The Protective Effect of Omeprazole Against Traumatic Brain Injury: An Experimental Study

Rafet Özay[1], Mehmet Erhan Türkoğlu[1], Bora Gürer[2], Habibullah Dolgun[1], Oya Evirgen[3], Berrin İmge Ergüder[4], Nazlı Hayırlı[3], Levent Gürses[1], Zeki Şekerci[1]

■ BACKGROUND: **The development of secondary brain injury via oxidative stress after traumatic brain injury (TBI) is a well-known entity. Consequently, the aim of the present study was to evaluate the role of omeprazole (OM) on rat model of TBI.**

■ METHODS: **A total of 24 male rats were used and divided into 4 groups as follows; control, trauma, OM, and methylprednisolone (MP). The trauma, OM, and MP groups were subjected to closed-head contusive weight-drop injuries. Rats received treatment with saline, OM, or MP, respectively. All the animals were sacrificed at 24 hours after trauma and brain tissues were extracted. The oxidant/antioxidant parameters (malondialdehyde, glutathione peroxidase, superoxide dismutase, nitric oxide) and caspase-3 in the cerebral tissue were analyzed, and histomorphologic evaluation of the cerebral tissue was performed.**

■ RESULTS: **Levels of MDA and activity of caspase-3 were significantly reduced in the OM and MP groups compared with the trauma group. Glutathione peroxidase and superoxide dismutase levels were increased both in the OM and MP groups compared with the trauma group. The pathology scores were statistically lower in the OM and MP groups than the trauma group.**

■ CONCLUSIONS: **The results of the present study showed that OM was as effective as MP in protecting brain from oxidative stress, and apoptosis in the early phase of TBI.**

INTRODUCTION

Traumatic brain injury (TBI) has become a global health problem, affecting more than 10 million people annually, and is most commonly caused by traffic accidents, falls from heights, and work accidents. According to the World Health Organization, TBI will become the third most common cause of mortality and disability by 2020.[1,2] The pathogenesis of TBI comprises primary and secondary injuries. The primary impact, caused by a mechanical trauma, results in disruption of vessels, neurons, and their axons. The secondary phase develops in cascades of oxidative stress, excitotoxicity, mitochondrial damage, inflammation, blood–brain barrier (BBB) damage, and cerebral edema.[3,4]

It is almost impossible to treat the damage resulting from the primary injury; therefore, most studies have focused on secondary brain injury.[5-7] Secondary injury is mostly the result of oxidative stress that itself is caused by aberrant accumulation of reactive oxygen species (ROS).[3,6] ROS accumulate within a few minutes of injury and further cause neuronal damage and death.[8] Oxidative stress also induces apoptosis through increased p53,

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

Abbreviations and Acronyms
AhR: Aryl hydrocarbon receptor
BBB: Blood–brain barrier
GPx: Glutathione peroxidase
H^+/K^+-ATPase: Hydrogen-potassium ATPase
IP: Intraperitoneal
MDA: Malondialdehyde
MP: Methylprednisolone
NO: Nitric oxide
OM: omeprazole
ROS: Reactive oxygen species
SOD: Superoxide dismutase
STAT3: Signal transducer and activator of transcription protein 3
TBA: Thiobarbituric acid
TBI: Traumatic brain injury

From the [1]Ministry of Health Diskapi Yildirim Beyazit Training and Research Hospital, Neurosurgery Clinic, Ankara; [2]Ministry of Health Fatih Sultan Mehmet Training and Research Hospital, Neurosurgery Clinic, Istanbul; [3]Ankara University Faculty of Medicine, Department of Histology and Embryology, Ankara; and [4]Ankara University Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

To whom correspondence should be addressed: Rafet Özay, M.D. [E-mail: rftozay@hotmail.com]

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cytochrome-c release, and caspase-3 activation.[9,10] However, the endogenous defense system consisting of free radical scavenger systems, enzymes, nonenzymatic antioxidants, and heme-binding proteins protect brain tissues from oxidative injury.[9,11] After TBI, the balance between the oxidative stress and the endogenous antioxidant mechanisms is disturbed.[11,12] The brain, with its high metabolic demands, is affected quickly by the disturbed cellular respiration and the resultant free radical injury.[5,12] Inflammation, excitotoxicity, and brain edema due to oxidative stress all lead to increased intracranial pressure and secondary brain injury, which is associated with increased morbidity and mortality if not promptly and effectively treated.[1,5,6]

