The effects of lornoxicam on brain edema and blood brain barrier following diffuse traumatic brain injury in rats

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BACKGROUND
In this experiment, the effects of lornoxicam on brain edema and the blood brain barrier (BBB) following diffuse traumatic brain injury (TBI) were studied.

METHODS
Twenty adult male Wistar albino rats were anesthetized, and experimental closed head trauma was induced by the Marmarou method. After head injury, the rats were randomly divided into two groups: Group I was the control group, to which 2 ml saline was administered intraperitoneally, and Group II was the lornoxicam group, to which 2 ml 1.3 mg kg⁻¹ lornoxicam was administered intraperitoneally. Twenty-four hours after head trauma, 99 mTc pentetate (DTPA) was injected at a dose of 37 MBq, and posterior planar images of each rat were obtained using an Infinia gamma camera. After imaging of BBB permeability, brain tissues were dissected from the cranium. The brain water content (BWC) of each sample was calculated using the wet-dry method.

RESULTS
The lesion/background (L/b) ratio of Group I was 3.76±0.46 and 3.02±0.66 for early (5th min) and late (60th min) imaging, respectively. In Group II, the L/b ratios were 3.52±0.96 and 2.63±0.63 for early and late imaging, respectively (p>0.05). BWC was 79.6±2.5% and 77.5±1.1% for Groups I and II, respectively (p<0.05).

CONCLUSION
In this rat model of TBI, lornoxicam reduced brain edema but did not affect BBB permeability.

Key Words: Blood brain barrier; brain edema; lornoxicam; traumatic brain injury.

AMAÇ
Bu çalışmada diffüz travmatik beyin hasarı (TBH) sonrası, lornoksikamin kan beyin bariyeri (KBB) ve beyin ödemi üzerine etkileri araştırıldı.

GEREÇ VE YÖNTEM
Yırtık erişkin erkek Wistar albino sıçana anestez uygulaması sonrası Marmarou yöntemi ile deneySEL kapali kafa travması oluşturuldu. Kafa travması sonrası sıçanların rando-nom arz olarak iki gruba ayrıldı: Grup I intraperitoneal yolla 2 mL salin uygulanan kontrol grubu ve Grup II intraperitoneal yolla 2 mL 1.3 mg kg⁻¹ lornoksikam verilen lornoksikam grubu. Kafa travmasından 24 saat sonra 99 mTc pentetate (DTPA) 37 MBq doza verildi ve her bir sıçanın posterior planar görüntülen bir Infinia gama kamera kullanılarak elde edildi. KBB permeabilitesinin görüntülenmesi sonrası sıçan dokuları kranyumdan disiskede edildi. Tüm örneklerin beyn su içeriği (BSI) ıslak-kuru metodu ile hesaplandı.

BULGULAR
Grup I lesion/background (L/b) oranları erken dönem (5. dk) 3,76±0,46 ve geç dönem (60. dk) 3,02±0,66 idi. Grup II L/b oranları erken dönem 3,52±0,96, geç dönem 2,63±0,63 olarak saptandı (p>0,05). BSC Grup I’de %79,6±2,5 ve Grup II’de %77,5±1,1 idi (p<0,05).

SONUÇ
Bu TBH sıçan modellinde lornoksikaminın beyin ödemini azaltığı ancak KBB geçirişenliğini etkilemediği görülmüştür.

Anahtar Sözcükler: Kan beyin bariyeri; beyin ödemi; lornoksikam; travmatik beyin hasarı.
Head trauma frequently results in death or in critical conditions that lead to long-term disability and rehabilitative treatment. Brain injuries are found in approximately 85% of victims who die in traffic accidents.[1] The overall mortality rate due to severe head trauma is 35%, and this is generally due to cerebral edema and increased intracranial pressure.[2]

The primary tissue damage caused by the mechanical effects of head trauma cannot be treated,[3] but efforts are made to modify the processes and minimize the consequences of secondary brain injury that occurs within minutes, hours, or days after the trauma.[4-6] Posttraumatic brain edema is one of the pathophysiologic events occurring late as a secondary injury mechanism, and is thought to be generated in part by vasogenic edema due to blood brain barrier (BBB) breakdown and in part by cytotoxic edema.[7]

Increases in brain cyclooxygenase-2 (COX-2) are associated with the central inflammatory response and delayed neuronal death, both of which cause secondary insults after traumatic brain injury (TBI).[8,9] Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be neuroprotective in models of brain injury.[10,11] Lornoxicam is a potent analgesic and NSAID that inhibits COX-1/COX-2 in a balanced fashion; it is well tolerated and suitable for parenteral administration.

