Nogo-A Inhibitory Peptide (NEP1-40) Increases Pan-Cadherin Expression Following Mild Cortical Contusion Injury in Rats

Nogo-A İnhibitör Peptidi (NEP1-40), Sıçanlarda Hafif Kafa Travması Sonrasında Pan-Cadherin Ekspresyonunu Artırıyor

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ABSTRACT

BACKGROUND: Nogo-A is a myelin-associated neurite outgrowth inhibitory protein that limits elongation of central nerve fibers, neuronal regeneration and plasticity. We investigated the effect of delivering an inhibitory peptide that neutralizes Nogo-A on neuronal recovery following mild cortical contusion injury

METHODS: 41 rats were allocated into the control and NEP1-40 treatment groups. PBS was applied following trauma over the parietal cortex after opening the dura in the control group. NEP1-40 solution was immediately applied following trauma after opening the dura in the treatment group. Each group was further divided into 3 subgroups and sacrificed on the third, eighth, and 21st days after injury. The brains were removed for analysis

RESULTS: Immunohistochemical staining of the injured cortex for pan-cadherin revealed a significant increase in staining in the NEP 1-40 treatment group at the 8th and 21st days after injury. Electron microscopic evaluation revealed better cytoarchitectural preservation in the axons of the animals treated with NEP 1-40

CONCLUSION: We observed improved preservation of injured neurons after topical application of NEP 1-40 following mild cortical injury. Pan-cadherin expression may correlate with the recovery of neurons and axonal bodies. Electron microscopical findings confirmed better preservation of neuronal structures after NEP1-40 treatment. Pan-cadherin is a good marker for neuronal recovery after cortical injury.

KEY WORDS: Mild cortical contusion injury, Motor recovery, NCAM, Neuroregeneration, Nogo-A, Pan-cadherin

ÖZ

AMAÇ: Nogo-A myeline bağımlı nörit büyüme inhibitör proteinidir ve santral sinir sisteminde hasar sonrasında sinir liflerinin büyümesini engeller. Bu çalışmada Nogo-A inhibitör peptidlerin hafif kafa travması sonrasında nöral iyileşmeye olan etkisi araştırılmıştır

YÖNTEMLER: Bu çalışmada, 41 Sprague Dawley sıçan, kontrol ve NEP1-40 ile tedavi edilen gruplar olmak üzere 2 gruba ayrıldı. Bütün hayvanlarda sağ parietotemporal kraniektomi ile korteks üzerinde hafif kortikal kontüzyon hasarı yapıldı. PBS taşıyıcı solüsyon olarak kullanıldı ve kontrol grubunda travma sonrasında sağ parietal korteks üzerindeki dura açılarak PBS uygulandı. Tedavi grubunda ise NEP1-40 korteks üzerindeki dura açılarak uygulandı. Her grup kendi içinde 3 ayrı gruba ayrılarak sıçanlar 3, 8 ve 21. günlerde feda edildiler ve beyinler analiz için çıkarıldı

BULGULAR: Hasarlı korteksin pan-cadherin (nöral hücrenin yapışma ve filizlenmede belirleyicisi) ile immünhistokimyasal boyanması NEP1-40 ile tedavi edilen grupta 8. ve 21. günde belirgin olarak anlamlı boyanma ile sonuçlandı. Elektron mikroskopik inceleme aksonların hücre mimarisinin NEP1-40 ile tedavi edilen grupta daha iyi korunduğunu gösterdi.

SONUÇLAR: Bu çalışmada hafif kafa travması sonrası topikal NEP1-40 ile tedavi edilen sıçanlarda hasarlı nöronların daha iyi korunduğunu gösterdik. Pan-cadherin ekspresyonu ile nöron ve aksonlardaki iyileşme arasında bir korelasyon olabilir. Elektron mikroskopisi bulgularımız tedavi edilen gruptaki nöronal korunmayı doğrulamıştır. Pan-cadherin kortikal hasar sonrasında nöronal iyileşmeyi gösteren iyi bir belirleyicidir.

ANAHTAR SÖZCÜKLER: Hafif kortikal kontüzyon hasarı, Motor düzelme, NCAM, Nörorejenerasyon, Nogo-A, Pan-cadherin

