EXPERIMENTAL STUDY

Neuroprotective effects of adalimumab on rats with experimental peripheral nerve injury: An electron microscopic and biochemical study

Ersin Polat, M.D.,[1] Ergün Dağlıoğlu, M.D.,[2] Güner Menekşe, M.D.,[3] Mehmet Serdar Dike, M.D.,[4] Çağatay Özdöl, M.D.,[5] Cezmi Çağrı Türk, M.D.,[5] Ali Erdem Yıldırım, M.D.,[2] Fatih Alagöz, M.D.,[2] Ali Dalgıç, M.D.,[2] Deniz Belen, M.D.[2]

[1]Department of Neurosurgery, Bafra State Hospital, Samsun-*Turkey*
[2]Department of Neurosurgery, Ankara Numune Training and Research Hospital, Ankara-*Turkey*
[3]Department of Neurosurgery, Ankara Training and Research Hospital, Ankara-*Turkey*
[4]Department of Neurosurgery, Ceyhan State Hospital, Adana-*Turkey*
[5]Department of Neurosurgery, Antalya Training and Research Hospital, Antalya-*Turkey*

ABSTRACT

BACKGROUND: Adalimumab, a new-generation anti-inflammatory agent, exerts its effect through tumor necrosis factor α (TNF-α), secreted from immune response cells such as macrophages and lymphocytes. TNF-α has been shown to play an important role in the processes of apoptosis and demyelination, and blockage of its activity may improve neural healing. Investigated in the present study is the probable neuroprotective influence of adalimumab in rats using a peripheral nerve injury model with biochemical and electron microscopic methods.

METHODS: Forty adult Wistar albino rats were randomly divided into control, sciatic nerve trauma, low-dose adalimumab, and high-dose adalimumab groups. Six rats from each group were assigned biochemical microscopy, and 4 were assigned electron microscopy. Neural injury was induced with clip compression following dissection of sciatic nerves. Adalimumab was simultaneously injected. The rats were sacrificed after 2 weeks of adalimumab treatment.

RESULTS: Nerve tissue lipid peroxidation values were found to be significantly decreased in both the low- and high-dose adalimumab treatment groups, compared to the group subjected only to sciatic nerve trauma.

CONCLUSION: Results demonstrate that adalimumab is an effective neuroprotective agent for neural healing, particularly in the early phase.

Keywords: Adalimumab; electron microscope study; lipid peroxidation; neuroprotection, peripheral nerve injury; rat; TNF-α.

INTRODUCTION

Peripheral nerve injuries are common, significant causes of long-term morbidity.[1] Incidence is approximately 2.8% of all cases of trauma.[2] Upon exposure to nerve compression, a spectrum of functional loss may occur, from slight weakness to total paralysis of muscles, or from total loss of sensation to mild paresthesias. Degree of severity is related to the involved nerve and location, level of pressure, and duration of compression. Nerve damage may continue, even after relief of compression.[3] Ischemic reperfusion injury is responsible for the majority of damage to nervous tissue,[4] and typically occurs due to free oxygen radicals and inflammatory reactions, which are caused by reduction reactions and neutrophilic infiltrations, respectively.[5] Contemporary management of peripheral nerve injuries includes the use of non-steroidal anti-inflammatory drugs, steroids, nerve-growth factors, thyroid hormones, growth hormone, adrenocorticotropic hormone, and insulin-like peptides.[6,7] Investigated in the present

Address for correspondence: Ergün Dağlıoğlu, M.D.
Ankara Numune Eğitim ve Araştırma Hastanesi,
Nöroşirürji Kliniği, Ankara, Turkey
Tel: +90 312 - 508 52 75 E-mail: ergundaglioglu@gmail.com

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study were the effects of a monoclonal antibody agent, adalimumab, on injured nerve tissue in rats, using an experimental clip compression injury model.

MATERIALS AND METHODS

Forty male Wistar albino rats, with body weights ranging from 180–210 g and aged between 3–5 months, were used. The rats were housed in cages (each accommodating 4) in a room with controlled temperature of 18–21°C and with automatic lighting (alternating 12-hour periods of light and dark). Food and water were available ad libitum.

