.**

■ CONCLUSIONS: **The results of the present study showed that DC inactivates transforming growth factor β1 and protects the brain tissue and neuronal cells after TBI.**

INTRODUCTION

Traumatic brain injury (TBI) is a problem that may cause disability and death of more than 10 million people worldwide if not treated promptly and effectively. TBI occurs after a mechanical shock to the skull from external mechanical forces for reasons such as traffic and industrial accidents, falls from height, and blows.[1-3] TBI is quintessentially divided into 2 categories. The first is the primary cerebral tissue injury that occurs after a mechanical shock to the skull in the initial phase and is called a primary injury (PI).[4] PI is impossible to cure but it may be possible to prevent it by various sanctions and practices (such as traffic rules).[1,4] In the second stage, secondary

Key words
- Antioxidants
- Apoptosis
- Decorin
- Methylprednisolone
- Neuroprotection
- Traumatic brain injury

Abbreviations and Acronyms
CS: Complement system
CSPG: Chondroitin sulfate proteoglycan
DC: Decorin
GPx: Glutathione peroxidase
IL: Interleukin
IP: Intraperitoneal
LP: Lipid peroxide
MDA: Malondialdehyde
MMP: Matrix metalloproteinase
MP: Methylprednisolone
NO: Nitric oxide
PI: Primary injury
PN: Peroxynitrite
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SCI: Spinal cord injury
SI: Secondary injury
SOD: Superoxide dismutase
TBI: Traumatic brain injury
TGF-β1: Transforming growth factor β1
TNF-α: Tumor necrosis factor α

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Table 1. Biochemical Reactions

Haber-Weiss Reaction	$O_2^- + Fe^{+++} + \rightarrow O2 + Fe^{++} \rightarrow FR$
Fenton reaction	$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH + OH^- \rightarrow HWR$
Peroxynitrite	$O_2^- + NO \rightarrow O\ NOO^-$
Superoxide dismutase	$Cu^{++}\text{-}SOD + O_2^- \rightarrow Cu^{+}\text{-}SOD + O_2$
Superoxide dismutase	$Cu^{+}\text{-}SOD + O_2^- + 2H^+ \rightarrow Cu^{++}\text{-}SOD + H_2O_2$
Glutathione peroxidase	$2H_2O_2 + GSH \rightarrow GSSG + 2H_2O$

injury (SI) occurs within the cerebral tissue, which is traumatized as a result of PI; it occurs depending on the release of harmful biochemical-neurophysiologic mediators and determines cell survival.[2-4] Most of the research conducted into TBI focuses on SI, in the hope that it can be treated.[2,4]

After TBI, it is believed that proinflammatory cytokines such as interleukin 1(IL-1), IL-6, tumor necrosis factor α (TNF-α), endothelial intercellular adhesion molecule 1, and transforming growth factor β (TGF-β) play pivotal roles.[5,6] In addition, activation of the complement system (CS), excessive secretion of intercellular adhesion molecule 1, and matrix metalloproteinases (MMPs) are associated with neuroinflammation and are involved in the disruption and permeability of the blood-brain barrier, edema formation, neurodegeneration, and apoptosis, after TBI.[7-9] Excessive production and secretion of cytokines occur as a result of activated microglia and astrocytes, neurons, and endothelial cells.[6] In the SI period, cellular ionic imbalance occurs (Ca^{++} and H^+ influx, K^+ efflux) at the traumatized site, and an increase in oxidative stress products as a result of mitochondrial damage is inevitable.[10-12] Rapidly increasing levels of intracellular Ca^{++} lead to the separation of a single electron from O_2, and superoxide (O_2^-) is produced, which means that the oxidative cascade is triggered.[13] Hydroxyl (OH^-) radicals are shown in the iron-catalyzed Fenton reaction in the presence of hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{++}) (Table 1).[14] O_2^-, which is released into the environment after trauma, acts as a reducing agent and provides electrons to ferric iron (Fe^{+++}), cycling it back to the ferrous state in the Haber-Weiss reaction. Subsequently, the Fenton reaction takes place again and the process leads to continuous increase of OH^- (Table 1).[15,16] When O_2^- reacts with nitric oxide (NO), reactive agents peroxynitrite (PN: $ONOO^-$) and OH^- occur (Table 1). PN decomposition results in the formation of markedly cytotoxic free radicals, which are nitrogen dioxide (NO_2) and carbonate radical (CO_3).[15,16] Consequently, oxidative stress occurs when there is a breakdown in the balance of antioxidant production with reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels. For that reason, preventive treatments against the inflammatory process in the early stage of TBI is important in breaking the oxidative stress cascade and the inhibition of apoptosis.[7,17]