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INTRODUCTION

The adult mammalian central nervous system (CNS) has a limited capacity for nerve regeneration and structural plasticity (2,4,9,15,49). The severe clinical consequences of CNS injury are due to the fact that, in contrast to most other tissues, the CNS lacks the ability to reconstitute itself by neuronal cell proliferation, and CNS neurons fail to regrow severed axons. One of the many explanations for the decreased anatomic plasticity after adult CNS injury is the presence of myelin-associated inhibitory factors that block neurite outgrowth (8,15-17,35,40). Currently the best-characterized cells mediating inhibitory signals in axonal growth are oligodendrocytes. Nogo-A is a myelin-associated neurite outgrowth inhibitory protein limiting neuronal regeneration and plasticity after CNS injury. It is a protein product of the Nogo gene (2,5,14-20,25,26,32,35,37,42,48), which is expressed on the cell surface of oligodendrocytes (33-35). The presence of glia-derived inhibitory factor Nogo-A has been suggested to provide a nonpermissive environment for elongation of nerve fibers (10,12,13,30). Following CNS lesions, reinnervation of denervated areas may occur via 2 distinct processes: regeneration of the damaged fibers or sprouting from adjacent intact fibers into the deafferented zone. Both regeneration and axonal sprouting are limited in the fully mature CNS of higher vertebrates but may be enhanced by neutralizing the neurite outgrowth inhibitory protein Nogo-A (3,4,8,21,29,35,36,51,52).

Neural cell adhesion molecules (NCAM) promote adhesion between the surfaces of neural cells during the acute inflammatory response to traumatic injury in the cerebral cortex (39,43). NCAM is found in the gray and white matter and expressed on glial cells, especially on reactive astrocytes and neurons that have completed their differentiation (22,28,41). N-cadherin is one of these adhesion molecules, and it is found outside the cellular plasma membrane, promoting hemophilic adhesions (46). Neurons lose their connections and transform into single cellular structures when antibodies to N-cadherin are applied (36,46,47). Recent studies demonstrate that the cadherin cell-adhesion molecules and their cytoplasmic partners can modulate axon-dendritic spine contacts (46). Cadherin activity is essential for synaptic plasticity and rearrangement (36,47).

In this experimental study, we aimed to investigate expression of adhesion molecules (pan-cadherins) from the nerve cells of the injured cerebral cortex and evaluate the injured cortical neurons for the presence of neuronal recovery, following treatment with NEP1-40.

MATERIALS and METHODS

Forty-one Sprague Dawley male rats in same age and weighing 150 to 180 grams were used. The animals were housed in individual polycarbon cages at the Baskent University of Animal Research Center in Ankara. All protocols were approved by the University of Baskent Animal Care and Use Committee (#:DA0211 and DA0213). Each rat was given at least 1 day to acclimate to its new surroundings, so as to reduce the effects of stress from transportation. Temperature was kept at 20°C ± 2°C and humidity was 50% ± 10% at the animal facility. Rats were fed ad libitum and remained under 12-hour light-dark conditions.

All animals were subjected to mild cortical contusion injury in which cortical impact was applied directly on the dura matter after right parietal craniectomy. Rats were randomly allocated into 2 groups: control animals (vehicle) and animals treated with NEP1-40 which is a 40-residue inhibitory peptide to Nogo-A. Phosphate buffered saline (PBS) solution which is used to dissolve NEP1-40 (0.1 mL) was immediately applied over the right parietal lobe after opening the dura in animals in the control group. NEP1-40 solution (0.1 mL) was immediately applied on the right parietal lobe after opening the dura in animals in the treatment group. Each group was further divided into 3 subgroups, and animals were sacrificed on the third, eighth, and 21st days after surgery. The brains were removed for analysis.

NEP1-40 preparation

100 μg; Human Nogo 1-40 peptide (NEP1-40) (Alpha Diagnostics Intl. Inc., San Antonio, Tex, USA), was diluted with 2.5 mL PBS (pH 7.5, 0.1-0.2% Bovine serum albumin (BSA), containing 0.05% sodium azide or merthiolate was divided by micropipettes into 25 tubes, each containing 0.1 mL NEP 1-40. The dose that was applied directly to each injured cortical area was 0.1 mL NEP 1-40.

Surgical procedure and head trauma

Anesthesia was achieved with an intraperitoneal injection of ketamine 60 mg/kg (Ketalar, Pfizer,

