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Effects of a Tumor Necrosis Factor-Alpha Inhibitor (Etanercept) on the Sciatic Nerve in a Hypoxic Ischemia-Induced Neonatal Rat Model

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Neonatal hypoxic-ischemic (HI) injury has been considered to have acute and long term deleterious effects on many tissues, including the peripheral nerve.
Objectives. In this study, the effects of a tumor necrosis factor-alpha (TNF-α) inhibitor (etanercept) on peripheral nerve damage and the ultrastructure of the sciatic nerve and gastrocnemius muscle in rats exposed to HI during the neonatal period were examined.
Material and Methods. In this study, 45 seven-day-old rats were used and they were divided into three groups. The right carotid arteries of the rats in the saline and etanercept groups were ligated and put in a hypoxia chamber containing 8% oxygen for two hours. Just after hypoxia, the etanercept group was given 10 mg/kg etanercept, but the saline group had only saline intraperitoneally. The sham group rats' carotid arteries were not ligated or put in hypoxia. The amplitude, area and latency of sciatic nerve compound motor action potential (CMAP), which mainly reflects axonopathy and myelinopathy, were measured using standard techniques in the seventeenth week following the HI. Sciatic nerve and gastrocnemius muscle were evaluated with a transmission electron microscope, and grading for myelin sheath damage was done to all groups.
Results. Neuropathy was seen in rats after HI. While treatment with etanercept showed a protective effect for the axons of sciatic nerve, demyelination could not be recovered with etanercept.
Conclusions. This study is the first in literature to show a partial interruption of the signal through the peripheral nerve fibers caused by axonal and myelin dysfunction continuation in rats exposed to HI after birth, in the 17th week (**Adv Clin Exp Med 2014, 23, 5, 705–713**).

Key words: action potentials, etanercept, peripheral nerves, neonatal ischemia, neonatal hypoxia.

Perinatal and neonatal hypoxic-ischemic (HI) insult has acute and long term deleterious effects not only on the brain but on many other tissues, including peripheral nerves [1, 2]. Many researchers have also reported that hypoxia/ischemia injury plays an important role in the development of peripheral neuropathy [3, 4]. Morphologically, ischemic nerves reveal various pathological abnormalities, including demyelination and remyelination, axonal degeneration and regeneration, focal, multifocal, or diffuse loss of nerve fibers, and endoneurial edema [5]. These pathological abnormalities occur not only as a result of trauma and related events but also in compression injuries, entrapments, tourniquet-induced peripheral nerve injuries, acute or chronic hypoxemia [6] and neonatal HI insult [1]. Schmelzer et al. [7] indicated that reperfusion injury, conduction block, and blood-nerve barrier disruption develop after severe ischemia of the peripheral nerve. Neonatal hypoxia-

ischemia in rats results from both carotid artery ligation and inhalation of 8% oxygen. Right carotid ligation leads to ischemia in the right brain hemisphere, whereas inhalation of low oxygen can result in generalized hypoxia including the peripheral nerves. The effects of focal cerebral and/or generalized hypoxia on peripheral axons were aimed to be evaluated in this study. It has been supposed that neonatal HI may cause motor axonal damage in the peripheral nervous system. It has been thought that preventing or treating brain and peripheral nerve injury together may be more effective in decreasing the complications of neonatal HI. Despite major improvements in perinatal medicine, the incidence of cerebral palsy caused by intrapartum asphyxia has remained unchanged, since the management strategies were supportive and not aimed at stopping the ongoing injury [8, 9].

Proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) which are released after nerve injury contribute to injury-induced peripheral nerve pathology and to the development of neuropathic pain [10–13]. TNF-α inhibitors may be administered subsequent to nerve injury where they have been shown to attenuate behavior indicative of neuropathic pain [14, 15].