The rats were randomly categorized into 4 groups: the control group, the trauma group, and the low- and high-dose adalimumab groups. Following overnight fast, the rats were weighed prior to surgical procedure, which was performed under general anesthesia. The rats were anesthetized intraperitoneally with mixture of 10 mg/kg xylocaine (Rompun® 2% solution; Bayer, Leverkusen, Germany) and 50 mg/kg ketamine hydrochloride (Ketalar® 5% solution; Parke-Davis Pharmaceutical Industries under license of Eczacıbaşı, İstanbul, Turkey). Once anesthetized, the rats were placed in prone position with extremities aside. Superficial sterilization was achieved using polyvinylpyrrolidone iodine (Polyod® 10% solution; Drogsan Pharmaceuticals, İstanbul, Turkey) to cover the sacral area and both lower extremities. Longitudinal skin incision was made in the right lower extremity at trochanter level. Following dissection, the sciatic nerve was exposed by vertically incising the gluteus maximus. The sciatic nerve was meticulously dissected, preserving the perineurium, without tractional injury. The nerve was compressed for 2 minutes using a Yasargil FE 693 temporary aneurysm clip with a closing force of 50 g/cm^2 (Aesculap, Inc., Corporate Parkway Center Valley, PA, USA). The injured region was proximally and distally marked with Prolene polypropylene thread (Ethicon, Inc., Somerville, NJ, USA). Procedure was repeated in each rat. Those in the low- and high-dose groups were intraperitoneally administered adalimumab 3 times (during surgery, and at 1 and 2 weeks of the injury) in 5 mg/kg and 50 mg/kg doses, respectively.

Two weeks later, the rats were sacrificed with high-dose anesthesia, and sciatic nerve segments were removed. Tissue samples were cut into 1-mm^3 pieces and kept in 0.1 M, phosphate-buffered, 2.5% glutaraldehyde solution (pH 7.4) for 2-hour fixation. After being washed 3 times with buffered solution, the samples were exposed to 1% osmium tetroxide for 1 hour as postfixation. They were later dehydrated in alcohol. Finally, samples processed with propylene oxide and blocs were prepared with embedding material, using the Araldite® CY212 kit (Huntsman Advanced Materials LLC, Salt Lake City, UT, USA). Blocks were polymerized in an oven of 560°C for 48 hours, after which semi-thin sections were obtained and stained with toluidine blue for examination under light microscopy. Thin sections were taken from the assigned region and stained with uranyl acetate-lead citrate for examination under microscope with EVO LS10 transmission attachment.

Measurement of thiobarbituric acid (TBA)-reactive components was used to ascertain lipid peroxidation, as defined by Mihara and Uchiyama.[8] Tissue samples were homogenized in potassium phosphate buffer solution 1:10 (w:v) using Dounce homogenization. After 0.5 mL homogenate was mixed with 3 mL of 1% phosphoric acid, 1 mL of 0.67% TBA was added. Tubes were suspended in boiling water for 45 minutes. After cooling, TBA components were separated into butanol, and 532 nm absorbance was determined. TBA reactive components-malondialdehyde complex base molar absorption value was determined as 0.156×10^5 M-1 cm-1, and level of lipid peroxide was calculated as nanomoles per gram of tissue.

RESULTS

Test results revealed differences in measured values among the groups. Analysis revealed a statistically significant change in peroxidation values in the trauma group, compared to the control group (p=0.02; Table 1). In addition, comparison revealed statistically significant differences between the control group and low-dose group (p=0.002), as well as between the low- and high-dose groups (p=0.009), but not between the control group and high-dose group (p=0.394). Results indicated that adalimumab effectively decreased lipid peroxidation with demonstrated positive effects on the healing of injured nervous tissue (high-dose vs low-dose comparison: p=0.009). The high-dose adalimumab group clearly benefitted the most, in terms of nerve healing (Table 1).

As a result of electron microscopy, sciatic nerve trauma induced phagocytic transformations in Schwann cells, particularly those on unmyelinated fibers (Fig. 1). It was hypothesized that low-dose adalimumab therapy would accelerate the healing process, resulting in fewer Schwann cells with phagocytic activity (Fig. 2a). Increase in number of mitochondria was observed in this group (Fig. 2b). The high dosage of adalimumab corresponded to more positive effects of healing, achieving a cross-sectional histological appearance quite

Table 1. Results of Mann-Whitney U test, used to compare differences among groups

Groups	p
Trauma and control	0.02
Trauma and low-dose adalimumab	0.026
Trauma and high-dose adalimumab	0.002
Low-dose adalimumab and control	0.002
High-dose adalimumab and control	0.394*
High-dose adalimumab and low dose adalimumab	0.009

*: Statistically significant.