Turkey) and xylazine 2% 10mg/kg (Rompun, Bayer, Turkey). Thilotears® gel (Liba, Turkey) was used to lubricate the eyes. During surgery, the animals were ventilated spontaneously; body temperature was maintained at 37°C using heated gel packs, the femoral artery was catheterized, and the blood gas values were analyzed at the presented in detail below (Table I). Preoperative doses of the antibiotic enrofloxacin (10 mg/ kg; Baytril K, Bayer, Turkey) was applied subcutaneously to all rats. The right hemispheric area was prepared with povidone-iodine (Betadine®, Pharma, Turkey), and the scalp was shaved. (For a detailed description of the experimental procedures see Lewen et al., Allen et al., Nilsson et al., and Marklund et al. (1,24,27,31). Briefly, rats were positioned in the prone position, the heads of the animals were fixed, and a craniotomy was made over the right parietal cortex, with its center 3.5 mm behind the bregma with the aid of surgical microscope (OPML – 9 FC Zeiss, Germany). The trauma was performed with a 21-g free-falling weight (in a cylindrical, hollow, Plexiglas tube) that was dropped from a height of 35 cm onto a piston resting on the exposed dura. This allowed maximal depression (1.5 mm) of the cortex. There was a 90° angle between the tube and brain tissue. The tube just touched to brain and there was no pressure due to the tube. The tube was used so that heights were identical for each animal and the tube also tube served as a guide for dropping the metal weight onto the dura. In this model, we calculated the potential energy for creating a mild cortical contusion injury as: Potential energy (PE) = mass (in kilograms) x the gravitational acceleration of the earth (9.8 m/sec^2) x height (in meters). When we applied this formula to our experimental data, we obtained PE = 0.021 kg (weight of the metal disc) x 9.8 m/sec^2 (constant) x 0.35 m (length of the tube) = 0.07 Joule. Thus, we transferred 0.07 Joule potential energy to the right parietal cortex for mild cortical injury. After opening the dura, PBS solution (0.1 mL) was immediately applied over the injured cortex in animals in the vehicle-treated group. After opening the dura, NEP1-40 solution (0.1 mL) was immediately applied over the injured cortex in the NEP1-40 treatment group following injury. The surgical site was sutured in layers, and an antibacterial spray (Pyedif®) (Sanofi-Dif, Turkey) was applied onto the skin incision. Immediately after surgery, all rats were given lactated Ringer solution (5-10 cc) subcutaneously and were maintained in an incubator until thermoregulation was re-established. Postoperative care was provided, and each animal received one additional subcutaneous injection of enrofloxacin (10 mg/kg).

Animals in both the vehicle and NEP 1-40 treatment groups were killed on the third, eighth, or 21st days postoperatively with a lethal dose of ketamine (150 mg/kg, Ketalar, Pfizer, Turkey) administered intraperitoneally. After transcardiac perfusion with 10% formalin, the brains were removed en bloc and immersed overnight in 10% formalin. The injured cortex over the parietal lobes, 3,5 mm behind the bregma was sectioned for immunohistochemical and electron-microscopic analysis.

Table I. Physiological variables in rats subjected to head trauma

Parameter	Experimental group	Time before and after head trauma			
		-30 min	**-15 min**	**15 min**	**30 min**
MABP(mmHg)	**Vehicle**	96±3	90±6	84±4	92±2
	NEP 1-40	95±5	90±5	89±1	90±9
pH	**Vehicle**	7.4±0.03	7.41±0.02	7.44±0.04	7.39±0.01
	NEP 1-40	7.39±0.02	7.42±0.03	7.45±0.01	7.40±0.02
pO2 (mm Hg)	**Vehicle**	88±3	84±4	87±3	87±3
	NEP 1-40	86±2	88±5	93±4	84±3
PaCO2 (mmHg)	**Vehicle**	44±1	42±5	41±5	39±3
	NEP 1-40	43±3	38±4	42±3	41±2

Blood gas values analysed at intervals are shown. Note all these values are within the normal physiological range. All values are expressed as means ± standard error. MABP, mean arterial blood pressure.

Immunohistochemical analysis

Formaldehyde-fixed and paraffin-embedded coronal sections of the traumatized brains were sectioned. After deparaffinization and rehydration, each section was immunostained using antibodies to cadherin (Pan Ab-4, rabbit polyclonal, Neomarkers). Immunohistochemistry procedures were carried out using the standard avidin-biotin complex method according to the following procedures: For both antigens, antigen retrieval was performed in a microwave oven in 10 mM citrate buffer + ethylene-diaminetetraacetic acid (EDTA) (pH, 6.0) at 700 Watts for 15 minutes. Endogenous peroxidase activity was then blocked with 0.3% H2O2 for 30 minutes. After incubation with 5% BSA in Tris-buffered solution (TBS: 50 mM Tris-HCl, 150 mM NaCl, pH 7.4) for blocking of nonspecific binding for 10 minutes, sections were incubated with primary antibodies for pan-cadherin (overnight) in a humidified chamber at room temperature. Sections were then incubated with peroxidase labeled polymer for 30 minutes, followed by 3'3-diaminobenzidine (DAB)+ substrate-chromogen solution. Next, they were counterstained with hematoxylin and mounted. Between steps, the slides were washed twice in TBS. For negative controls, the primary antibodies were omitted and nonimmune serum was used instead. For positive controls, we stained tissue sections considered to be suitable according to the manufacturer's protocol. We used a tonsil section for pan-cadherin.

A neuropathologist blinded to the study protocol studied all histological sections. The extent and intensity of pan-cadherin expression was semiquantitatively evaluated. Scoring was classified into the following 3 groups: 0: no expression; 1: moderate expression; 2: marked expression.