Although many reports have studied the morphological and biochemical outcomes [16–18], and the long-lasting behavioral changes [19, 20] occurring in rats after neonatal hypoxia-ischemia, little emphasis has been placed on assessing the long--lasting influence on peripheral nerves. Furthermore, there has been no study that investigates the effects of TNF-α inhibitor (etanercept) on peripheral neuronal damage in a neonatal rat model of HI. In this study, we evaluated whether HI had an influence on peripheral nerves in rats when they became 4 months old after having been exposed to HI on the 7th day after birth. Additionally, in this study, the effects of etanercept on peripheral nerve damage in rats that were exposed to HI on the 7th day after birth were examined in the 17th week.

Material and Methods

The Preparation of the Animals and Surgical Procedure

In this experimental study, 7-day-old Wistar male rat pups (n = 45) were used and they were spontaneously delivered. It is known that the most widely used model of neonatal asphyxial brain injury, the 7-day-old rat, in many ways has brain maturity equivalent to that of an early 3rd trimester human fetus [21]. At our institution, the Experimentation Ethics Committee on animal research in the Faculty of Medicine approved all the procedures. The procedures of the European Convention in order to protect Vertebrate Animals used in Experimental and other Scientific Studies have been followed.

The rats were randomLy allotted into one of the 3 experimental groups: a saline treated (saline) group, a TNF-α inhibitor treated (etanercept) group and a sham (sham) group, each containing 15 animals.

Isofluran inhalation was used for less than 5 min to anesthetize the rat pups in the saline and etanercept groups. In these groups, hypoxia-ischemia was induced according to the Levine–Rice model [22]. This model was used in our previous study [2]. A median incision was made in the neck. Under microscopic magnification, the right common carotid artery was dissected and ligated with a 6-zero silk suture [2]. After the wound was sutured, the animals were allowed to have a 1 h recovery and feeding period. Except for the sham group, the rats were then placed into a plastic chamber and exposed to a continuous flow of 8% oxygen – 92% nitrogen for 2 h. The carotid arteries of the rats in the sham group were located without ligation and then they were placed into an open chamber without any supplemental oxygen for the same intervals. The chambers were partially submerged in a water bath at 33 ± 1 °C to maintain a constant thermal environment. Immediately after hypoxia, while the etanercept group was administered intraperitoneally (i.p.) 10 mg/kg etanercept (Enbrel 25 mg flakon, Wyeth) which was dissolved in saline (0.5 mL), the saline group had only saline (0.5 mL i.p.). After the hypoxic period, the rats were survived in the room conditions till the 17th week [2].

Electrophysiological Recording

Electrophysiological recordings (compound motor action potential – CMAP) across the nerve segment were made using a BIOPAC MP 100 acquisition system (Santa Barbara, USA). Prior to the electrophysiological recordings, the rats were anesthetized with 80 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi Pharmaceutical Co.-Warner Lambert, Istanbul, Turkey) and 8 mg/kg xylazine (Rompun, Bayer Pharmaceutical Co, Istanbul, Turkey), administered intramuscularly. The hind limbs of the rats were placed in a standard position during the electrophysiological recordings, then the hind limbs were shaved. CMAPs were recorded in all 3 groups. Standardized nerve conduction study techniques were used to record CMAP of the gastrocnemius muscle [23]. Briefly, bipolar

stimulating electrodes (Medelec small bipolar nerve electrodes, 6894T, Oxford, UK) were placed around the sciatic nerve at the sciatic notch. The supramaximal stimulus consisted of a single square pulse (intensity 10 V, duration 0.5 ms). The sciatic nerve was stimulated from the most distal site of stimulation by bipolar electrode. CMAPs were recorded from the gastrocnemius muscle by surface disc electrodes (Medelec, number 017K006, Oxford, UK) which were always positioned on the distal 1/3 of the leg. The ground electrode was placed on the other thigh, to which the stimulation was not applied and so the CMAPs were not recorded. During the study, the body temperature of the rats was maintained at 37°C using a heating pad, and continuously monitored by rectal probe digital thermometer. BIOPAC Acknowledge Analysis Software (ACK 100 W) was used to determine amplitude, area, duration and distal motor latency (DML) (thus, conduction velocity) of CMAP. The amplitude of a given CMAP was defined as the height in millivolts from baseline to the peak of the negative phase. The DML (in ms) was determined as the interval of time between the onset of the stimulus and that of the response. The area was measured under the curve from the first negative deflection to the first baseline crossing and the duration was measured from the first negative deflection to the first baseline crossing.