The antiulcer activity of benzimidazole-derivative drugs usually is believed to be via acid suppression after an irreversible interaction with hydrogen-potassium ATPase (H^+/K^+-ATPase), which is the terminal proton pump of the parietal cell.[13-15] Omeprazole (OM) is a member of benzimidazole family and has been shown to block H^+/K^+-ATPase in the first 4 hours after administration.[8,15] Despite its inhibitory effects on gastric acid secretion, less is known about diverse effects of OM other than its proton pump inhibitory actions, such as carbonic anhydrase inhibitory, anti-inflammatory, antioxidant, and apoptotic cell death control activities.[13-15] In vitro investigations demonstrated that OM also inhibits release of tumor necrosis factor-alpha and interleukin-6 from the human microglial cell culture and human monocytes culture, which contributes to the neuroprotective effect of OM towards the microglial, astrocyte, and monocytic toxicity.[16] In addition, it was proven previously that OM reduces interferon-γ–induced neurotoxicity through the inhibition of the signal transducer and activator of transcription protein 3 (STAT3) signaling pathway.[16] Tumor necrosis factor-alpha, interleukin-6, interferon-γ, and STAT3 signaling pathways are known as mediators with a critical role in the development of inflammatory, oxidative stress, and apoptosis processes.[4,6,9,12]

OM is one of the most prescribed drugs worldwide, and its safety and efficacy have been well established. However, it has never been investigated previously for TBI. The aim of this study is to investigate whether OM treatment would reduce the post-TBI–related oxidative stress, neuroinflammation, and apoptosis in rats after experimental TBI.

MATERIALS AND METHODS

Experimental Groups

Animal care and all experiments were carried out according to the Council of European Communities, Directive dated November 24, 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.

The groups of rats were as follows:

- 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 in the section "Anesthesia and Trauma Procedure." After craniectomy, brain samples were removed 24 hours after injury.
- Group 3: OM ($n = 6$); similar to group 2, but the rats received a single intraperitoneal (IP) dose of 20 mg/kg OM (Loseprol, Pharmada, Turkey) immediately after TBI.
- Group 4: methylprednisolone (MP) ($n = 6$); similar to group 2, but rats received a single IP dose of 30 mg/kg MP (Prednol, Mustafa Nevzat, Turkey) immediately after TBI.

The dosages of OM and MP used in this study were obtained from previous studies.[17,18]

Anesthesia and Trauma Procedure

All rats were kept under environmentally controlled conditions at 22–25°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, 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 to maintain the body temperature at 37°C. A moderate brain injury model, described by Marmarou et al.,[19] and modified by Ucar et al.,[20] 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 in diameter and 3 mm in thickness was fixed to the cranium with the use of bone wax between the 2 sutures in the midline. Trauma was applied at the point in which 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 was sutured before termination of anesthesia. All the animals were anesthetized with the aforementioned agents at 24 hours after trauma. Before the rats were euthanized, 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* for 20 minutes. No protease inhibitor was used for tissue homogenization. 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 via 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 heteropoly-molybdenum blue by the

copper-catalyzed oxidation of aromatic amino acids. Absorbance measurements were made at 700 nm with a spectrophotometer. The protein concentration of the sample was determined with a protein calibrator.[18,21]

Tissue Malondialdehyde (MDA) Analysis

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.[22] Tissue MDA levels were determined by a method based on the reaction with thiobarbituric acid (TBA). In summary, the samples were mixed with 2 volumes of cold saline solution containing 0.001% butylated hydroxytoluene (200 µL of 0.01% butylated hydroxytoluene solution in methanol) and 0.07% sodium dodecyl sulfate (20 µL of 7% sodium dodecyl sulfate). Then, 1 mL of samples was added to 500 µL of 0.01 N H_2SO_4 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 minutes. After cooling, an equal volume (2 mL) of n-butanol was added to each test tube and mixed. The mixture was centrifuged at 4000*g* for 10 minutes at room temperature. The absorbance of the organic layer in 1 mL of cell was read at 535 nm (Molecular Devices Corporation, Sunnyvale, California, USA). MDA concentrations were expressed as nmoles per milligram tissue wet weight.

Tissue Glutathione Peroxidase (GPx) Analysis

GPx activity was measured by following changes in nicotinamide adenine dinucleotide phosphate absorbance at 340 nm.[23] Extinction coefficients of nicotinamide adenine dinucleotide phosphate were used for GPx in the activity calculations (IU, international unit). 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.[24] 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.[25] 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 nitroblue tetrazolium reduction rate. SOD activity was expressed as U/mg-protein.

Tissue Nitric Oxide (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.[26] 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.