Electron Microscopic Technique

For electron microscopic examination, all tissues were fixed in PBS containing 2.5% glutaraldehyde for 2-3 hours; they were then postfixed in 1% osmium tetroxide (OsO4) and dehydrated in a series of graded alcohol baths (25%, 50%, 75%, 95%, and absolute alcohol). After passing through propylene oxide, the specimens were embedded in Araldyte CY 212, DDSA (2-dodecenyl succinic anhydride), benzyldimethyl amine, and dibutyl phthalate. Semithin sections were cut and stained with toluidine blue and examined with a light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with the LEO 906E EM transmission electron microscope (TEM).

Statistical evaluation

Statistical Package for the Social Sciences (SPSS) version 11.0 was used for statistical analysis. Data were evaluated using the Wilcoxon signed rank test to compare changes among the individual groups. A P value less than .05 was considered statistically significant.

RESULTS

Rats in all groups returned to normal activity after recovery from the anesthesia. They started to walk around in their cage, and they were fully alert to external stimuli. They showed no behavioral effects or neurological deficits after mild cortical contusion.

Hematoxylin-and-eosin–stained coronal sections of rat brain at the area of trauma showed reactive gliosis and prominent inflammatory cell (mainly histiocytes) infiltration. Membranous immunore-activity was considered positive for pan-cadherin. Thus, cytoplasm-only stainings were ignored.

Immunohistochemical (pan-cadherin) results

Strong expression of pan-cadherin was observed in reactive astrocytes and inflammatory cells in all groups. Ependymal cells also exhibited a positive reaction for pan-cadherin (to some extent) in all groups. No significant pan-cadherin staining was observed in any of the vehicle-treated groups or on the third day in the NEP-1-40–treated group. On the eighth (Figure 1A) and 21st (Figure 2A) days, the neuronal cells of the cortical regions of the animals in the vehicle-treated groups showed no membranous positivity for pan-cadherin. On the eighth (Figure 1B) and 21st (Figure 2B) days, the neuronal cells (particularly those of the cortical regions) of animals in the NEP-1-40–treated groups exhibited marked membranous positivity for pan-cadherin. The immunostaining pattern was more striking on the 21st day compared with the eighth day (Figure 1B) for animals in the NEP-1-40–treated group (Figure 2B). Regarding semiquantitative pan-cadherin expression on the eighth day and 21st days, analyses revealed that membranous positivity for pan-cadherin was significantly greater for animals in the NEP-1-40–treated groups than it was for animals in the vehicle-treated groups (P < .05). (Table II) shows the semiquantitative pan-cadherin results in detail.
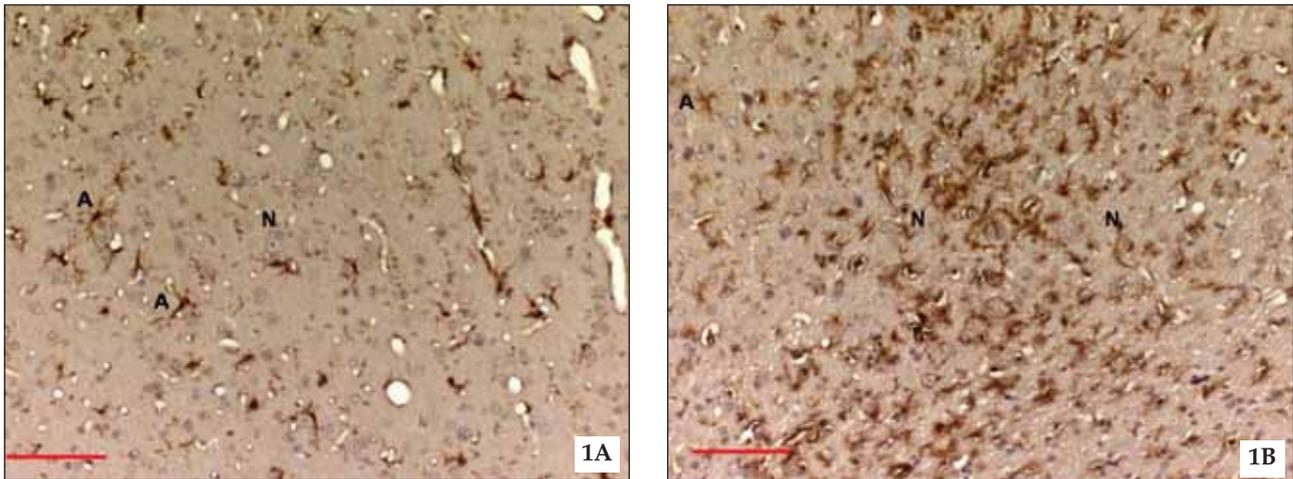
Figure 1: *Immunohistochemistry results for pan-cadherin staining in the eighth day in the vehicle-treated group. Neuronal cells in the cortical regions of the brain show no membranous positivity for pan-cadherin (A). Neuronal cells, particularly those in the cortical regions of the brain, exhibited a moderate degree (1+) of membranous positivity for pan-cadherin in the NEP-1-40–treated group (B) (x20) (bar: 100 micrometers) (A: reactive astrocyte; N: neuron; E: ependymal cell).*
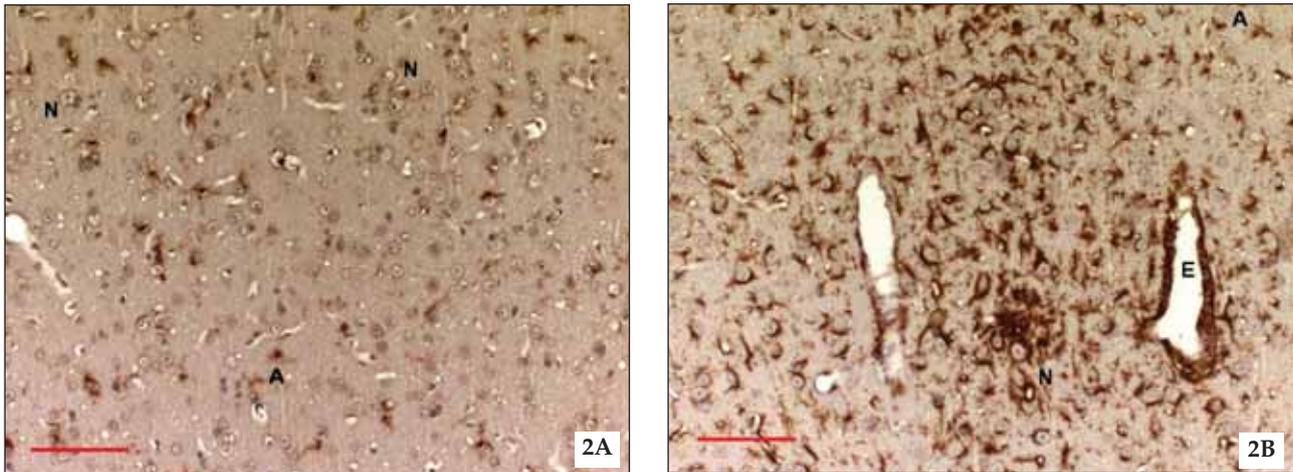