After electrophysiological recording, all pups were euthanized by decapitation. The sciatic nerve and gastrocnemius muscle were removed in all groups of animals after the electrophysiological recording for ultrastructural evaluation.

Electron Microscopic Evaluations

The ultrastructures of the sciatic nerve and gastrocnemius muscle fibers were observed using a transmission electron microscope. For transmission, electron microscopic evaluation of these tissue samples were prefixed with 2.5% glutaraldehyde and then postfixed with 1% osmium tetroxide. They were dehydrated in a graded alcohol series, cleared with propylene oxide and embedded in epoxy resin (EMBed-812 Embedding Kit; Electron Microscopy Sciences). Sections were cut by a microtome (Leica UCT-125, Leica Microsystems GmbH, Vienna, Austria). Semi-thin sections of 0.5–1 μm thickness were stained with toluidine blue. After the semi-thin sections were examined, ultrathin sections were cut into 50–70 nm and these sections were contrasted with uranyl acetate and lead citrate. The sections were examined and photographed using an electron microscope (Jeol JEM1011, Tokyo, Japan). All micrographs of the sciatic nerve sections were taken at 3000 times magnification randomLy from the sciatic nerve for all three groups.

Table 1 Ultrastructural grading system of myelinated axons

Grade 0	Normal
Grade 1	Separation in myelin configuration
Grade 2	Interruption in myelin configuration
Grade 3	Honeycomb appearance
Grade 4	Collapsed myelin forming ovoids

Also in this study, myelin damage of the sciatic nerve was evaluated. The grading was done to 4 samples from each of the groups. These samples were randomLy chosen from each group used for grading. During this procedure, 50 myelinated axons from each sample (total 200 myelinated axons from each of the groups) were evaluated ultrastructurally at 3000 times magnification. For evaluating myelin damage of the nerve fibers, a myelin sheath grading was performed as described before by Kaptanoglu and coworkers [24] and summarized in Table 1. Grade 0 represents the normal morphology. Grade 1 consists of just separation in myelin configuration, while grade 4 indicates collapsed myelin forming ovoids in addition to all the pathologies explained in grades 1 through 3. The grading system is designed to grade the most severe histopathological findings and named according to the worst degree of damage seen at that view. The investigators who performed this grading were kept unaware of the experimental design.

Statistical Analysis

The data was processed and analyzed using the statistical package STATISTICA 6.0. Descriptive statistics (mean ± standard deviation) were calculated in each group for all the parameters of CMAP (amplitude, area, distal motor latency, total duration, duration of depolarization and duration of repolarization). Descriptive statistics of the CMAP variables are shown in Table 2. All variables (CMAP parameters and ultrastructural data), according to the Shapiro-Wilks test, showed a normal distribution. One-way analysis of variance (ANOVA) was used to test the mean differences between all the parameters of CMAP and the groups for ultrastructural data. Following these processes, a Tukey *post hoc* test was used to determine the significant differences between pair-wise groups. The results were accepted statistically significant at $p < 0.05$.

Table 2 Descriptive statistics (mean ± SD) for compound motor action potential parameters studied

Experiments	Variables					
	amplitude (mV)	area (mVms)	distal motor latency (ms)	total duration (ms)	duration of depolarization (ms)	duration of repolarization (ms)
Sham (sham) (n = 15)	5.46 ± 2.09	0.0051 ± 0.0025	3.39 ± 1.65	7.17 ± 2.07	1.87 ± 0.72	1.33 ± 0.58
Saline-treated (saline) (n = 14)	2.26 ± 1.54[a]	0.0028 ± 0.0025[a]	4.77 ± 1.20[a]	6.54 ± 2.25	1.25 ± 0.28[a]	1.94 ± 0.55[a]
Etanercept-treated (etanercept) (n = 14)	5.51 ± 2.16[b]	0.0037 ± 0.0021	5.46 ± 1.17[a]	6.12 ± 2.37	1.07 ± 0.22[a]	1.46 ± 0.84

[a] Significantly different from sham group: $p < 0.05$.
[b] Significantly different from saline group: $p < 0.05$.
n – the number of rats in each group.