In this experimental study, the effects of lornoxicam on the BBB and development of brain edema in a rat model of diffuse TBI are elucidated.

**MATERIALS AND METHODS**

The research protocol was approved by our University’s Committee on the Humane Care of Laboratory Animals. Twenty adult male Wistar albino rats, each weighing 220-250 g, were maintained under controlled environmental conditions (temperature 22 °C, humidity 65%, and light-dark cycle 12 hour (h):12 h) for a minimum of 4 days. All rats were fasted for 18 h before the experiments but were allowed free access to water until 20-30 minutes (min) before the start of the experiment. All experiments were started between 10 a.m. - 11 a.m.

After anesthesia with thiopental (30 mg kg⁻¹ intraperitoneal [IP]), a median line scalp incision was made and the periosteum opened. A 10 mm diameter metal disc was glued to the cranium in the midline at the intersection of the coronal and lambdoid sutures. Diffuse closed head injury was induced by the method of Marmarou et al.[12] using a 450 g - 2 m weight-height impact onto the metal disc on the intact skull of the rats.

Twenty-four hours after head injury, the rats were randomly placed into one of two groups: Group I (n=10) served as the control group, to which 2 ml saline was administered IP, and Group II (n=10) was the lornoxicam group, to which 2 ml 1.3 mg kg⁻¹ lornoxicam was administered IP. Twenty-four hours after the head trauma, 99mTc pentetate (DTPA) was injected via a 24-gauge cannula in the femoral vein at a dose of 37 MBq, and posterior planar images of each rat in the supine position were obtained with an Infinia gamma camera (GE Healthcare, Tiram, Hacelmel, Israel) using a low-energy, high-resolution parallel-hole collimator with a 20% energy window centered at 140 keV. The field of view was a 256x256 matrix and had a scale of 5 zoom. Two images were obtained from each rat: one 5 min after isotope injection (early) and one 60 min after isotope injection (late). The ratios of proportional background were estimated using region-of-interest (ROI) analysis. ROIs were drawn to brain-to-background ratios (lesion/background [L/b]).

After imaging of BBB permeability, rats were sacrificed by cervical dislocation, and brain tissues were removed from the craniumatraumatically. Tissue samples were weighed separately and then dried to a constant weight at 105 °C for 24 h. The brain water content (BWC) of each sample was calculated using wet-dry method: BWC% = 100x (wet weight - dry weight)/wet weight.

Statistical analyses were performed using Mann-Whitney U-test for significant differences between two groups. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows® version 14.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± SD. P values less than 0.05 were considered to be statistically significant.

**RESULTS**

Regarding BBB analysis, L/b in Group I was 3.76±0.46 (early) and 3.02±0.66 (late) and in Group II was 3.52±0.96 (early) and 2.63±0.63 (late) (p>0.05). Differences between groups at each time point were not significant, but BBB permeability decreased between the early and late periods within groups (p<0.05, Table 1).

Regarding brain edema, BWC was 79.56±2.5% in Group I and 77.47±1.05% in Group II (p<0.05, Table 1). Early and late brain-to-background ratios are shown in Table 1. Examples of bio-distribution of Tc99m DTPA in dynamic and static (60th min) images are shown in Figure 1.

**DISCUSSION**

In this experimental model, we found a significant decrease in brain edema and non-significant decrease in BBB permeability when lornoxicam was adminis-
tered after TBI. The Marmarou mechanism for producing brain injury creates clinical features of diffuse axonal injury, biochemical characteristics of trauma, and brain edema 6-24 h after the trauma. While the Marmarou model may not create all of the pathophysiological changes that are observed in head trauma patients, it is a standard model for testing therapies (some even later tested in Phase III trials in humans) for preventing or treating secondary brain injury after TBI.