Decorin (DC; Sigma-Aldrich, St Louis, Missouri, USA) is a member of the small leucine-rich proteoglycans family; a single glycosaminoglycan chain is linked to its core protein.[18,19] It plays an important role in the cell cycle and extracellular matrix organization.[18] By means of its molecular binding property, DC inactivates TGF-β1, CS, and TNF-α in the extracellular matrix.[20-22] DC also modulates the in vivo activity of matrix molecules such as collagen, fibronectin, and thrombospondin.[19,20] Synthesis of DC is developmentally regulated in the brain as well as in many tissues.[23,24] In addition, it was previously shown that DC prevents the tumoral cell cycle and angiogenesis by effecting epidermal growth factor receptor and also has beneficial effects on various injuries, including TBI and spinal cord injury (SCI).[25-28]

The aim of the present study was to evaluate the role of DC on a rat model of TBI, in terms of its antioxidant and antiapoptotic properties. In addition, the effects of DC on traumatized cerebral tissue are histomorphologically examined, and its effectiveness is compared with methylprednisolone (MP) treatment.

METHODS

Experimental Groups

Animal care and all experiments were carried out in line with the European Communities Council. We used the directive dated 24 November 1986 (86/609/EEC) for the protection of animals for experimental use. The study was conducted on 24 male Wistar albino rats weighing 250–300 g. All experimental procedures were approved by the animal research ethics committee of Gazi University and the study was conducted at the animal breeding and experimental research laboratory center of the same university.

Group 1. Sham ($n = 6$); skin incision only. The rats underwent a skin incision and nontraumatic brain samples were obtained 24 hours after surgery.

Group 2. Trauma ($n = 6$); the rats underwent TBI as described later. After craniectomy, brain samples were removed 24 hours after injury.

Group 3. DC ($n = 6$); similar to group 2, but the rats received a single intraperitoneal (IP) dose of 0.01 mg/kg DC immediately after TBI.

Group 4. MP ($n = 6$); similar to group 2, but rats received a single IP dose of 30 mg/kg MP (Prednol [Mustafa Nevzat, Istanbul, Turkey]) immediately after TBI.

The dosage of DC and MP used in this study was obtained from past studies.[29,30]

Anesthesia and Trauma Procedure

All rats were kept under environmentally controlled conditions at 22°C–25°C, with appropriate humidity and a 12-hour light cycle and granted free access to food and water. The animals were anesthetized by an IP injection of 10 mg/kg xylazine (Rompun [Bayer, Istanbul, Turkey]) and 50 mg/kg ketamine (Ketalar [Parke Davis, Istanbul, 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.,[31] and modified by Ucar et al.,[32] 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 2 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 hour after trauma. Before the rats were killed, their brains were extracted immediately without causing 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 histopathologic analysis.

Biochemical Procedures

Tissues were homogenized in physiological saline (1 g in 5 mL) and centrifuged at 4000*g*/20 minutes. The upper layer of clear supernatant was removed and used in the analysis. The supernatant samples were adjusted before the analysis 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-Ciocalteu 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 by using a protein calibrator.[33,34]

Tissue Malondialdehyde Analysis

Malondialdehyde (MDA) is formed from the breakdown of polyunsaturated fatty acids and serves as an important and reliable index for determining the extent of peroxidation reactions.[33,34] We determined tissue MDA levels using a method based on the reaction with thiobarbituric acid. MDA concentrations were expressed as nmole/milligram wet tissue weight.

Tissue Glutathione Peroxidase Analysis

Glutathione peroxidase (GPx) activity was measured by following changes in NADPH absorbance at 340 nm.[35] Extinction coefficients of NADPH were used for GPx in the activity calculations (IU). The results were expressed as IU/mg protein.

Tissue Superoxide Dismutase Analysis

Total (Cu-Zn and Mn) superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined according to the method of Sun et al.[36] The principle of the method is based on the inhibition of the nitroblue tetrazolium 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 nitrobluetetrazolium reduction rate. SOD activity was expressed as IU/mg protein.

Tissue NO Analysis

The level of NO was estimated by the method based on the diazotization of sulfanilic acid by NO at acidic pH and subsequent coupling to N-1-napthyl-ethylene diamine (Griess reaction) as described previously.[37] Because 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 results were expressed as μmol/mg protein and IU/mg protein, respectively.