Results

In the sham group, no rats died in the course of the study. However, 1 rat in the etanercept and 1 in the saline group died during hypoxia.

Electrophysiological Data

Typical records of CMAP in the sham group, and saline- and etanercept-treated HI groups are shown in Fig. 1. The means and standard deviations for amplitude, area, distal motor latency, depolarization and repolarization times of CMAP, and total duration of CMAP in all groups are summarized in Table 2. As seen in Table 2, an increase in repolarization duration and motor latency of CMAP, and a decrease in depolarization duration, area and amplitude of CMAP were seen in rats after HI ($p < 0.05$).

As seen in Table 2, there were no statistically significant differences between the sham and etanercept-treated groups regarding CMAP amplitude and area. Also, etanercept applied just after HI prevents the decrease in the amplitude ($p < 0.05$) but not area of CMAP in rats with HI, showing that CMAP amplitude was only protected by the treatment with etanercept. In fact, as shown in Table 2, the mean area of the etanercept group rats was higher than the saline group. However, this result was not statistically significant.

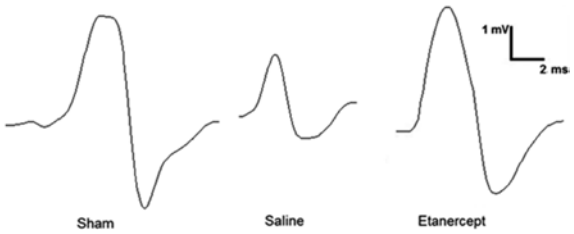

Fig. 1. Sample records of the compound motor action potential (CMAP) in sham (sham), saline-treated (saline) and TNF-α inhibitor-treated (etanercept) group. Calibrations for all traces are shown in the upper right; vertical bar = 1 mV; horizontal bar = 2 ms

In this study, motor dysfunction, defined by a significant increase in DML, was also recorded. In the saline group, the latency of CMAPs prolonged significantly from 3.39 ± 1.65 ms to 4.77 ± 1.20 ms, when compared to the sham group ($p = 0.031$). The etanercept treatment did not prevent these prolongations, and statistical differences were not found, when compared to the saline group (Table 2).

As seen in Table 2, in the saline group, the duration of depolarization of CMAPs decreased significantly from 1.87 ± 0.72 ms to 1.25 ± 0.28 ms, when compared to the sham group ($p = 0.006$). The etanercept treatment did not prevent these decreases, and statistical differences were not found compared to the sham group (Table 2).

Also, as seen in Table 2, in the saline group, the duration of repolarization of CMAPs prolonged significantly from 1.33 ± 0.58 ms to 1.94 ± 0.55 ms, when compared to the sham group ($p = 0.034$). The etanercept treatment prevented these prolongations, and no statistical differences were found when compared to the sham group. Also, as seen in Table 2, there were no statistically significant differences between all groups regarding CMAP total duration ($p > 0.05$).

The Electron Microscopic Findings

Qualitative Analysis

The axons, axonal myelin and Schwann cells of the sciatic nerve showed normal ultrastructural features in the sham group (Fig. 2). Degenerative changes were observed in the axonal myelin in the