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 min at 30°C. The results were expressed as U/mg protein.[18,21]

Histologic Examination and Analysis

The brain tissue samples were removed immediately and post fixed in 10% neutral-buffered formalin, dehydrated through a graded series of ethanol, and embedded in paraffin for histologic examination. Paraffin blocks cut into 5-µm thick sections, stained with hematoxylin–eosin, and were examined by light microscopy (Leica DM3000; Leica Microsystems Inc., Buffalo Grove, New York, USA). Histopathologic changes of the acute-phase period (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.[18,21,27]

For observations of semi-thin 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. Semi-thin (0.7-µm thick) sections of brain tissue were stained with %1 toluidine blue and examined with the aid of light microscope (Leica DM3000). The semi-thin 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 with a light microscope (Leica DM3000) with an LCD camera and transferred to a screen (Acer Inc., New Taipei City, Taiwan). The counting area consisted of 15 fields of about 100 × 100 µm² 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. Then, damaged neurons were counted. Non-neuronal 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.

Statistical Analysis

Data analysis was performed with SPSS for Windows, version 11.5 (SPSS Inc., Chicago, Illinois, United States). Whether the distribution of continuous variables was normal or not was determined by use of the Shapiro-Wilk test. The Levene test was used for the evaluation of homogeneity of variances. Data are shown as mean ± SD, median (interquartile range), or median (min–max), where applicable. The mean differences between groups were analyzed with one-way analysis of variance, and the Kruskal-Wallis test was applied for comparisons of the median values. When the *P* value from one-way analysis of variance or Kruskal-Wallis test statistics were statistically significant, post-hoc Tukey HSD or Conover's

non-parametric multiple comparison test were used to determine which group differed from which others. A P value less than 0.05 was considered statistically significant.

RESULTS

Tissue MDA Analysis

Mean tissue MDA levels were significantly different ($P < 0.001$) between the control and trauma groups. We therefore concluded that tissue MDA levels increased due to the lipid peroxidation increase after TBI. We also found a statistically significant difference between the OM and MP groups and the trauma group ($P = 0.003$ for both) (Figure 1A).

Tissue GPx Analysis

The mean tissue GPx levels were significantly different between the control and the trauma groups ($P < 0.001$), indicating decreased tissue GPx levels after TBI due to increased oxidative stress. There was a statistically significant difference between the trauma group and the OM and MP groups ($P < 0.001$ for both) (Figure 1B).

Tissue SOD Analysis

SOD levels also were decreased significantly 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 OM and the MP groups ($P < 0.001$ for both) (Figure 1C).

Tissue NO Analysis

NO levels were statistically significantly greater in the trauma group than the control group ($P < 0.001$). MP treatment showed statistically significant reduction in the NO levels ($P < 0.001$). NO levels were lower in the OM group than the trauma group but without statistical significance ($P > 0.05$) (Figure 1D).

Tissue Caspase-3 Analysis

The mean caspase-3 activity was 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 OM and MP groups ($P < 0.001$ for both) (Figure 1E). Biochemical analyses of the study are summarized in Table 1.

Histologic Results

Light microscopic examination of the cerebral cortex prepared from the control group showed 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 were observed, indicating the damage. 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 areas of spongiosis with degenerative changes (Figure 2B, Figure 3A–D). In the OM group, degenerative neuropil changes and spongiosis were observed less frequently (Figure 2C, Figure 3B–E). In the MP group, the morphological 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 OM and MP groups (Figure 2C–D). The trauma group had statistically greater pathology scores than the OM and the MP groups ($P < 0.001$ for both). The percentage of degenerated neurons in the trauma group (28.8 ± 6.5) was significantly greater than the MP group (12.2 ± 3.8) ($P < 0.001$) and also greater than the OM group (21.3 ± 6.7) but without a statistically significant difference ($P > 0.05$). Our results histopathologically demonstrated that both OM and MP protected the brain from TBI (Figures 2 and 3). Histopathologic parameters and pathology scores are summarized in Table 2.

DISCUSSION

The results of the present study have shown that OM decreased elevated caspase-3 activity and MDA levels while increasing the activity of antioxidant enzymes such as GPx and SOD in rat brain in the early phase of post-TBI. OM demonstrates neuroprotective effect by restoring the endogenous system, inhibiting the increased level of inflammatory cytokines, reducing lipid peroxidation, and inhibiting apoptosis.[5,6,18] To the best of our knowledge, this is the first study to describe the neuroprotective effect of OM against TBI in rats in the English-language literature. Although its optimal dosing and timing in the setting of TBI with various injury severities remains elusive, its wider therapeutic window makes it a good candidate for dose-escalation studies.