Tissue Caspase-3 Analysis

The Caspase-3 Colorimetric Detection Kit (907-013 [Assay Designs, Ann Arbor, Michigan, USA]) was used. 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-nitro-aniline) 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 minute at 30°C. The results were expressed as U/mg protein.[29,34]

Histologic Examination and Analysis

The brain tissue samples were immediately removed and postfixed in 10% neutral buffered formalin, dehydrated through a graded series of ethanol, and embedded in paraffin for histologic examination. Paraffin blocs cut into 5-μm-thick sections and stained with hematoxylin-eosin were examined by light microscopy (Leica DM3000). Histopathologic changes of the acute phase (1–2 days) after brain injury such as perineural edema, perivascular edema, satellitosis (perineural oligodendroglia), spongiosis (neuropil edema) and hemorrhage were evaluated in each rat. A scoring system was used to record the histopathologic changes after brain injury as 0 (none), 1 (mild), 2 (moderate), and 3 (severe).[29,38]

For observations of semithin sections, brain specimens were fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH, 7.4) for 4–6 hours at 4°C, postfixed in 1% osmium tetroxide for 2 hours, dehydrated in an ascending alcohol series, and embedded in araldite. Semithin (0.7 μm thick) sections of brain tissue were stained with %1 toluidine blue and examined with the aid of a light microscope (Leica DM3000). The semithin sections were selected for each rat to quantify the number of damaged neurons in the cerebral cortex. The histologic images at ×40 magnification were obtained using a light microscope (Leica DM3000) with a liquid crystal display camera and transferred to a screen (ACER). The counting area consisted of 15 fields of about 100 × 100 μm^2 each, randomly selected from the cerebral cortex on each section. Damaged neurons were identified by a shrunken and intensely basophilic cytoplasm and a pyknotic nucleus with no discernible nucleolus.[38] Then, damaged neurons were counted. Nonneuronal cells such as glial cells (distinguished from neurons by cell size, nuclear shape, cytoplasm, location, and characteristic staining patterns of chromatin), pericytes, and endothelial vascular cells were excluded.

A senior histologist blinded to the all groups carried out all analysis.

Table 2. Biochemical Measurements

Variables	Control	Trauma	Methylprednisolone	Decorin	*P* Value
Tissue malondialdehyde (nmol/mg)	1.41 (0.62)[a]	3.08 (1.26)[a,b,c]	1.45 (0.30)[b]	1.30 (0.57)[c]	**<0.001**
Tissue glutathione peroxidase (IU/mg protein)	0.08 (0.03)[a]	0.03 (0.04)[a,b,c]	0.10 (0.03)[b]	0.09 (0.02)[c]	**0.005**
Tissue superoxide dismutase (U/mg protein)	12.41 (0.31)[a,e]	6.16 (1.39)[a,b,c]	10.47 (1.94)[b,d,e]	12.01 (2.42)[c,d]	**<0.001**
Tissue NO (IU/mg protein)	10.37 (1.81)[a,e,f]	28.00 (8.06)[a,b,c]	12.75 (2.56)[b,e]	11.62 (3.37)[c,f]	**<0.001**
Tissue caspase-3 (IU/mg protein)	0.26 ± 0.09[a]	0.53 ± 0.12[a,b,c]	0.31 ± 0.10[b]	0.29 ± 0.08[c]	**<0.001**

Biochemical measurements and statistical analysis: [a]control versus trauma ($P < 0.001$); [b]trauma versus methylprednisolone ($P < 0.05$); [c]trauma versus DC ($P < 0.01$); [d]methylprednisolone versus decorin ($P < 0.05$); [e]control versus methylprednisolone ($P < 0.01$); [f]control versus decorin ($P < 0.05$). Statistically significant *P* values are indicated in bold.

RESULTS

Antioxidant Panel

Table 2 summarizes the results of the antioxidant enzymes panel.

Tissue MDA Analysis. Mean tissue MDA levels were statistically significantly different (P < 0.001) between the control and trauma groups. We therefore concluded that tissue MDA levels increased as a result of the lipid peroxidation increase after TBI. We also found a statistically significant difference (P < 0.001 for both) between the DC versus trauma groups and MP versus trauma groups, indicating that both DC and MP provided protection against increased MDA in the brain (Figure 1A).