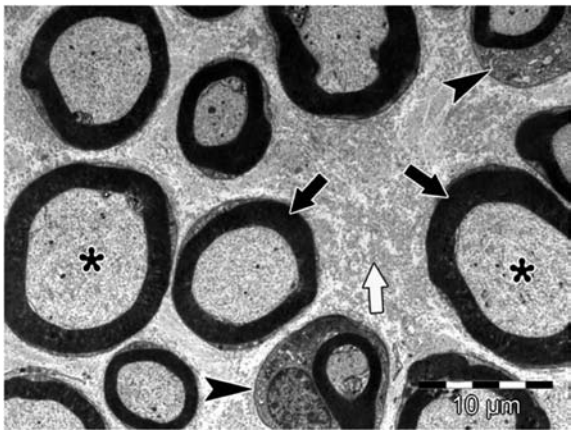

Fig. 2. Electron microscopic image of rat nerve fibers in the sham group. Nerve fibers are normal ultrastructurally. Myelin sheath (black arrow), axon (asterisk), Schwann cell (arrowhead), endoneurial connective tissue (white arrow) (×3000)

saline-treated group. There was also axonal shrinkage and endoneurial edema in the saline group (Fig. 3a–b). The grading 0–3 of damage showed that there was no significant difference when comparing the etanercept group to the saline group (Table 3 and Fig. 3c–d), but the amount of myelin damage increased in the etanercept group for grade 4.

As regards the gastrocnemius muscle, fibers have normal morphologic characteristics in all groups (Fig. 4a–c). The nuclei of the cells were regularly outlined and exhibited normal chromatin content. Myofibrils exhibited a regular sarcomere organization. Cytoplasmic organelles and capillary structures between the myofibrils showed a normal structure.

Quantitative Analysis

Table 3 indicates the results of the quantitative grading of damage found after 600 myelinated axons in 3 groups were examined. All of the