Neuronal/astrocytic mitochondrial dysfunction causes ROS accumulation and has a major role in neuroinflammation and TBI pathophysiology.[6,11] The brain, with its high oxygen consumption rate, large amounts of polyunsaturated fatty acids, and transition metals such as iron, which is involved in free radical production catalysis, and its own high level of reactive radical formation is especially sensitive to the oxidative injury.[5,11] There are many endogenous antioxidants that try to prevent oxidative damage under physiological conditions, including SOD, GPx, and catalase.[28] These endogenous antioxidant mechanisms become overwhelmed with TBI, leaving ROS to damage the cell membrane through lipid peroxidation, protein and DNA oxidation, and mitochondrial electron-transport chain inhibition.[19,28] Molecular damage in conditions with increased oxidative stress also decreases antioxidant enzyme activity.[29] The present study revealed that after moderate TBI, SOD and GPx levels decreased significantly, possibly as the result of increased oxidative stress. OM leads to a significant increase in these antioxidant enzymes, and this could be an indicator of the antioxidant activity of these drugs.

Microglia are immunocompetent cells, and their activation leads to the secretion of various chemokines, cytokines, and neurotransmitter receptors such as glutamate.[30] Glutamate is the main central nervous system excitatory amino acid.[31] TBI activates a neuroinflammatory cascade that leads to excitoneurotoxic lesions in the brain, with activated microglia playing a major role.[32] Prolonged release and binding of these substances to neural receptors activates the N-methyl D-aspartate receptors, which causes an increase in the intracellular calcium levels. Excessive glutamate release leads to excessive Ca^{2+} influx play