Figure 2: *Immunohistochemistry results for pan-cadherin staining on the 21st day in the vehicle-treated group. Neuronal cells in the cortical regions of the brain show no membranous positivity for pan-cadherin (A). At day 21, neuronal cells, particularly those in the cortical regions of the brain, exhibited marked (2+) membranous positivity for pan-cadherin in the NEP-1-40–treated groups (B). Note: positive staining was observed in reactive astrocytes in all groups (x 20) (bar: 100 micrometer) (A: reactive astrocyte; N: neuron; E: ependymal cell).*

Table II. Results of pan-cadherin stainings of the rats in groups

	Vehicle group	NEP 1-40 group
Sacrificed on the 3rd day	No pan-cadherin staining	No pan-cadherin staining
Sacrificed on the 8th day	No pan-cadherin staining	1,0,0,1,1,1,1
Sacrificed on the 21st day	0,0,1,0,0,0	2,2,1,2,2,2,1,2

Electron microscopy

The most-prominent immunohistochemical stainings were observed on the 21st day and TEM was therefore used to compare animals in the NEP-1-40–treated group and animals in the vehicle-treated group only for the 21st day. For animals in the vehicle-treated group, TEM demonstrated massively shrunken and compacted dark neurons (Figure 3A). Dilatation of the Golgi and endoplasmic reticulum cisternae was evident in this group (Figure 3B, 3C). Axonal structures showed diffuse
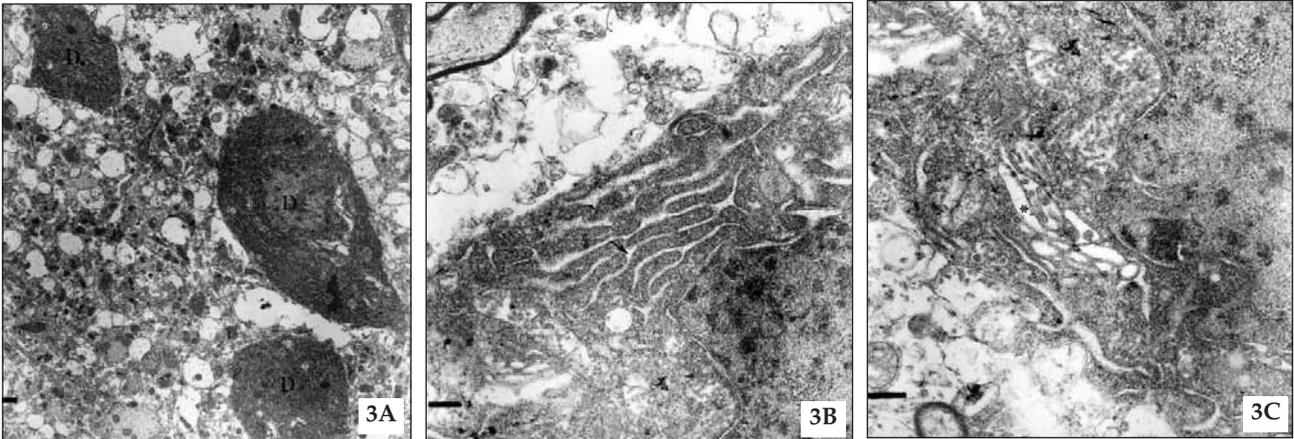
Figure 3: Transmission electron microscopy findings in those brain areas on the 21st day in the vehicle-treated group show massively shrunken and compacted dark neurons in the sections (A) (bar: 1 micrometer), dilatation of the Golgi cisternae (*) (B) (bar: 400 nanometer), and endoplasmic reticulum cisternae (arrow) (C) (bar: 400 nanometer). D: dark neurons.