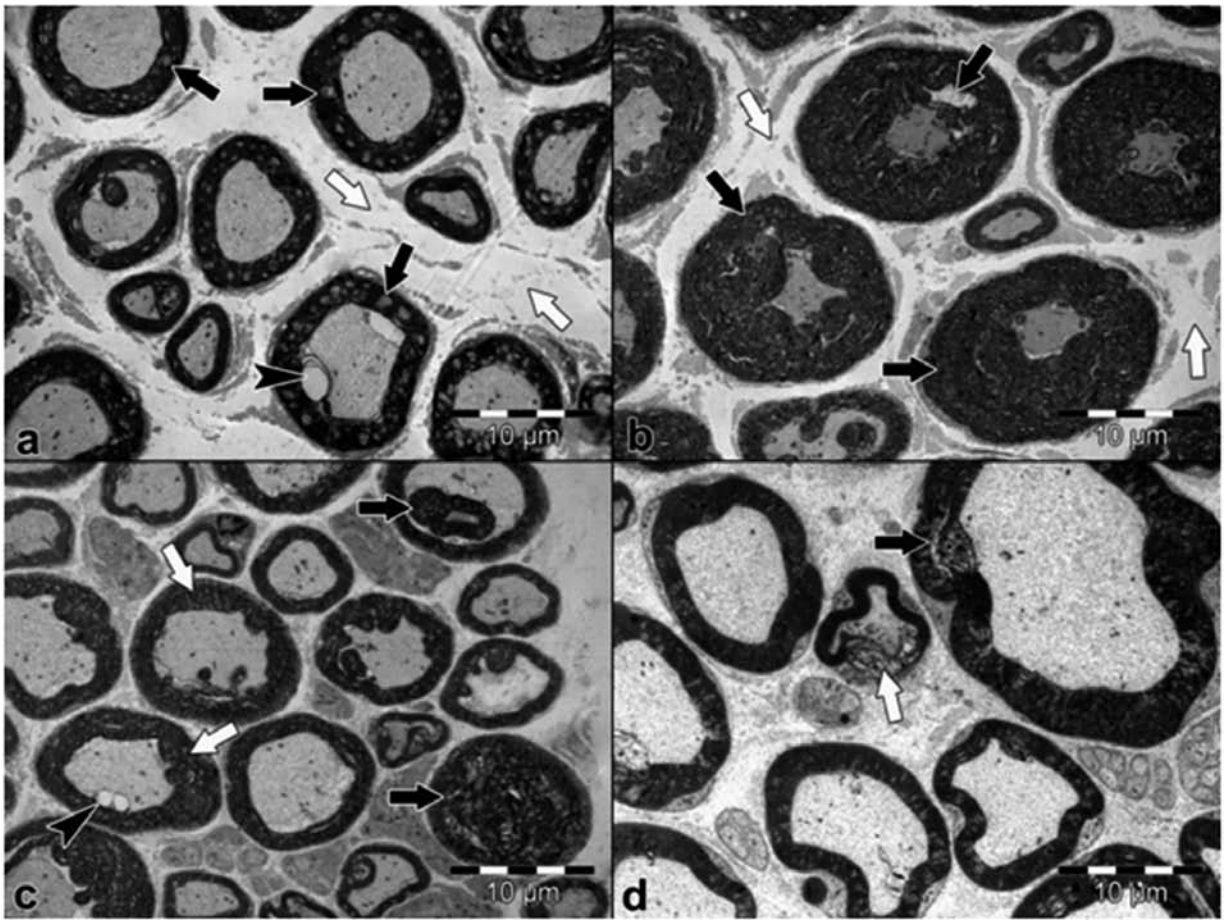

Fig. 3a–d: a. Electron microscopic image of rat nerve fibers in the saline group. The black arrow shows the separation of myelin representing grade 1. Axonal shrinkage (arrowhead), endoneurial edema (white arrow). b. Electron microscopic image of rat nerve fibers in the saline group. The black arrow shows collapsed myelin, forming ovoids, representing grade 4. White arrow shows endoneurial edema. c. Electron microscopic image of rat nerve fibers in the etanercept group. The white arrow shows the honeycomb appearance representing grade 3 and the black arrow shows collapsed myelin, forming ovoids representing grade 4. Axonal shrinkage (arrowhead). d. Electron microscopic image of rat nerve fibers in the etanercept group. The black arrow shows the separation of myelin representing grade 1 and the white arrow shows interruption in myelin configuration representing grade 2 (×3000)

Table 3. Ultrastructural grading scores for the experimental groups

Experimental Groups	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Sham	100.00 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Saline	40.5 ± 20.42[a]	26.0 ± 19.39[a]	7.0 ± 4.16[a]	13.5 ± 8.54[a]	13.0 ± 18.87[a]
Etanercept	17.5 ± 9.15[a]	17.0 ± 19.90[a]	5.0 ± 2.58[a]	8.0 ± 3.65[a]	52.5 ± 18.36[a,b]

a – clinical importance according to the sham group.
b – $p < 0.05$ compared to the saline group.

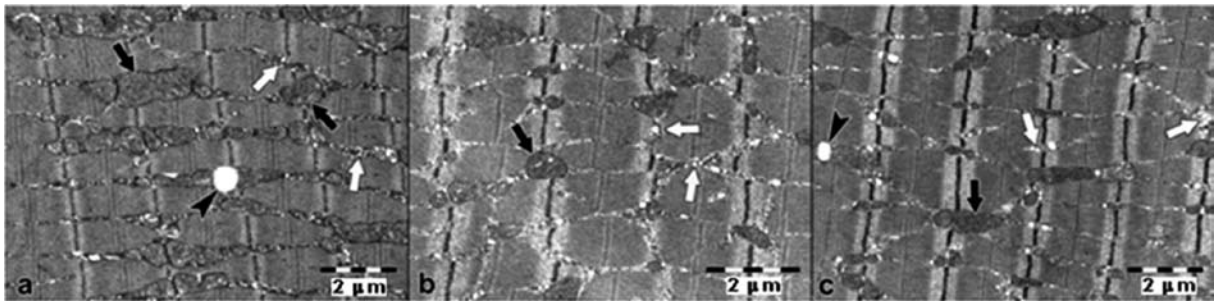

Fig. 4a–c: The gastrocnemius muscle fibers have normal morphological characteristics. a. The sham group (×12000), b. The saline group (×15000), c. The etanercept group (×12000). Mitochondria (black arrow), sarcoplasmic reticulum cisternaes (white arrow), lipid vacuoles (arrowhead)

myelinated axons were found to be normal (grade 0: 100.0 ± 0.00%), as expected, when the biopsies taken from the sham group were examined. Because the standard deviation of GD0-GD4 scores in the sham group were 0.0, a statistical comparison with the other groups can not be made and p-value can not be given. Therefore, we can say that there was clinical importance in the saline and the etanercept groups according to the sham group. As seen in Table 3, in the saline group, myelin injury developed and the myelin proportion showing normal morphology decreased, while the amount of myelin damage that has higher grade (i.e., worse) increased. No significant differences were found between the biopsies from the etanercept group and the saline group, but the amount of myelin damage showing higher grades increased.