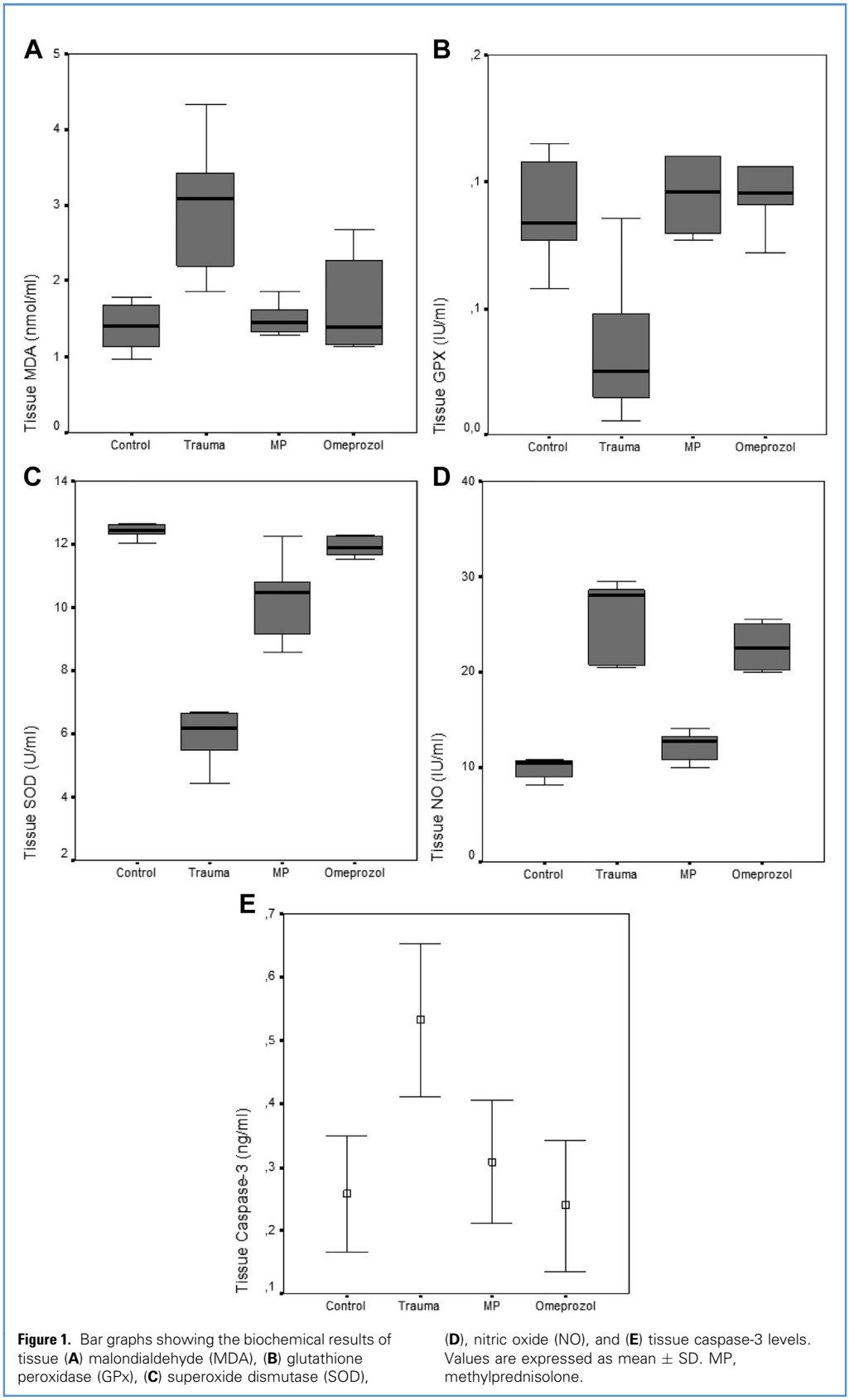

Figure 1. Bar graphs showing the biochemical results of tissue (**A**) malondialdehyde (MDA), (**B**) glutathione peroxidase (GPx), (**C**) superoxide dismutase (SOD), (**D**), nitric oxide (NO), and (**E**) tissue caspase-3 levels. Values are expressed as mean ± SD. MP, methylprednisolone.

Table 1. Biochemical Measurements

Variables	Control	Trauma	Methylprednisolone	Omeprazole	*P* Value
Tissue MDA, nmol/mL	1.41 ± 0.62*	3.08 ± 1.26*†‡	1.45 ± 0.30†	1.39 ± 1.31‡	**0.003**
Tissue GPx, IU/mL	0.08 ± 0.03*	0.03 ± 0.04*†‡	0.10 ± 0.03†	0.09 ± 0.02‡	**0.002**
Tissue SOD, U/mL	12.41 ± 0.31*§‖	6.16 ± 1.39*†‡	10.47 ± 1.94†§¶	11.89 ± 0.61‡‖¶	**<0.001**
Tissue NO, IU/mL	10.37 ± 1.81*§‖	28.0 ± 8.06*†	12.75 ± 2.56†§¶	22.50 ± 5.12‖¶	**<0.001**
Tissue caspase-3, ng/mL	0.26 ± 0.09*	0.53 ± 0.12*†‡	0.31 ± 0.10†	0.24 ± 0.10‡	**<0.001**

Bold values indicate statistically significant.
MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase; NO, nitric oxide.
*Control versus trauma ($P < 0.001$).
†Trauma versus methylprednisolone ($P < 0.01$).
‡Trauma versus omeprazole ($P < 0.01$).
§Control versus methylprednisolone ($P < 0.05$).
‖Control versus omeprazole ($P < 0.01$).
¶Methylprednisolone versus omeprazole ($P < 0.05$).

important roles in the development of the post-TBI excito-neurotoxicity. In addition to inflammatory mediators, an increasing level of extracellular H^+ ions at the site of injury lowers the pH of the extracellular fluid. The acidic pH and hydrogen ions created by various metabolic processes activates acid-sensitive ion channels and have a negative influence on cell survival and metabolism.[7,33]

Cerebral perfusion of traumatized brain tissue was impaired when metabolic imbalance was not effectively treated.[4-6] Consequently, one result of cerebral ischemia is acidosis, and the factors