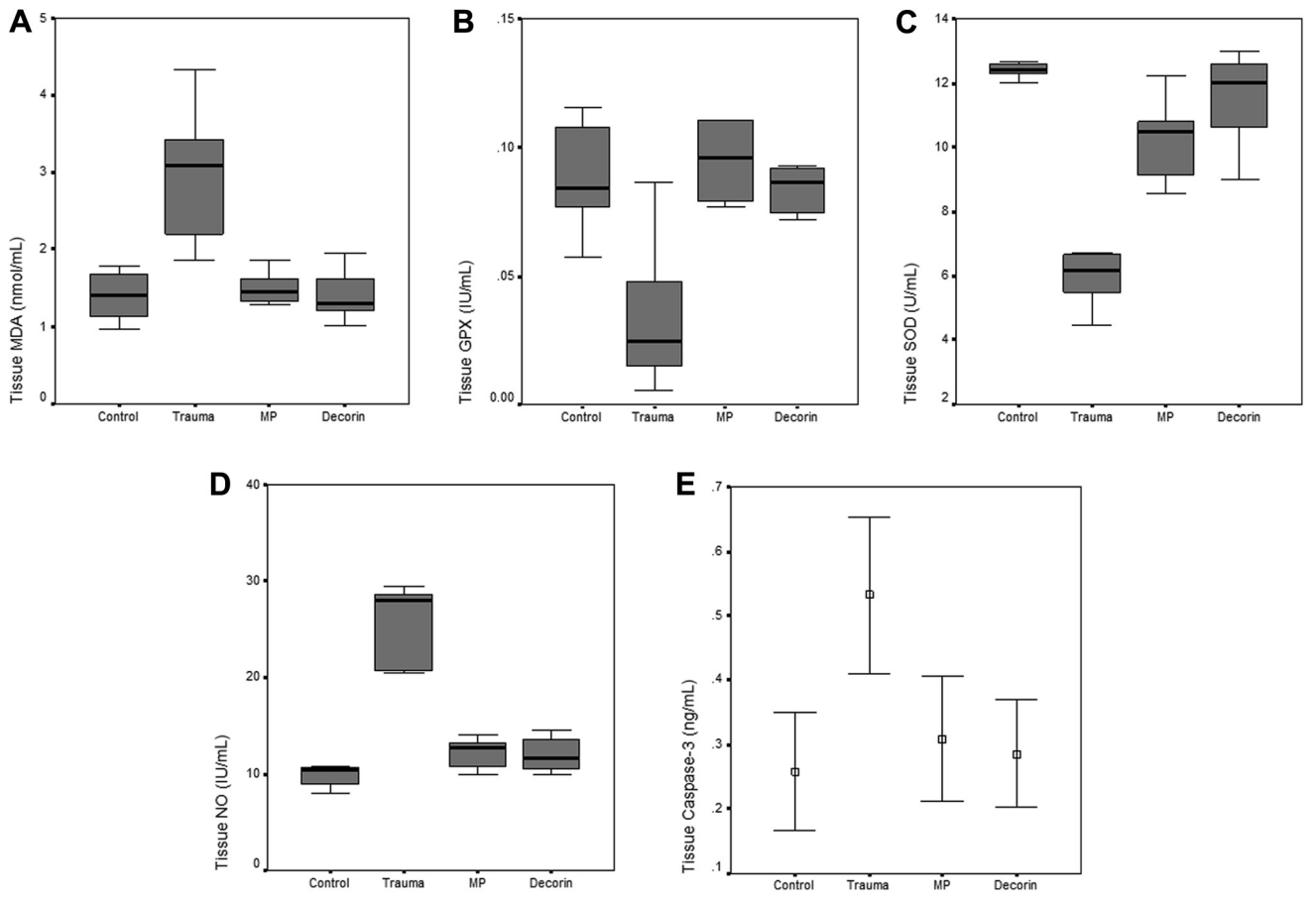

Figure 1. Bar graphs showing the biochemical results of tissue malondialdehyde (**A**), glutathione peroxidase (**B**), superoxide dismutase (**C**), tissue NO (**D**), and tissue caspase-3 (**E**) levels. Values are expressed as mean ± standard deviation. GPX, glutathione peroxidase; MDA, malondialdehyde; MP, methylprednisolone; SOD, superoxide dismutase.