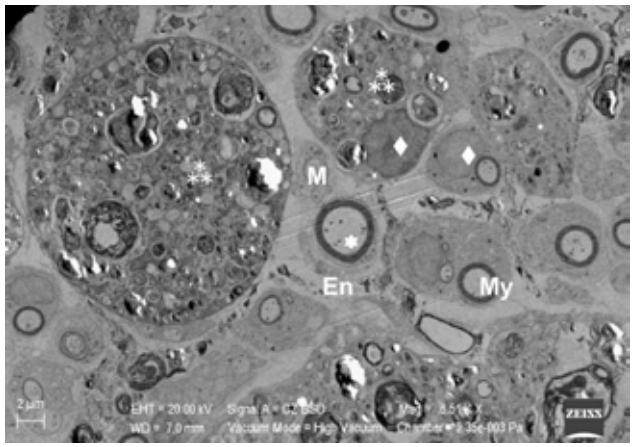

Figure 1. Electron microscopic examination of the trauma group. My: Myelinated nerve fibers; M: Unmyelinated nerve fibers; En: Endoneurium; ♦: Schwann cell nucleus; ✸: Axoneme; ⁂: Degenerated Schwann cells, including autophagic vacuoles.

similar to that of the control group. However, the high dosage was also shown to increase number of mitochondria and oxidative stress (Figs. 3a, b). While high-dose adalimumab may accelerate the regeneration process, it may also have negative effects on structure of myelinated nerve fibers.

DISCUSSION

Factors affecting the healing process following peripheral nerve injury also determine clinical improvement. In addition to primary injury of the peripheral nerve, nerve compression causing secondary ischemic injury is a well-known factor that affects the healing process.[9] Through this process, inflammatory reaction has beneficial and harmful effects on nerve tissue. Extensive degeneration in the axons and myelin sheath follows injury before being regenerated during the healing process. Cytokines, particularly TNF-α, play a major role in inflammatory reactions. TNF-α has important pro-

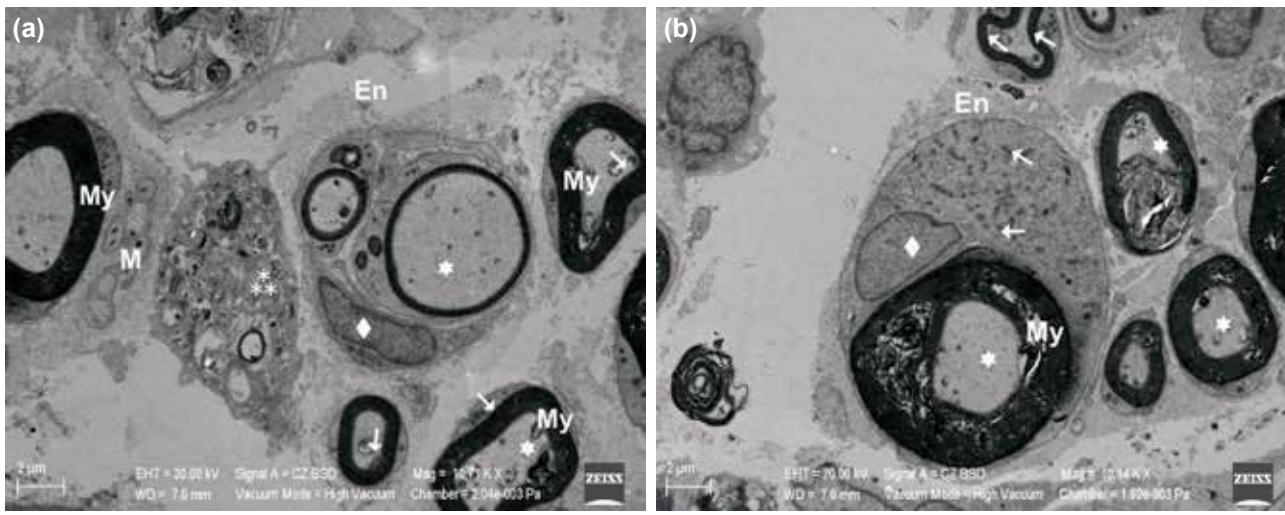

Figure 2. (a) Electron microscopic examination of low-dose adalimumab group. **(b)** Another section of low-dose group. My: Myelinated nerve fibers; M: Unmyelinated nerve fibers; En: Endoneurium; ♦: Schwann cell nucleus; ✸: Axoneme; ⁂: Degenerated Schwann cells, including autophagic vacuoles; ➘: Mitochondria in axon and Schwann cells.

