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A Peptide Derived from Activity-Dependent Neuroprotective Protein (ADNP) Ameliorates Injury Response in Closed Head Injury in Mice

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ABSTRACT

Brain injury induces disruption of the blood-brain barrier, edema, and release of autodestructive factors that produce delayed neuronal damage. NAPSVIPQ (NAP), a femtomolar-acting peptide, is shown to be neuroprotective in a mouse model of closed head injury. NAP injection after injury reduced mortality and facilitated neurobehavioral recovery ($P < 0.005$). Edema was reduced by 70% in the NAP-treated mice ($P < 0.01$). Furthermore, in vivo magnetic resonance imaging demonstrated significant brain-tissue recovery in the NAP-treated animals. NAP treatment decreased tumor necrosis factor-α levels in the injured brain and was shown to protect pheochromocytoma (PC12 cells) against tumor necrosis factor-α-induced toxicity. Thus, NAP provides significant amelioration from the complex array of injuries elicited by head trauma.

Traumatic brain injury is a major cause of mortality and morbidity in the 15- to 24-year age group in the Western world (Waxweiler et al., 1995). It is also considered a risk factor for late onset of Alzheimer's disease (Schofield et al., 1997). Mechanical brain trauma leads to functional impairment and elicits widespread cell death in the ipsilateral hemisphere (Ommaya, 1995; Povlishock and Christman, 1995; Gennarelli, 1996). To date, there is no effective drug for the treatment of brain-injured patients. Thus, understanding and developing neuroprotective agents that inhibit the posttraumatic cell death are of importance (McIntosh et al., 1998).

The present study investigates the neuroprotective properties of a femtomolar-acting, 8-amino-acid peptide derived from activity-dependent neuroprotective protein (ADNP, 828 amino acids, pI of 5.99) (Bassan et al., 1999). ADNP is regulated by vasoactive intestinal peptide (VIP) (Bassan et al., 1999). First discovered in the intestine (Said and Mutt, 1970), the 28-amino-acid VIP was later found in abundance in neurons of the central and peripheral nervous system, providing neuromodulator, neurotransmitter, growth factor, and neuroprotective functions (Gozes and Brenneman, 1989; Said, 1996; Gozes et al., 1999). VIP gene expression increases during synapse formation, exhibits regulation by synaptic activity, and declines with age (Gozes and Brenneman, 1989). VIP was originally shown to possess neuroprotective activity in electrically blocked neuronal cultures (Brenneman and Eiden, 1986), apparent only in the presence of glial cells (Brenneman and Gozes, 1996; Gozes and Brenneman, 1996).

VIP treatment of astrocytes for 3 h produced an increase in ADNP mRNA (Bassan et al., 1999) and the secretion of growth factors (e.g., ADNF, 14 kDa, pI of 8.3; Brenneman and Gozes, 1996; Gozes and Brenneman, 1996). ADNP and ADNF share a short peptide motif: NAPVSIPQ (NAP) in ADNP and SALLRSIPA (ADNF-9) in ADNF, which exhibit immunological similarity and femtomolar neuroprotection in cell culture (Gozes et al., 1997; Brenneman et al., 1998; Bassan et al., 1999). In adult rats, significant amounts of NAP were shown to reach the brain after intranasal administration (Gozes et al., 2000) and improved performance in the water maze in animals (previously subjected intracere-

ABBREVIATIONS: ADNP, activity-dependent neuroprotective protein; VIP, vasoactive intestinal peptide; ADNF-9, Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SALLRSIPA); NAP, Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (NAPVSIPQ); CHI, closed head injury; TNFα, tumor necrosis factor α; NSS, neurological severity score; MRI, magnetic resonance image; NMDA, *N*-methyl-D-aspartate; APP, amyloid precursor protein; FOV, field of view; TR, repetition time; TE, time to echo; MTS, (3-(4,5-dimethylthiazol-2-yl-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium.

bral injection of the cholinotoxin ethylcholine aziridium). After traumatic brain injury (Chen et al., 1996), the blood-brain barrier is transiently disrupted, suggesting increased availability of NAP to provide protection against secondary damage in this condition.

An experimental model of closed head injury (CHI) in the rat and mouse has been developed and studied mechanistically (Chen et al., 1996). A major role has been proposed for inflammatory cytokines and reactive oxygen species in early post-traumatic pathology (Shohami et al., 1994, 1996; Beit-Yannai et al., 1996). The current study was designed to assess the efficacy of NAP in this mouse model of CHI. On the basis of multiple measurements, significant neuroprotection was demonstrated with NAP treatment. Reduction in TNFα production and protection against TNFα toxicity contribute to the neuroprotective mechanism provided by NAP.