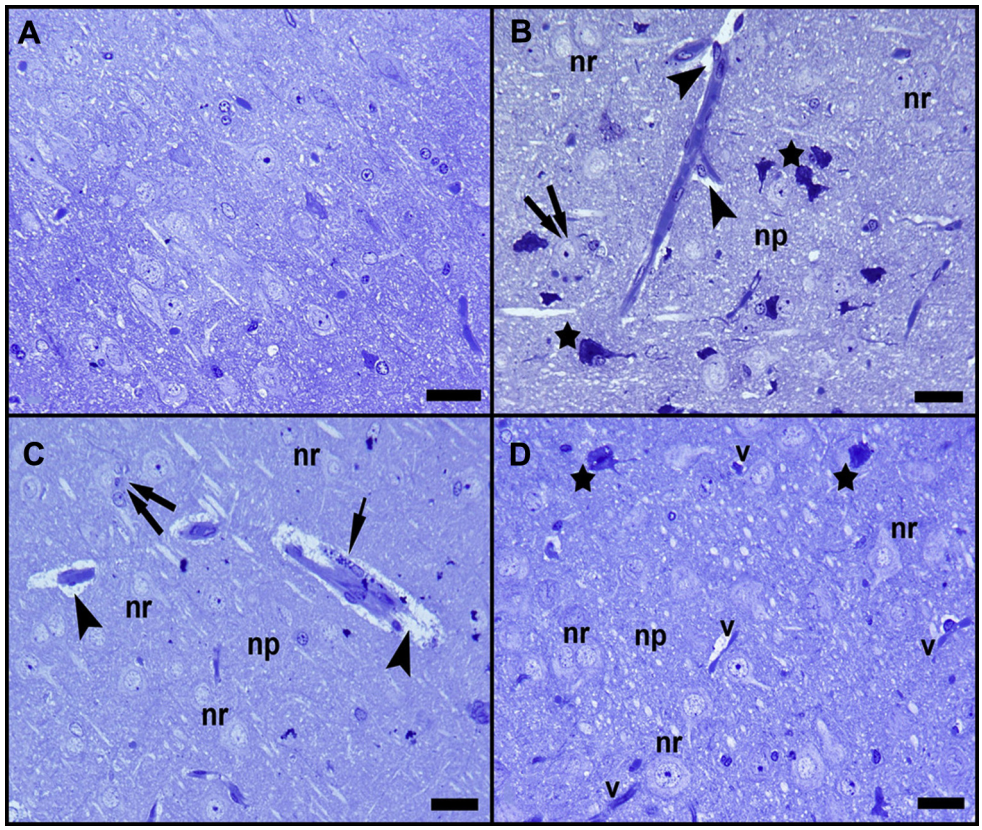

Figure 2. Histologic alterations in control and brain trauma groups after 24 hours. Control (**A**) normal appearing brain parenchyma; SF (**B**) damaged contracted neurons with pyknotic nucleus and darkly stained cytoplasm (*stars*) and normal looking neurons (*nr*) in neuropil (*np*); DC (**C**) mast cells with metachromatic granules were seen close to the blood vessels (*arrow*), satellitosis (*double arrows*), perivascular edema (*arrowheads*); methylprednisolone (**D**) showing less perivascular (*v*) and perineural edema. (**A**–**D**) ×40 obj.; toluidine blue staining; *Bars* represent 40 μm.

Tissue GPx Analysis. The mean tissue GPx levels were statistically significantly different between the control and the trauma groups ($P < 0.005$), indicating decreased tissue GPx levels after TBI as a result of the much increased oxidative stress. There was also a statistically significant difference between the trauma group and the DC ($P < 0.005$) and MP groups ($P < 0.005$) (Figure 1B), indicating that both substances increased antioxidant GPx activity, thus protecting the brain from oxidative stress.

Tissue SOD Analysis. SOD levels were also significantly decreased in the control group compared with the trauma group ($P < 0.001$). The difference was statistically significant when the trauma group was compared with the DC and MP groups ($P < 0.001$ for both) (Figure 1C), showing that DC and MP protected the brain from oxidative stress by increasing SOD activity.

Tissue NO Analysis. Tissue NO levels were statistically significantly higher in the trauma group than in the control group ($P < 0.001$). MP and DC treatments showed statistically significant reduction in NO levels ($P < 0.001$) (Figure 1D).

Tissue Caspase-3 Analysis. The mean caspase-3 activity was statistically significantly different between the control and trauma groups ($P < 0.001$), indicating that TBI increases caspase-3 activity in damaged tissue. There was also a statistically significant difference between the trauma group and the DC and MP groups ($P < 0.001$ for both) (Figure 1E), indicating that both substances prevent apoptotic cell death after TBI and prevent increased activity of caspase-3.

Histologic Results

Light microscopic examination of the cerebral cortex prepared from the control group showed the normal cellular composition with neurons and neuroglial cells. The brain parenchyma neuropil and the blood vessels were normal (Figure 2A). In the trauma group, neurons with darkly stained and shrunken cytoplasm with a pyknotic nucleus indicating the damage were observed. Perivascular and perineural edema caused by astrocytic foot process swelling around blood vessels and neurons were evident. The brain parenchyma neuropil contained microcystic spaces without inflammation and focal