degeneration and massive infoldings of the axolemma and the myelin sheath. The periaxonal space was filled with myelin debris (Figure 4A). Periaxonal fluid, lamellation of the myelin sheath, and myelin sheath infoldings were observed in severely degenerated axons in animals in the vehicle-treated group (Figure 4B). In animals in the vehicle-treated group, the distances between the individual neurofilaments were reduced, and the axons were compacted (Figure 5A). On the 21st day, animals in the NEP-1-40–treated group showed no dark neurons, and the axonal ultrastructure was preserved (Figure 4C). The distances between individual neurofilaments had increased to normal size in animals in the NEP-1-40–treated group (Figure 5B).

DISCUSSION

In this study, we have evaluated the presence of neuronal recovery and the expression of adhesion molecules (pan-cadherins) after mild cortical contusion injury following treatment with NEP1-40 which is a 40-residue inhibitory peptide to Nogo-A. We confirmed mild parenchymal damage to animals in the vehicle-treated groups, and we observed recovery of the nervous tissue following NEP 1-40 treatment after eighth days and 21st days of the treatment. On the third day, however, no difference was apparent for animals in either the vehicle or the treatment group. Immunohistochemical pan-cadherin stainings at 8 and 21 days after injury showed increased activity with NEP 1-40 treatment. TEM observations supported the findings of pan-
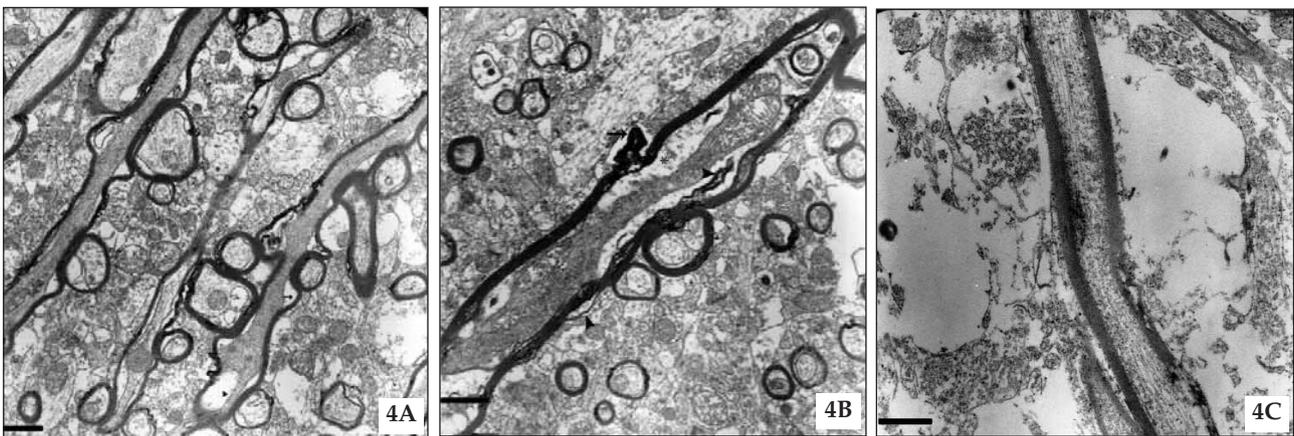