Materials and Methods

Animals and Trauma. Male Sabra mice (Hebrew University strain) weighing 35 to 45 g were used. The study was approved by the Institutional Animal Care Committee of Hadassah Medical Center and the Hebrew University. CHI was induced under ether anesthesia, as previously described (Chen et al., 1996). Briefly, a metal rod weighing 333 g is allowed a free fall from a prefixed height (at 3 cm for a mouse weighing 28–32 g) over the exposed skull covering the left hemisphere in the midcoronal plane. The tip of the rod is covered with silicone, and it delivers the impact to the head that is fixed on the bottom plane of the trauma device. Sham-operated mice were anesthetized, their skull exposed, but trauma was not induced. After CHI the mice were assigned to vehicle or NAP treatment.

Administration of NAP. Fifteen minutes after CHI, mice were injected (subcutaneously) with NAPVSIPQ (Bassan et al., 1999) (synthesized by Peptide Technologies, Bethesda, MD) at a dose of 0.25 to 0.3 μg/g of body weight or with the vehicle (dimethyl sulfoxide diluted in saline 1:20). NAP was previously shown to reach the brain (Gozes et al., 2000). Moreover, the blood-brain barrier was previously shown to be disrupted soon after trauma, and extravasation of the albumin-bound dye Evans blue was enhanced 5- to 6-fold at 4 h post CHI (Chen et al., 1996). It is therefore assumed that NAP can readily cross the blood-brain barrier to reach the brain parenchyma under the same conditions.

Neurobehavioral Evaluation. Mice ($n = 74$) were observed for 14 days after injury and the neurological severity score (NSS) was assessed at 1 h, and 1, 2, 7, and 14 days after injury. The NSS used in the present study is a modification of the original one described in our report on the CHI model and used in a number of studies (Shohami et al., 1995, 1996; Beit-Yannai et al., 1996; Chen et al., 1996, 1997). The number of tasks had been reduced from 25 to 10.

TABLE 1

NSS for head-injured mice

For each failed task the mouse receives 1 point. Maximum = 10 (failure in all tasks), minimum = 0 (success in all tasks).

| Task	NSS
	points
Presence of mono- or hemiparesis	1
Inability to walk on 3-cm-wide beam	1
Inability to walk on 2-cm-wide beam	1
Inability to walk on 1-cm-wide beam	1
Inability to balance on 0.5-cm-wide beam	1
Inability to balance on 0.5-cm-diameter round stick	1
Failure to exit 30-cm circle within 2 min	1
Inability to walk straight	1
Loss of startle reflex	1
Loss of seeking behavior	1
Maximum total	10
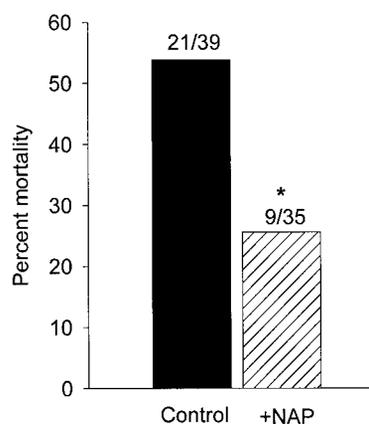
Fig. 1. NAP protects against mortality after closed head injury. A total of 74 mice was subjected to CHI and assigned to two experimental groups, one receiving vehicle (control, $n = 39$) and the other, NAP ($n = 35$). Upon termination of the experiment (2 weeks after CHI) the mortality rate in each group was calculated [control = 21 (53.8%) and NAP-treated = 9 (25.6%) dead animals]. The chi square test (*$P < 0.005$) was used for statistical comparison.

The different tasks are used to evaluate motor ability, balancing, and alertness of the mouse. One point is awarded for failing to perform a particular task (Table 1). When a mouse was dead, it was excluded from the NSS evaluation of that particular day on (and was not scored arbitrarily as 10).

Brain Edema. Mice were sacrificed 24 h after injury, the time of peak edema formation (Chen et al., 1996), and brain cortical samples of ~20 mg were cut from the left (traumatized) and right (contralateral) hemispheres, from the site bordering the lesion. Samples were weighed before and after drying in a desiccated oven for 24 h at 100°C. Water content was calculated as %H_2O = (wet weight − dry weight)/wet weight $\times$ 100.