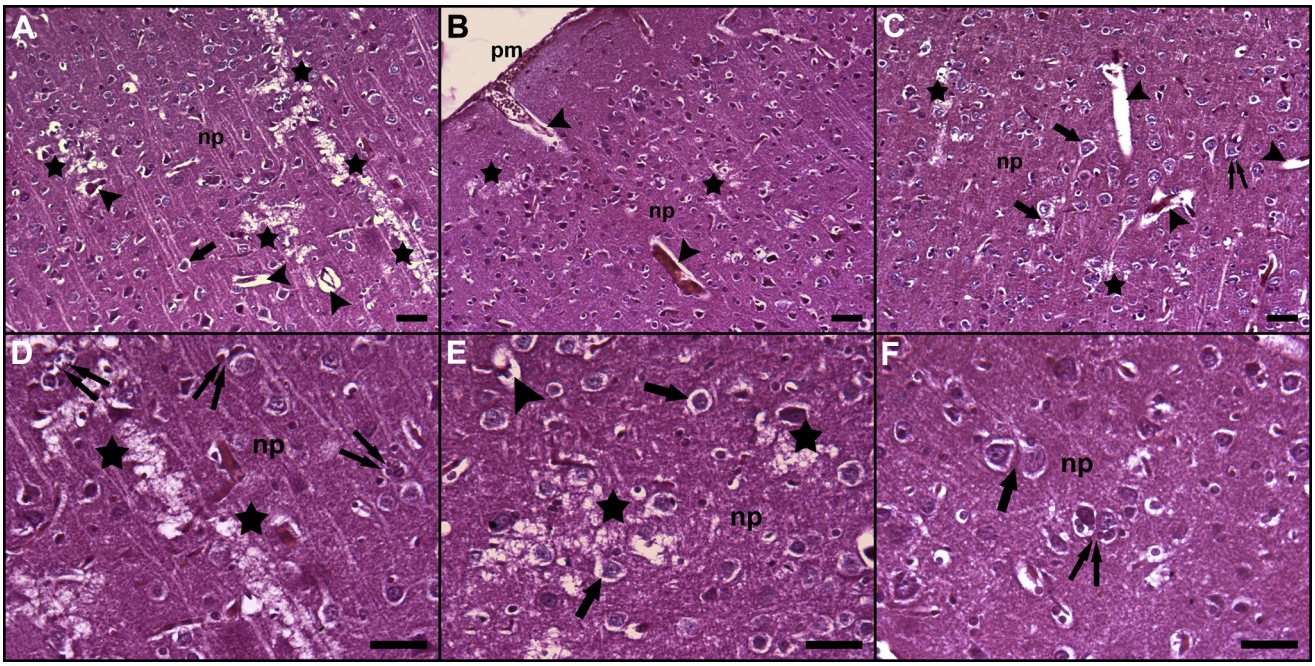

Figure 3. Histologic alterations in brain trauma groups after 24 hours. Trauma (**A, D**) and DC (**B, E**) Perivascular edema (*arrowheads*), perineural edema (*arrow*), marked focal neuropil (*np*) spongiosis with degenerative changes (*stars*), damaged neurons surrounded by oligodendrocytes-satellitosis- (*double arrow*); Photomicrograph of the MP (**C, F**) showing less perivascular (*arrowhead*) and perineural (*arrow*) edema and microvacuolize areas (*stars*). (**A**–**C**) ×20; (**D**–**F**) ×40; hematoxylin-eosin staining; *Bars* represent 40 μm.

areas of spongiosis with degenerative changes (Figure 2B, Figure 3A–D). In the DC group, degenerative neuropil changes and spongiosis were observed less frequently (Figure 2C, Figure 3B–E). However, in the MP group, the morphologic pattern of the cerebral cortex parenchyma and cellular structure was similar to the control group (Figure 2D, Figure 3C–F). Mast cells with metachromatic granules were seen close to the blood vessels in the MP groups (Figure 2C,D). The trauma group had statistically higher pathology scores than the DC and MP groups ($P < 0.001$). The degenerated neuron levels in the trauma group were significantly higher than the in DC group and were also higher than in the MP group ($P < 0,005$). The percentage of degenerated neuron numbers in DC and MP groups was lower than in the trauma group. Our results histopathologically showed that both DC and MP protected the brain from TBI (Figures 2 and 3).

Histopathologic parameters, the number of neurons, and pathology scores are summarized in Table 3.

DISCUSSION

The results of our study have shown that DC both reduced increased caspase-3 activity, NO, and MDA levels and increased the activity of antioxidant enzymes such as GPx and SOD in the post-TBI rat cerebrum. It has been also histopathologically proved through the results of this presented study that DC protects the brain from TBI.

The ROS description often refers to radicals derived from oxygen, which consists of O_2^-, OH^-, and peroxyl (RO_2^-) molecules. However, there are also nonradical molecules within the ROS family, including hydrogen H_2O_2 and PN.[10,11] In the last 30 years, the time course of cerebral ROS and RNS production has been well identified in organized experimental models of TBI. In these studies, it has been shown that O_2^- and OH^- immediately increased after trauma and both have remained at high levels for at least 1 hour.[6,39,40] Similarly, neuronal NO synthetase, which can produce a precursor of PN (NO and O_2^-), is upregulated within 24 hours after TBI.[16] Therefore, total brain NO concentrations are increased in the first 30 minutes and then is decreased in the next 6 hours period after TBI. The second upregulation of NO levels is seen between 6 and 24 hours and this might continue for days.[41] The immediate posttraumatic burst of OH^- is followed by increases in the lipid peroxidation product such as phosphatidylcholine hydroperoxide within 1 hour after injury.[40] Lipid peroxidation is defined as the oxidative degradation of lipids that occurs when oxygen radicals react with polyunsaturated fatty acids, which yields lipid radicals (LOO^-) and alkoxyl radical (LO^-).[16,40] When lipid peroxides (LPs) are destroyed, aldehydes occur, most of which are biologically active, and the process results in the degradation of cell and membrane integrity.[2,10] MDA is produced in the end of the peroxidation of fatty acids which have 3 or more double bonds.[10] MDA levels are