The BBB is a highly selective barrier that prevents the passage of many substances from the blood into the extracellular fluid of the brain, or into the brain cells, and vice versa. The increase in BWC after trauma is thought to be related to vasogenic edema due to disruption of the BBB. Although use of the spectrophotometric measurement of Evans blue in brain tissue for BBB analysis is very popular, its methodological problems in previous studies may have contributed to over-estimates of tracer levels in the brain. In our study, 99mTc pentetate, a non-diffusible tracer for the evaluation of BBB permeability, was used for assessing the integrity of the BBB. 99mTc pentetate penetrates the BBB only if the BBB has been disrupted. 99mTc-DTPA was also used in the past for brain scintigraphy to detect brain infarcts as well as brain metastases.

Following TBI, arachidonic acid leaks from cell membranes and is converted into prostaglandins by cyclooxygenase. In animal experiments, prostaglandins have been found to increase in brain tissue after trauma. COX-2 is a primary inflammatory mediator that converts arachidonic acid from damaged membranes into vasoactive prostaglandins, producing reactive oxygen species in the process. Following TBI, these free radicals damage neural membranes, white matter, and the tight junctions that form the BBB. Peroxidative reactions may also be implicated in the progressive vascular damage that affects autoregulation and leads to arteriolar spasm and thrombosis. Thus, products of COX-2 likely play a role in secondary responses that may result in increased intracranial pressure, vasospasm, and ischemia, resulting in worsened outcomes.

Although COX-2 induction following TBI may result in selective beneficial responses, chronic COX-2 production may contribute to free radical-mediated cellular damage, vascular dysfunction, and alterations in cellular metabolism. These may cause secondary in-

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<th>Table 1. Brain-to-background ratios and BWC of groups (mean±SD)</th>
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<tr>
<td>Group I (control) n=10</td>
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<tr>
<td>Brain-to-background activity ratios (L/bg)</td>
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<td>Early (5 min)</td>
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<td>Brain water content (BWC)</td>
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* p<0.05, when compared with between groups.

Fig. 1. (a) In vivo single-photon dynamic imaging of biodistribution of Tc99m DTPA. (b, c) The biodistribution and uptake of Tc99m DTPA were confirmed by in vivo static imaging (60th min).

(Color figure can be viewed in the online issue, which is available at www.tjtes.org).
juries to the brain that worsen behavioral outcome. If the initial phase of COX-2 expression is beneficial, then delayed pharmacological treatment with steroids or COX-2-specific inhibitors could result in better outcomes, and may lead to new clinical treatment paradigms.

Many studies have been performed in recent years using COX inhibitors to prevent cerebral damage after head trauma. Major targets of COX inhibition are cerebral vasculature, COX-2 release from neurons and neuroinflammatory response. Studies have found that all three targets can be modified with appropriate treatment. Indomethacin significantly reduces the incidence of post-ischemic BBB disruption in the early period but does not have a significant effect on post-ischemic brain edema. In vivo, rofecoxib has been found to prevent excitotoxic neuronal damage. Meloxicam, a COX-2 inhibitor, has been found to preserve BBB permeability, decrease anti-inflammatory activity, and decrease brain edema in a model of diffuse TBI.

The central nervous system inflammatory reaction occurring after aneurysmal subarachnoid hemorrhage and intracerebral hemorrhage involves the upregulation of numerous cytokines and prostaglandins. After intracerebral hemorrhage, COX inhibition with celecoxib, a selective COX-2 inhibitor, decreases brain edema, inflammation, and perihematomal cell death by decreasing generation of prostaglandin E2.

This is the first study in the literature to test the effects of lornoxicam on brain edema and BBB permeability in an animal model of TBI. In this model, lornoxicam reduced brain edema but did not have a significant effect on BBB permeability.

Conflict-of-interest issues regarding the authorship or article: None declared.

REFERENCES