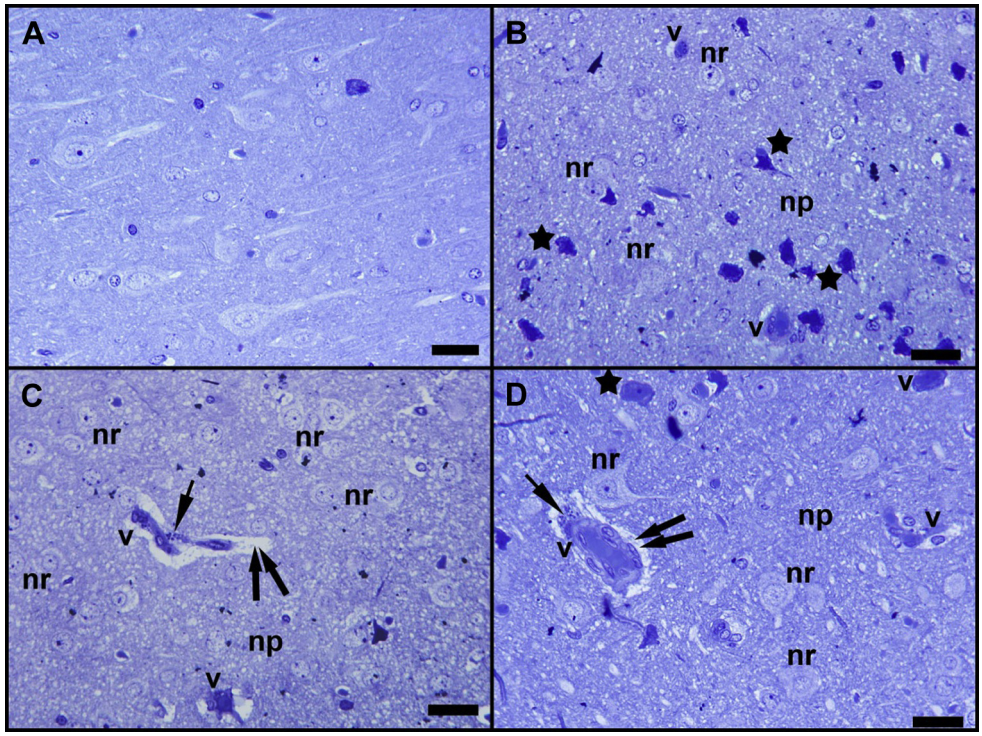

Figure 2. Histologic alterations in control and brain trauma groups after 24 hours. (**A**) Control: normal-appearing brain parenchyma; (**B**) trauma (saline solution)-damaged contracted neurons with pycnotic nucleus and darkly stained cytoplasm (*stars*) and normal-looking neurons (nr) in neuropil (np); (**C**) Omeprazole: perivascular edema (*double arrow*); (**D**) methylprednisolone showing less perivascular (v) edema; (**C**–**D**) Mast cells with metachromatic granules were seen close to the blood vessels (*arrow*). (**A**–**D**), ×40 objective; Toluidine blue staining; Bars represent 40 μm.

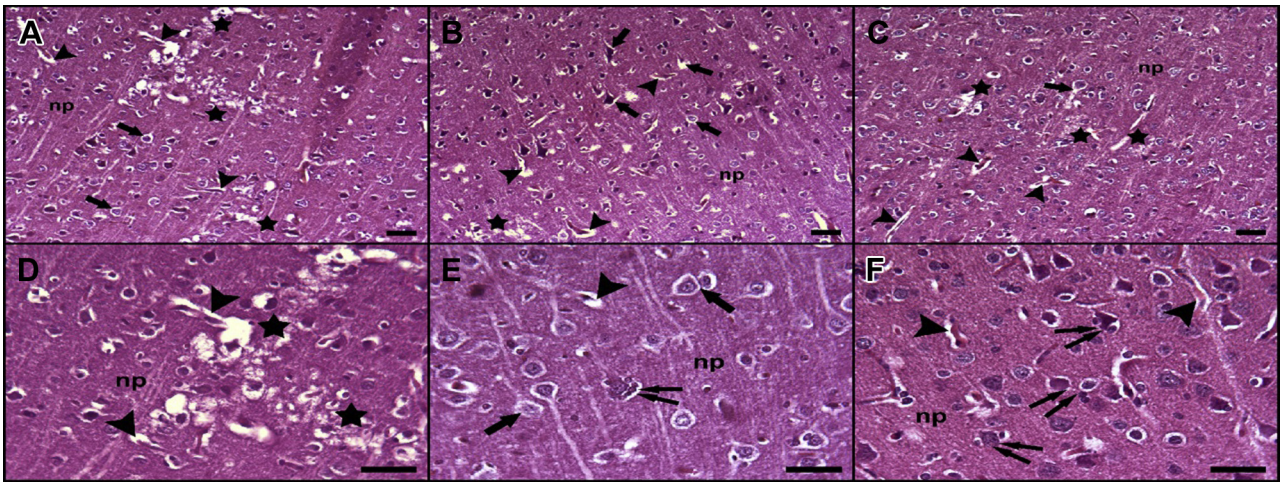

Figure 3. Histologic alterations in brain trauma groups after 24 hours. (**A**, **D**) Trauma (saline solution) Perivascular edema (*arrowheads*), perineural edema (*arrow*), marked focal neuropil (np) spongiosis with degenerative changes (*stars*); (**B**, **E**) Omeprazole and (**C**, **F**) methylprednisolone: damaged neurons surrounded by oligodendrocytes and satellitosis (*double arrow*), showing less perivascular and perineural edema and microvacuolar areas; (**A**–**C**) ×20 objective; (**D**–**F**), ×40 objective; (**H**–**E**), staining; Bars represent 40 μm.