Discussion

In this study, ligation of the right common carotid artery and exposure to hypoxia by a continuous flow of 8% oxygen – 92% nitrogen for 2 h resulted in electrophysiological and histological abnormalities in the peripheral nerve of the neonatal rats. This study demonstrates that HI decreased significantly in the amplitude and area of CMAP in the gastrocnemius muscle, suggesting a partial interruption of the signal through the peripheral nerve fibers, mainly reflecting axonal dysfunction and a significant increase in the DML of CMAP, mainly reflecting myelin sheath damage (motor dysfunction). However, etanercept, which was administered shortly after hypoxia, showed a preventive effect on the axonal dysfunction after HI. On the other hand, treatment with etanercept did not have a preventive effect on motor dysfunction after HI.

In addition to electrophysiological abnormalities in the peripheral nerve of the neonatal rats, degenerative changes in the electron microscopic evaluations were observed in the axonal myelins in the saline group. Also, there was axonal shrinkage and endoneurial edema in this group. No significant differences were found between the biopsies from the etanercept group and the saline group in the grading 0–3 of damage, but the amount of myelin damage that shows higher (i.e., worse) scores (grade 4) increased in the etanercept group (Table 3). Thus, it has been found that the electrophysiological results and detailed analysis made by using the quantitative scoring of the axonal myelin of sciatic nerve tissue apparently supported each other. On the other hand, in this study, no histological changes in the skeletal muscle cells were observed using electron microscopy in the saline group.

A reliable transmission in the central and peripheral nervous systems has saltatory conduction to travel down the myelinated axon. This conduction is defined as an action potential moving in discrete jumps down a myelinated axon. This uninterrupted process is outlined as the charge passively spreading to the next node of Ranvier to

depolarize it to threshold which will then trigger an action potential in this region which will then passively spread to the next node and so on. However, the need for a continuous energy supply to maintain appropriate ion concentrations to support uninterrupted action potential generation places central fibers in a potentially vulnerable state [25]. Ion gradients cannot be re-established for proper action potential generation when energy demand exceeds supply under injury conditions such as anoxia/ischemia or trauma. Ionic dysregulation within the injured axon occurs causing an influx in Na^+ and Ca^{2+} and an efflux of K^+ [26]. Thus, conduction of action potential fails. LoPachin and Lehning [27] reported an increase in axoplasmic Ca^{2+} in a wide range of experimental nerve injury models which causes acute or chronic axon degeneration. In our study, possible pathways/ /mechanisms to the alterations seen after HI can be the levels of axoplasmic Ca^{2+} increase in response to *in vitro* ischemia in myelinated axons. This situation needs to be addressed in future research.

Morphological and biochemical changes associated with HI brain insult have been extensively studied in the experimental model of perinatal asphyxia [20]. A few studies dealing with the long-term behavioral alterations after HI insult in neonatal rats have demonstrated obvious sensorimotor deficits, learning and memory impairment [19, 28]. However, peripheral neuronal changes in adulthood associated with neonatal HI insult have not been studied enough in the experimental model of perinatal asphyxia. Therefore, in our study, the late effects of hypoxia-ischemia on adult rats (17-weeks) were investigated taking into account the action potential parameters and ultrastructure of the sciatic nerve and gastrocnemius muscle.