Figure 4: Transmission electron microscopy findings on the 21st day in the vehicle-treated group: axonal structures were diffusely degenerated. Massive infoldings of the axolemma (arrow head) and myelin sheaths were observed (arrow). The periaxonal space was filled with myelin debris (curved arrow) (A) (bar: 1 micrometer). Periaxonal fluid (*), lamellation of the myelin sheath (arrow heads), and myelin sheath infoldings (B) (bar: 1 micrometer). A preserved axonal ultrastructure at the 21st day in the NEP-1-40–treated group (C) (bar: 400 nanometer).
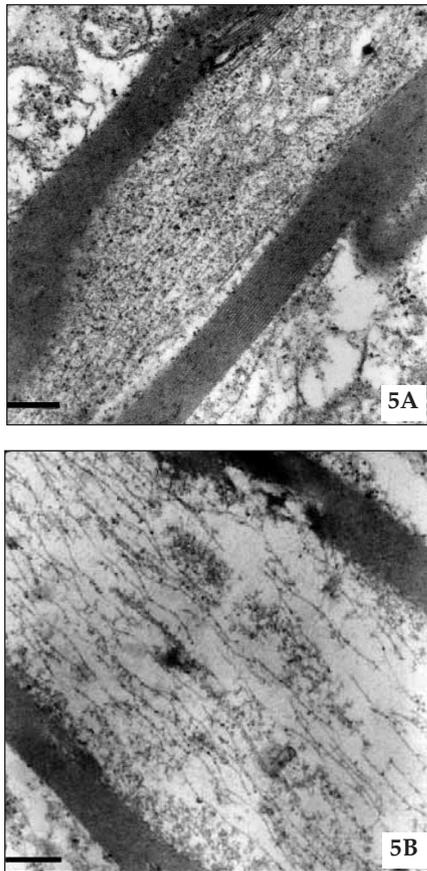
*Figure 5: Compacted axon. Note that the distances between the individual neurofilaments are reduced, and axons are compacted at the 21st day in the vehicle-treated group (**A**). A normal axonal cytoskeleton at the 21st day in the NEP-1-40–treated group (**B**) (bars: 200 nanometer).*

cadherin stainings, and we observed preservation of the nerve fibers, axoplasm, and cytostructure in animals in the treatment groups. By inhibiting the Nogo peptide, we promoted neuronal recovery and increased the amount of adhesion molecules at the trauma site. We conclude that the increased staining for pan-cadherin after the third week may be related to neuronal regeneration.

In this study we used cortical-contusion–injury model. We calculated the transferred energy in joules, which is different from previous studies using the same model (1,24,27,31). Using different tube lengths and different metal weights, it is possible to generate different contusion injuries; this model is inexpensive, simple, and easy to administer.

Within the first 3 weeks after lesioning, adult mammalian CNS axons show an initial growth response. However, sprouting axons extend only for very short distances before they cease growing and either degenerate or end locally at the lesion border (38). Although there are some exceptions to this rule, most sprouting axons in the adult mammalian CNS fail to enter the lesion site (4,44). It is clear that the lesion area itself is of prime importance in the context of regeneration failure. The observation that cultured neurons can extend neurites into optic nerve explants, even in the presence of optimal trophic factor conditions (40), has led to the recent belief that adult mammalian CNS neurons cannot regenerate owing to the predominance of neurite growth-inhibiting molecules (4,44). Tremendous effort has been made to identify neurite growth-inhibiting molecules (10,44). It has been hypothesized that myelin-associated molecules become exposed during the injury response and subsequently abort regeneration (4,44). Different attempts have been made to reduce or neutralize these proteins. These treatments have resulted in substantial elongation of regrowing corticospinal and brainstem descending projections. The studies further suggest that myelin proteins represent a major, nonpermissive component of CNS tissues that may serve as border markers that restrict the growth of neurites to appropriate regions and layers in the CNS, and that they also may exert an inhibitory role in the context of regenerative neurite growth. Antibodies against the myelin proteins have been used to neutralize the growth-inhibitory activity of these proteins (4,44,50). Continuous application of these antibodies by implanting living mouse-antibody–secreting hybridomas, either on top of a lesion or into the lateral ventricles, has been demonstrated to elongate some corticospinal nerves, the optic nerve, and septo-hippocampal sprouts considerable distances distal to the lesion site (4,44,50). Subsequent work has shown that coadministration of neurotrophic factors enhance sprouting of lesioned fibers, which correlates with significant recovery of certain motor functions (44).