influencing its severity include the blood flow reduction degree and the blood glucose level.[30] Ischemic injury becomes more severe with a pH less than 6.4, which constitutes severe acidosis.[33] Neuronal death ensues, with the mediation of glutamate and H_2O_2 exposure, and develops through several mechanisms, such as acid-sensing calcium channels and ferrous iron release.[33,34] Glutamate transporters also increase the intracellular H^+ and extracellular OH^-, causing pH changes.[35] A pH of 7.0-6.5 constitutes milder acidosis and will cause less severe ischemic injury and neuron death mediated by glutamate.[7,30] Previous studies have reported that sites of brain damage have high concentrations of extracellular K^+, leading to increased superoxide production mediated by Ca^{2+}.[35,36] Extracellular K^+ and K^+ binding protein are needed for the activity of H^+/K^+-ATPase, an enzyme found in many tissues, both epithelial and non-epithelial (including microglia), with a mediating role for cellular active K^+ uptake together with H^+ efflux, resulting in intracellular pH and K^+ regulation.[16,37-39] Previously, it had been shown that OM can penetrate the BBB.[39] OM also has been reported to irreversibly block microglia H^+/K^+-ATPase, similar to its effect on the gastric mucosa.[7] OM significantly may decrease intracellular acidification of microglia by H^+/K^+-ATPase inhibition after TBI and possesses neuroprotective activity.

Polyunsaturated fatty acid side chains are very sensitive to free radicals and are found in large amounts in the brain cell membrane, causing lipid peroxidation products to increase quickly

Table 2. Histopathologic Parameters

Variables	Control	Trauma	MP	Omeprazole	*P* Value
Perineural edema	0 (0–1)*†‡	3 (3–3)*§‖	1.5 (1–2)†§¶	2 (2–2)‡‖¶	<0.001
Perivascular edema	1 (1–2)*†‡	3 (3–3)*§‖	2 (1–3)†§	2 (2–3)‡‖	<0.001
Satellitosis	0 (0–0)*†‡	3 (2–3)*§‖	1 (1–2)†§¶	2 (2–2)‡‖¶	<0.001
Spongiosis	0.5 (0–1)*†‡	3 (3–3)*§‖	2 (1–3)†§	2 (1–3)‡‖	<0.001
Hemorrhage	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	1.000
Pathology score	2 (1–3)*†‡	12 (11–12)*§‖	6.5 (6–9)†§¶	8 (7–10)‡‖¶	<0.001
Degenerated Neurons	6.3 ± 2.7*‡	28.8 ± 6.5*§	12.2 ± 3.8§¶	21.3 ± 6.7‡¶	<0.001

*Control versus trauma ($P < 0.001$).
†Control versus methylprednisolone ($P < 0.001$).
‡Control versus omeprazole ($P < 0.001$).
§Trauma versus methylprednisolone ($P < 0.001$).
‖Trauma versus omeprazole ($P < 0.001$).
¶Methylprednisolone versus omeprazole ($P < 0.01$).

after injury.[40] One of the main causes of the secondary injury is the lipid peroxidation via oxidative injury.[41,42] MDA can be used as an index of lipid peroxidation, as it develops after the breakdown of myelin, glial, and neural membranes.[5,42] Chancal et al.[43] demonstrated that OM reduced the oxidative stress by reduction of MDA and restoration of the depleted GPx, catalase, and SOD. We found much greater MDA levels after TBI in the trauma group than the control group; treatment with OM decreased the levels of MDA in a significant manner. In addition, OH^- is one of the main factors in oxidative damage. OM decreases the chemotaxis of neutrophils, contributes to the reduction of cytokines, and reduces the oxidative stress through scavenging OH^- radicals, which prevents oxidative damage due to the lipid peroxidation after TBI.[8,42] Thus, the antioxidative effect of OM may be considered as one of the mechanisms of its neuroprotective effect.

One theory about the increased neuronal NO with trauma is NOS expression stimulation by cytokines and the resultant NO-synthase family-mediated NO production from L-arginine.[44,45] It has been demonstrated previously that total brain NO concentrations increased immediately, in the first 30 minutes, in the rat TBI animal model and then decreased.[12,46] This decrease was possibly due to substrate depletion and ended in a relative NO deficiency period for about 6 hours. This was then followed by an NO increase in the late stage, which sometimes lasted for days.[44,45,47] NO is a well-known mediator of BBB damage and cerebral edema.[44,46] However, controversially it has been suggested that the effect of exogenous NOS inhibitor and/or L-arginine administration reduces contusion volumes and improves cerebral blood flow in traumatic brain tissue.[47-49] There was a statistically significant increase within the first 24 hours after TBI in the total NO levels in our trauma group compared with the control group. Treatment with MP decreased total NO levels significantly, but OM did not have such an effect. OM may not have a significant effect on NO levels in early TBI, or the number of animals might be small to show a significant difference.