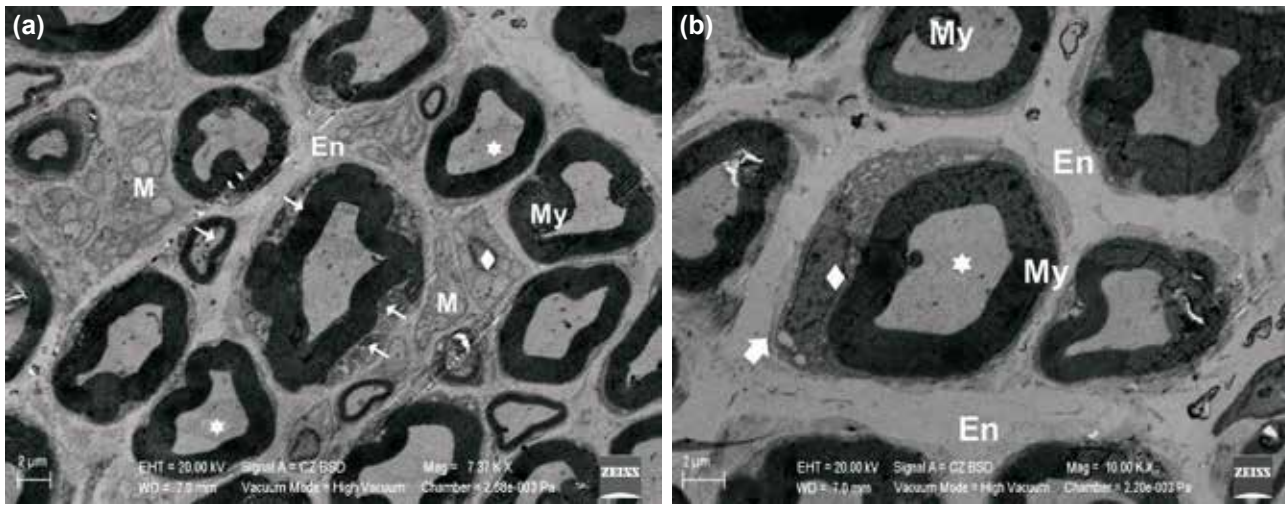

Figure 3. (a) Electron microscopic examination of high-dose adalimumab group. **(b)** Another section of high-dose group. My: Myelinated nerve fibers; En: Endoneurium; ♦: Schwann cell nucleus; ✸: Axoneme; ➘: Mitochondria in axon and Schwann cells; ⬅: Tubulus of rough endoplasmic reticulum.

inflammatory properties in the peripheral immune system, regulating antigen-presenting cellular activities and autoreactive T cells in apoptosis.[10–13] In overall pathogenesis of inflammatory demyelination, invasion by autoreactive T cells, in addition to interactions between adhesion molecules and metalloproteinases, is important.[8,14,15] Results of clinical trials concerned with autoimmune arthritis, ulcerative colitis, and Crohn's disease (in which increased inflammatory reaction and increased TNF-α levels are accounted for in pathogenesis), have supported the assumption that decreased levels of TNF-α may have a positive effect on nerve healing.[16] In that respect, adalimumab-positive, anti-inflammatory effectiveness, as shown in cases of psoriasis, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis, would have similar beneficial impact on inflammatory nervous system demyelination.[17] This was also demonstrated in an experimental autoimmune encephalitis model; with inhibition of TNF-α, demyelinating lesions in CNS showed regression.[18]