To our knowledge, Lenzlinger and colleagues (23) were the first to report an in vivo study supporting a beneficial effect of Nogo-A inhibition on cognitive function in a rat experimental model of traumatic brain injury (TBI). In this study, functional improvement was achieved with the intervention beginning 24 hours after the injury, making this beneficial treatment strategy potentially interesting for use in brain-injured patients. Lenzlinger and

coworkers reported significantly increased sprouting of corticospinal tract fibers following TBI. In this study, the Nogo monoclonal antibody significantly attenuated cognitive deficits after TBI. The major difference in our study is that we produced a mild cortical contusion instead of considerable parenchymal damage and we have applied the NEP 1-40 peptide immediately following cortical injury instead of 24 hours after head injury as was done in Lenzlinger's study. Thus, early treatment might be a factor that promoted neuronal recovery in our study. Emeric and colleagues (8) reported anatomic plasticity in the motor cortex of adult rats after a unilateral aspiration lesion to the sensorimotor cortex and treatment with monoclonal antibody (mab) to IN-1 (Nogo-A), which permits neurite-outgrowth from the intact, opposite cortex into deafferented subcortical targets. After a 6-week survival period, a dramatic increase in movements of the lesion-impaired forelimb was demonstrated using intracortical stimulation in animals treated with Nogo-A mab compared to controls. These results showed that after adult cortical lesioning, treatment with Nogo-A mab induces functional reorganization of the motor cortex.

Electron microscopy of injuries that do not cause considerable parenchymal damage have demonstrated formation of massively shrunken and compacted neurons called dark neurons. Head injuries initiate the formation of dark neurons. This common response to the injury of individual neurons is a dramatic compaction of undamaged, ultrastructural elements in the cytoplasm and the nucleus (7,45,53). In our study, dark neurons were observed in animals in the vehicle-treated groups; however, following NEP 1-40 treatment, formation of dark neurons were prevented. Owing to this, we concluded that the Nogo-A monoclonal antibody promoted neuronal recovery. Another consequence of experimental TBI is a dramatic reduction in the distances between the neurofilaments (cytoskeletal compaction) inside several axonal segments (11). In the current study, cytoskeletal compaction of neurofilaments were also relieved after treatment.

The neural cell adhesion molecule (NCAM) and N-cadherin from the cadherin superfamily are regulators of the cell surface interactions between neurons (22,28,41,47). In cultured systems, the NCAM and N-cadherin may promote neurite outgrowth in some cases on the surfaces of Schwann

cells. Recent studies suggest that the NCAM may act differentially from other adhesion modules during this neurite outgrowth process (22,28,41,46). The NCAM is expressed on fasciculating axons and nonmyelinating Schwann cells. The NCAM mediates axon–axon interactions and axon–Schwann-cell interactions. Myelination downregulates this molecule and the levels of the NCAM progressively increase in the cerebrospinal fluid of acute multiple sclerosis patients paralleling the progressive clinical improvement after the attack (28).

Members of the cadherin superfamily are important in interactions between Schwann cells and axons (47). N-cadherin is a Ca2+-dependant intercellular adhesion molecule identified on growth cone surfaces in vitro (36). In vivo, N-cadherin has been shown to be expressed on normal unmyelinated fibers and on the regenerating fibers of the peripheral nervous system (6). N-cadherin expression was location specific; intense immunoreactivity was identified on the plasma membrane surface of axon–axon and axon–Schwann cell contacts, but not where axons or Schwann cells were in contact with basal lamina. There also is evidence that sorting axons into different pathways may depend on N-cadherin expression such as that which takes place during brain development (36,46,47).

In the current study, we demonstrated recovery of injured axons following topical application of NEP 1-40 immediately after mild cortical injury. Significant N-cadherin expression was observed in rats following NEP 1-40 application in contrast to rats in control and vehicle-treated groups. Therefore, we hypothesize that NEP 1-40 treatment promotes neuronal recovery after injury and that neuronal axons regenerate and adhere new neurons, or make new connections, that bridge the injury site. In this study we think that, inhibition of the Nogo peptide by NEP 1-40 may stimulate sprouting of new axons, and this may be a key factor in neuroregeneration. In rats treated with NEP 1-40, expression of N-cadherin was greater after 3 weeks than it was at 1 week, and this substantiated the finding that the level of nerve connection and axonal sprouting increases with time. In our study, neuronal recovery was readily visible 3 weeks after injury.

Neuronal regeneration depends on an interplay between extrinsic cues and intrinsic properties of the

lesioned neuron. Although the mechanisms that are responsible for regeneration failure in the adult mammalian CNS are not completely understood, research in this area is promising for brain injured patients. Inhibition of Nogo-A permit injured CNS nerve fibers to regenerate over long distances by neutralizing myelin-associated growth-inhibitory proteins in spinal cord and brain. This may be an important step toward a potential treatment because the Nogo-A monoclonal antibody could be applied to the injured cortex during initial emergency surgery immediately after open head injury or by intradural infusion pumps in the subacute stages of cortical injury.

CONCLUSION

CNS neurons can regrow when provided a suitable environment. Injured neurons may recover after mild cortical injury following topical application of NEP1-40. Pan-cadherin expression may correlate with the recovery of the neurons or the axonal bodies and can be used as a good marker of neuronal recovery.

Acknowledgments

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