Moderate cerebral contusion injury after TBI in rats causes extensive neuronal apoptosis and the peak is approximately 24 hours' postinjury.[21,50] Interleukin-converting enzyme caspase-3 has been used as a trusted marker for apoptotic cell death.[21,22,50,51] Pretreatment with OM has been shown previously to perfectly block stress-induced DNA fragmentation related to apoptosis. This indicates an antiapoptotic role that can prevent the cell death observed with gastric ulcers.[8,52] It also has been shown that the OH^- generated from H_2O_2 can cause oxidative damage to the DNA through the Haber-Weiss reaction.[53] OM is also believed to block oxidative DNA damage induced by OH^- due to scavenging OH^-.[8] The present study demonstrated that the trauma group had much greater tissue caspase-3 activity, indicating increased apoptosis, than the control group after TBI. OM lowered caspase-3 activity and proved its neuroprotective effect against monocytic and microglial damage.

The aryl hydrocarbon receptor (AhR) is expressed in various regions of the brain, and there are several reports demonstrating that the AhR mediates various biological processes such as cell differentiation and apoptosis in addition to inflammatory processes in many different cells.[54-56] Substantially, it plays a major role in the modulation of inflammation, and its activation is important in inhibiting the inflammatory response.[57] OM activates AhR and therefore protects against oxidative injury in association with decreased ROS generation and chemoattractant protein expression.[58,59] Due to biochemical and histopathologic results of this study, OM may have potential neuroprotective effects through the induction of enzymes via AhR, which will be resulted in detoxification of the ROS-generated lipid peroxides and hydroperoxides. OM could therefore potentially be used as a therapeutic agent to prevent TBI-dependent secondary brain injury.

The histopathologic assessment criteria used for each rat included perineural and perivascular edema, spongiosis and satellitosis, and hemorrhage. The brain morphology was normal in the control group, whereas there was marked perineural and perivascular edema, satellitosis, spongiosis, and hemorrhage in the trauma group. We observed mast cells containing metachromatic granules near the blood vessels in the OM and MP groups, but there were less perivascular and perineural edema, degenerative changes in the neuropil, and spongiosis than the trauma group. The trauma group had significantly greater pathology scores than the OM and MP groups. However, the percentage of damaged neurons in the trauma group was greater than the OM group but without a statistically significant difference. This might be due to relatively smaller number of animals in each group. It also has been reported that surgical manipulations during tissue resection and inadequate tissue fixation can create neurons with a darkly basophilic cytoplasm and shrunken perikarya as artifacts.[27] It can be difficult to distinguish such handling artifacts from actual neuronal injury, as perfect fixation is hard to ensure with brain tissue. The handling-artifacts during the tissue fixation period might be effected the statistical results of the part of degenerated neurons which were not significant between the OM and trauma groups.

Pharmacokinetic properties of OM have similarities between rats and humans. OM is metabolized to 2 major metabolites, 5-hydroxyomeprazole and OM sulfone.[13] Measurements with both metabolites have shown that plasma levels of OM increase rapidly after its administration either intraperitoneally or intravenously. Although its plasma elimination half-life is relatively shorter, its inhibitory action lasts up to approximately 48–72 hours.[13,60] In addition, it was demonstrated that OM can pass through the BBB rapidly.[13,16,39] For that reason, we estimated that the desired effects of OM might be obtained if it is used in the early phase of TBI. The most effective dose of OM was determined as the 20 mg/kg for gastric secretory studies in rats.[61] However, there are no sufficient data regarding its optimal dosing for in vivo studies of neuroprotective activity. Therefore, rat gastric secretory studies were accepted as a reference for the dose of OM in our study, which also parallels to dosing in human gastric ulcer studies.[13,61] In studies related to TBI, oxidative and nitrosative damage was immediately induced after primary injuries, and apoptosis was reported to occur within the first 24 hours.[8,62,21,41,49,50] It has also been shown and emphasized that BBB deterioration in the TBI occurred within the first 24 hours, and antioxidant therapy should be initiated early in this

process.[5,11,18,45,63] Hence, we have limited study period to 24 hours postinjury to address early biochemical and histopathologic changes after TBI, considering previous studies. This study could pave the way for future studies to explore the effects of OM and other proton-pump inhibitors on intracellular acidosis, STAT3 pathway, AhR, scavenging of OH^-, and activated microglia in experimental TBI models and therefore potentially make promising contributions to the medical literature. In addition, it would also be interesting to investigate whether there is an additive effect of combination of OM with MP or other antioxidant agents.

CONCLUSIONS

Considering the neuroprotective efficiency of OM observed in this study, it is suggested that OM may be considered for further evaluation in preclinical and clinical studies against post-TBI. This destroying effect could be attenuated by the diverse effect of OM other than its proton-pump inhibitory actions, such as inhibition of carbonic anhydrase and STAT3 signaling pathway, activation of AhR, scavenging of OH^-, and anti-inflammatory, antioxidant, and apoptotic cell death control activities. Further studies will be necessary to determine the optimal dosage and clinical potential of OM.

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