Magnetic Resonance Image (MRI) Experiments. MRI experiments were performed on a wide-bore 8.4T spectrometer (Bruker, Karlsruhe, Germany) equipped with a mini-imaging accessory (Mini 0.5; Bruker) capable of producing pulsed gradients of up to 20 gauss/cm in three directions. MR images were acquired with a commercial radio frequency transmit/receive head coil having an inner diameter of 3.8 cm. Mice were subjected to controlled head injury. Two groups were studied: 1) control group ($n = 5$) and 2) NAP-treated ($n = 7$). Images were acquired at 22 ± 2 h and 14 days after
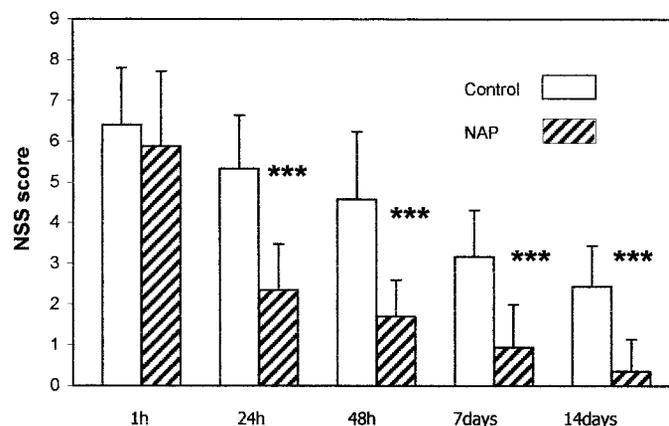
Fig. 2. NSS. Ten different tasks were used to evaluate motor ability, balancing, and alertness. One point was awarded for failing to perform a particular task (Table 1). Mice that died during the 14-day period were excluded from the study (they were not arbitrarily scored as 10). At 1 h, $n = 29$ and 42 in the control and ANP-treated groups, respectively, and at 14 days, $n = 10$ and 15 (***$P < 0.005$, Student's t test).
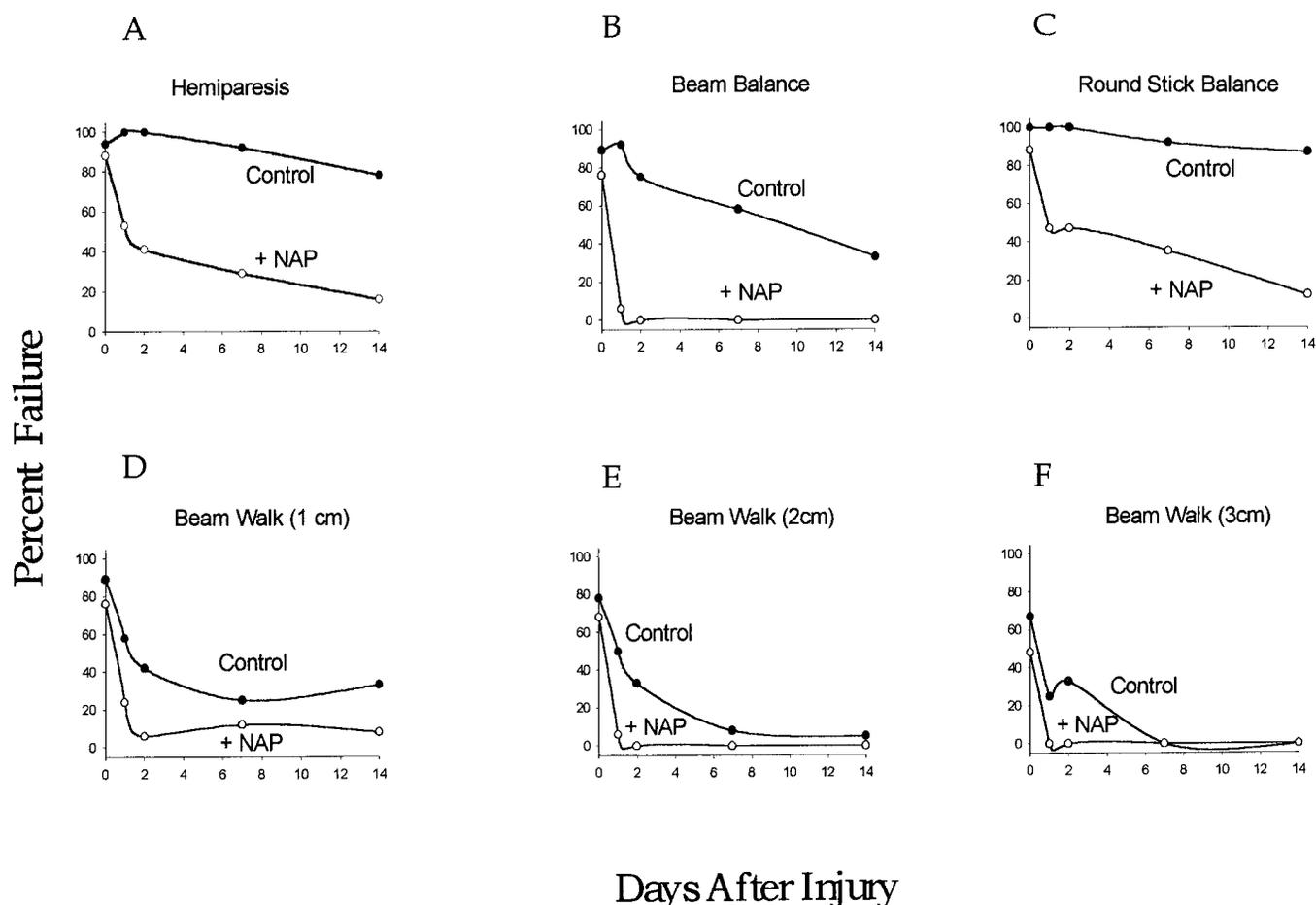
Fig. 3. NAP-facilitated clinical recovery after CHI. Mice were tested for selected motor abilities over 14 days after traumatic brain injury. Six tests are depicted in the figure and the percentage of failure of the control animals (●) and the NAP-treated animals (○) is shown. The chi square test (*$P <$ 0.005) was used for statistical comparison. Hemiparesis, beam balance, and round stick balance were significantly improved by NAP treatment from 24 h up to 14 days ($P < 0.005$). The three tests on beam widths (3, 2, 1 cm) showed significant improvement only at 24 and 48 h after injury. By 7 and 14 days control mice had recovered to an extent similar to that of the NAP-treated animals.