Table 3. Histopathologic Parameters

Variables	Control	Trauma	Methylprednisolone	Decorin	*P* Value
Perineural edema	0 (0–1)[a,b,c]	3 (3–3)[a,d,e]	1.5 (1–2)[b,d]	2 (1–2)[c,e]	**<0.001**
Perivascular edema	1 (1–2)[a,b,c]	3 (3–3)[a,d,e]	2 (1–3)[b,d]	2 (1–3)[c,e]	**0.002**
Satellitosis	0 (0–0)[a,b,c]	3 (2–3)[a,d,e]	1 (1–2)[b,d,f]	2 (1–3)[c,e,f]	**<0.001**
Spongiosis	0.5 (0–1)[a,b,c]	3 (3–3)[a,d,e]	2 (1–3)[b,d]	2 (1–3)[c,e]	**<0.001**
Hemorrhage	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	1.000
Pathology score	2 (1–3)[a,b,c]	12 (11–12)[a,d,e]	6.5 (6–9)[b,d,f]	8 (4–10)[c,e,f]	**<0.001**
Degenerated neurons	6 (4–11)[a,b,c]	29 (20–40)[a,d,e]	12 (7–17)[b,d]	13 (1–40)[c,e]	**0.003**

[a]Control versus trauma ($P < 0.001$); [b]control versus methylprednisolone ($P < 0.001$); [c]control versus decorin ($P < 0.001$); [d]trauma versus methylprednisolone ($P < 0.001$); [e]trauma versus decorin ($P < 0.01$); [f]methylprednisolone versus decorin ($P < 0.05$).

well correlated with the degree of LP; therefore, these are being used as an indicator of the LP.[6,42] There was a statistically significant increase within the first 24 hours after TBI in the total MDA and NO levels in our trauma group compared with the control group, but DC and MP administration decreased this increase in a significant manner. Based on the results of the present study, it could be asserted that DC protects neuronal membrane from LP and it might blockade NO synthetase via an unexplained biochemical mechanism.

There are many endogenous antioxidants such as SOD, GPx, and catalase that aim to prevent oxidative damage under physiologic conditions.[33-36] SOD converts O_2^- into H_2O_2 and O_2 in the low pH environment and presence of the elements such as Cu^+ - Mn^{++} - Fe^{++} and Ni^{++} (**Table 3**).[14] On the other hand, H_2O_2 which is formed by the dismutation of superoxide radicals is detoxified by converting into H_2O by GPx (**Table 1**).[6] It was previously shown that molecular damage in conditions with increased oxidative stress also decreases antioxidant enzyme activity.[6,42] SOD and GPx are decreased significantly in the post-TBI period in our moderate head trauma model, possibly because of the increased oxidative stress. DC and MP administration lead to a significant increase in these antioxidant levels, and this could be an indicator of the antioxidant activity of these drugs.

It is reported that the most effective method to overcome oxidative stress is to prevent interaction of the lipid and alkoxy radicals with the polyunsaturated fatty acids that exist in the structure of the target cell membrane.[2,4] One of the best antioxidant treatment options used for this purpose is high-dose MP therapy. The beneficial effects of MP treatment on LP have been proved in clinical studies and experimental SCI models.[4,16] In the early studies, it was suggested that the antioxidant activity of MP is related to its lipophilicity.[16] However, it has been subsequently shown that neuroprotective activity does not depend only on the lipophilicity but also that MP has supportive effects on the aerobic metabolism in SCI. In addition, MP decreases intracellular Ca^{++} levels and has a preventive effect against the loss of neurofilament.[43,44] It is also reported that these neuroprotective effects of MP are shown only when high doses are used and are unconnected with the antiinflammatory activity of MP and its steroid receptors in experimental models as well as clinical studies.[44,45] As in previous studies, the results of our study have also shown that MP therapy reduces MDA levels, known as the LP index. Furthermore, it has been proved through our study results that MP reduces NO levels increased as a result of TBI and supports antioxidant enzyme activity (SOD and GPx).