In this study, the severity of myelin damage to the sciatic nerve was evaluated. The grading 0–3 of damage showed that there was no significant difference between the etanercept group and the saline group (Table 3). However, the amount of myelin damage increased in the etanercept group for grade 4. It is known that the soluble and transmembrane of TNF has two biological forms. It was reported that transmembrane TNF signaling was essential in preserving myelin integrity and compaction and, most importantly, in promoting remyelination [29]. Nevertheless, it was demonstrated that the anti-inflammatory effect of etanercept is not sufficient to stimulate recovery, indicating that it is the protective effect of transmembrane TNF signaling to ultimately drive the positive outcome in chronic disease [29]. In our study, the inhibition by etanercept of transmembrane TNF signaling probably caused myelin dysfunction continuation in rats. In other words, non-selective inhibition of both forms of TNF with etanercept did not result in myelin preservation as well as remyelination. Therefore, it has been found that the amount of myelin damage increased in the etanercept group compared to the saline group for grade 4.

Hypoxia is a central feature of ischemic, inflamed, and infected tissues and a principal determinant of the pathophysiology of local and generalized systemic inflammatory responses in these conditions [30]. Also, exposures to hypoxia are important components in the pathophysiology of local and systemic inflammatory responses [31]. Indeed, Lahat et al. [32] reported that hypoxia enhances lysosomal TNF-degradation in mouse peritoneal macrophages. In addition, Hempel et al. [33] showed that hypoxia increases the release of the cytokines IL-1 (interleukin-1) beta and TNF-α in human alveolar macrophage and that this increase may be due to decreased PGE2 (prostaglandin E2) synthesis during hypoxia.

In this study, it has been found that the exposure to hypoxia for 2 h that decreased the amplitude of CMAP by 57.79 ± 25.65% relative to the sham amplitude ($p < 0.05$), recovered to 57.35 ± 38.80% after treatment with etanercept. Also, in our previous study [1], ligation of the right common carotid artery and exposure to hypoxia by a continuous flow of 8% oxygen-92% nitrogen for 1 h resulted in electrophysiological abnormalities in the peripheral nerve of the rats. However, in that study [1], the amplitude of CMAP recorded from the rats treated with saline after hypoxia for 1 h decreased by 21.12 ± 10.29% compared to the sham group ($p < 0.05$) and recovered to 24.17 ± 18.40% after treatment with a platelet-activating factor (PAF) antagonist (ABT-491). Thus, the more long-term exposure to hypoxia in rats, as expected, is found to increase nerve damage.

In contrast to the central nervous system, nerve fibers of the peripheral nervous system are able to regenerate and reinnervate distal targets [34]. Regenerative and repair processes of the peripheral nerve begin almost immediately after injury [35]. Kato et al. [11] reported that immediate therapy with the TNF-α antagonist etanercept, administered systemically (i.p.) and locally (epineurially) after peripheral nerve crush injury in adult rats, enhances the rate of axonal regeneration. Our results confirm that HI has important neuropathic effects on peripheral nerves. This effect was observed especially with axonal myelin and the endoneurium. By using electrophysiological testing, in this study, it has been found that immediate etanercept therapy enhances axonal regeneration after neonatal HI.

In this study, a statistically significant difference was found in the saline group compared to the sham group for depolarization and repolarization

durations. Accordingly, the decrease in CMAP depolarization duration in saline compared to the sham group suggested that the opening-closing kinetics of Na^+ channels accelerated. On the other hand, treatment with etanercept accelerated this decrease in the depolarization duration more and so it did not have a preventive effect on the Na^+ channel kinetic after HI.

In addition, an increase in repolarization duration in the saline group compared to the sham group suggested that the opening-closing kinetics of K^+ channels slowed down. As we could not find any difference between the sham and the etanercept groups regarding repolarization duration, we suggest that etanercept treatment alters the opening and closing kinetics of voltage-gated potassium channels. Our results implicate the voltage-gated potassium channels as additional etanercept targets, opening up new perspectives for the pharmacological prevention of peripheral neuropathy.

The authors concluded that on the basis of the results, this study implies that neonatal HI insult causes axonal and myelin sheath damage to peripheral nerves, but the TNF-α inhibitor etanercept has a preventive effect only on axonal dysfunction but not motor dysfunction after HI.

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