In the present study, Schwann cells, particularly on non-myelinated fibers in the sciatic nerve, underwent pronounced phagocytic transformation, an indicator of the natural healing process. Results indicated that even with lower doses of adalimumab, the number of Schwann cells with phagocytic functions decreased, and the healing process was simultaneously accelerated. Moreover, it was interesting to note that increase in mitochondria could be induced, even with relatively small doses. With higher doses of adalimumab, regeneration was precipitated, so that a structural appearance comparable to that of the control group could be achieved on histological examination, particularly in non-myelinated nerves. With regard to healing, there is an obvious difference between myelinated and non-myelinated fibers, which may be due to penetration level of the drug into target tissues. Following the relief of peripheral nerve compression, ease in circulation provides required nutrients and oxygen to the nervous tissue. Subsequently, reperfusion injury can ensue, with free oxygen radicals and consequent increase in lipid peroxidation.[19,20] Present results indicate that adalimumab has statistically significant, dose-related beneficial effects on decrease in lipid peroxidation, acting as a neuroprotective agent. The present is the first study to report neuroprotective activity parallel to anti-inflammatory effects in an alternative mechanism to previously reported cyclooxygenase and lipoxygenase pathways.[20,21]

Conclusion

The present study demonstrated that adalimumab caused particular changes in phagocytic activity, with positive effects on the healing process of nervous tissue. Beneficial effects on lipid peroxidation and anti-inflammatory activity support the adoption of adalimumab, which has neuroprotective properties, as a future candidate for treatment of patients with peripheral nerve injury.

Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Deneysel periferik sinir hasarı yapılan sıçanlarda adalimumabın nöroprotektif etkileri: Elektron mikroskobik ve biyokimyasal bir çalışma

Dr. Ersin Polat,[1] Dr. Ergün Dağlıoğlu,[2] Dr. Güner Menekşe,[3] Dr. Mehmet Serdar Dike,[4] Dr. Çağatay Özdöl,[5] Dr. Cezmi Çağrı Türk,[5] Dr. Ali Erdem Yıldırım,[2] Dr. Fatih Alagöz,[2] Dr. Ali Dalgıç,[2] Dr. Deniz Belen[2]

[1]Bafra Devlet Hastanesi, Nöroşirürji Kliniği, Samsun
[2]Ankara Numune Eğitim ve Araştırma Hastanesi, Nöroşirürji Kliniği, Ankara
[3]Ankara Eğitim ve Araştırma Hastanesi, Nöroşirürji Kliniği, Ankara
[4]Ceyhan Devlet Hastanesi, Nöroşirürji Kliniği, Adana
[5]Antalya Eğitim ve Araştırma Hastanesi, Nöroşirürji Kliniği, Ankara

AMAÇ: Yeni nesil bir antienflamatuvar ajan olan adalimumab, etkisini makrofaj ve lenfositler gibi hücresel immün yanıt elemanlarından salınan tümör nekrozu faktörü (TNF-α) üzerinden gösterir. Özellikle apoptoz ve demiyelinizasyon gibi süreçlerde önemli rol oynayan TNF-α aktivitesinin önlenmesi ile nöral iyileşmede artış gözlenebilir. Bu çalışmada, sıçan deneysel siyatik sinir hasarı modelinde adalimumabın nöroprotektif etkinliği elektron mikroskobik ve biyokimyasal olarak incelendi.

GEREÇ VE YÖNTEM: Çalışmada toplam 40 adet Wistar albino cinsi sıçan sham, travma, düşük doz adalimumab ve yüksek doz adalimumab olmak üzere rastgele dört gruba ayrıldı. Her gruptan altı sıçan biyokimyasal ve dört sıçan elektron mikroskobik analizde kullanıldı. Siyatik sinirleri diseke edilen sıçanlara klip kompresyonu ile periferik sinir hasar modeli uygulandı. Siyatik sinirleri diseke edilen sıçanlara klip kompresyonu ile periferik sinir hasar modeli uygulandı ve eş zamanlı adalimumab tedavisi uygulandı. İki haftalık adalimumab tedavisi sonrası sıçanlar sakrifiye edildi.

BULGULAR: Düşük doz ve yüksek doz grubunun her ikisinde de yapılan incelemelerde adalimumabın, sinir dokusu lipid peroksidasyon değerlerini istatistiksel olarak anlamlı biçimde azalttığı görüldü.

TARTIŞMA: Bu çalışmanın sonuçları adalimumabın nöral doku iyileşmesinde özellikle erken döneminde etkili nöroprotektif bir ajan olduğunu göstermiştir.

Anahtar sözcükler: Adalimumab; elektron mikroskobik çalışma; lipid peroksidasyonu; nöroprotektif etki; periferik sinir hasar; sıçan; TNF-α.

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