TGF-β1 overwhelms mitochondrial function through its inducing effects on ROS-generating enzymes, NADPH oxidases.[46,47] Besides, synthesized ROS directly destroy proteins of tight junctions and indirectly activate MMPs that contribute to disruption of the blood-brain barrier after TBI. Activated MMPs lead to an increase of cellular/serum vascular endothelial growth factor level, which leads to apoptosis and neuroinflammation via the activation of caspase-1/3 and IL-1β release.[26,48] Moreover, the smad pathway, mitogen activated protein kinase pathways, and Rho-GTPase pathway mediate ROS synthesis, which is activated by TGF-β.[49,50] Subsequently, ROS induce secretion and activation of TGF-β1, which causes a vicious cycle.[49] TGF-β1 also suppresses the expression of the antioxidant enzymes, including SOD, catalase, glutaredoxin, and glutamate cysteine ligase, which leads to a decrease in glutathione concentration.[47,49] TNF-α has a similar role to TGF-β in mediating neuronal death in the acute phase of TBI.[51] In addition, TNF-α shows its effects by inducing ROS and RNS production.[6] A drug that has an inhibiting effect on TNF-α and TGF-β might be a valuable treatment option for TBI. It has been reported that for both TGF-β and TNF-α to become active, they should be freed from chondroitin sulfate proteoglycans (CSPGs) by MMPs.[52] MMPs, which are excessively synthesized and secreted after TBI, separate these inflammatory mediators from CSPGs.[21,52] DC, which is known as a member of the CSPG family, has antifibrotic and antiinflammatory activities in many tissues including the brain because it is a natural inhibitor of TGF-β and TNF-α.[21,24,52] DC keeps TGF-β and TNF-α in an inactive state by connecting these mediators to itself via its core protein in the extracellular matrix.[21,52,53] In addition, DC specifically blocks TβR-I/II receptors; therefore, it inhibits the intracellular signaling that is formed by TGF-β through the Smad 2/3 complex.[53] Supporting this hypothesis, it was previously reported that DC treatment abrogates inflammation and prevents oxidative stress and apoptosis; therefore, it dissolved excessive scar formation in traumatized tissue and increased functional recovery in an experimental SCI model.[28,51] The results of our study have shown

that oxidative stress markers (MDA, NO) are decreased in rats treated with DC. This effect of DC might be related to its suppressing activated forms of TGF-β and TNF-α. In addition, it has been determined by all our results that antioxidant enzymes (SOD and GPx) are increased. Thus, it could be estimated that they are consumed less than in the nontreatment trauma group.

Experimental and clinical studies mention an important role for the increased local expression of complement pathway molecules such as C1 and C3 in contributing to inflammation and neuronal/axonal disruption within the traumatized neuronal tissue.[54] The classic activation of CS is triggered through interaction of the C1q complex with antigens including M and G type.[22,54] The immune reactive cells, another accomplice of oxidative stress and apoptosis, migrate to the traumatized site as a result of the activation of local CS.[54] Sema3A receptors, which code an immunoglobulin-like protein (Ig-like C2-protein), create immune reactivity in the early period after TBI, similar to C1q activation.[55] The neuroprotective efficacy of treatments that provide C1 inhibition and Sema3A receptor suppression has been shown experimentally.[56,57] DC inhibits C1 complex activity by binding to C1q and suppresses Sema3A by means of ErbB4 receptor activation.[22,56] Beneficial effects of DC might be associated with the blocking of C1q and suppression of Sema3A receptors in this presented study. Consequently, it is possible to say that the treatment of DC application in the early period after TBI may prevent neuronal functional loss and other late period complications, such as the formation of excess scarring.

Programmed cell death (apoptosis) is a normal physiologic process serving different functions such as resolution of immune response or damaged tissue reshaping.[10,12] However, moderate cerebral contusion injury after TBI causes neuronal apoptosis within the first 24 hours, without waiting for the physiologic process.[12,34] The IL-converting enzyme caspase-3 has been used as a trustable marker for apoptotic cell death.[34] The results of our study showed that the trauma group had higher tissue caspase-3 activity, indicating increased apoptosis, than did the control group after TBI. DC, in a manner similar to MP, decreased caspase-3 activity, protecting the brain from the effects of apoptosis. Antiapoptotic properties of DC might be related to its antiinflammatory activities (inhibition of C1q, Sema3A, TGF-β, and TNF-α) and its effects on vascular endothelial growth factor and NADPH oxidase suppression. According to these biochemical results, the trauma group had statistically higher pathology scores than did the DC and MP groups in addition to a higher percentage of degenerated neurons. The DC and MP groups have less perivascular and perineural edema, fewer degenerative changes in the neuropil, and less spongiosis than the trauma group.

CONCLUSIONS

The findings of the current study suggest that TGF-β1 plays a prominent role in development and severity of posttraumatic injury via apoptosis, neuroinflammation, ROS-generating enzyme system, and lipid peroxidation. This destroying effect could be attenuated by blocking the TGF-β1 signaling pathway. DC inactivates TGF-β1 and protects the brain tissue and neuronal cells after TBI. Further studies are necessary to determine the optimal dosage and clinical potential of DC.

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