injury. For MRI, the mice were anesthetized with Equtessin (0.2 ml/kg) and placed in a fixing device to prevent head movements.

The MRI protocol included first coronal multislice T_1 weighted images (256 × 128 matrix size, FOV of 3 × 3 cm, TR/TE = 500/15 ms). To control head position, we acquired a saggital T_1 weighted image that was positioned at the higher edge of the fissura rhinalis from which we chose five slices for the T_2 weighted images. After the correct head position was achieved, T_2 weighted images were acquired (256 × 128 matrix size, FOV of 3 × 3 cm, TR/TE = 3000/60 ms, and two averages and a slice thickness of 1.5 mm).

TNFα Measurement. A brain tissue sample of 20 mg was removed from the cortex adjacent to the site of injury at the left (traumatized) hemisphere and was assayed for TNFα levels by enzyme-linked immunosorbent assay kit (Genezyme Diagnostics, Cambridge, MA), and expressed in nanograms per milligram of protein. Samples were taken at 0, 4, and 8 h post CHI, the period during which TNF is up-regulated (Shohami et al., 1994).

PC12 Cells. PC12 cells were maintained in high-glucose Dulbecco's modified Eagle's medium, including heat-inactivated horse serum (8%), fetal calf serum (8%), and glutamine (1 mM). Cells were seeded in 96-well plates (2 × 10^4 cells/well) in medium containing heat-inactivated horse serum (2.5%) and fetal calf serum (2.5%). TNFα (100 ng/ml) was added upon seeding with or without NAP and incubated for 48 h. Cell viability was measured by the MTS assay, a colorimetric assay for mitochondrial function of living cells (Promega, Madison, WI) (Haviv and Stein, 1999).

Results

NAP Reduces Mortality in Head-Injured Mice. Mice ($n = 74$) were subjected to CHI and assigned to two groups [control, vehicle-injected ($n = 39$) and experimental, NAP-injected ($n = 35$)]. Treatment was given 15 min after injury and the mice were evaluated for 14 days. The overall mortality in the control and NAP-treated groups was significantly reduced (chi square test, $P < 0.005$) by more than 50%, as can be seen in Fig. 1.

NAP Facilitates Clinical Recovery from Head Injury. To assess the functional impairment after trauma, a scoring system (NSS) was used based on the ability of the mice to perform 10 different tasks (Table 1). These tests evaluate the motor ability, balancing, and alertness of the mouse. One point is given for failing to perform each of the tasks; thus, a normal, uninjured mouse scores 0. The severity of injury is defined by the initial NSS, evaluated 1 h post CHI, and referred to as NSS1. The NSS1 determines the severity of the trauma and is a reliable predictor of the late outcome. Thus, fatal or near-fatal injury is defined in mice having an NSS1 of 9 to 10, severe injury in mice with an NSS1 of 7 to 8, moderate injury with NSS1 of 5 to 6, and mild injury in mice with an NSS1 of <4.

The control ($n = 18$) and NAP-treated ($n = 26$) mice did not differ in the severity of trauma because their NSS1s were 6.4 ± 1.4 and 5.9 ± 1.84, respectively. It should be noted that in the subgroup of NSS1 of 9 to 10, the protective effect of NAP was most pronounced [91 versus 58% mortality in control and NAP-treated mice, respectively (Student's t test, $P < 0.005$).

Furthermore, the NAP-treated group showed a significantly faster recovery during the 14-day evaluation period (Fig. 2, $P < 0.005$), at the end of which the nontreated mice still had some neurological deficits, whereas the NAP-treated mice had recovered almost completely.

Because NSS is a composite parameter, the data were analyzed for its individual components and the improvement in the ability to perform specific tasks along the time axis at 1 h, and 1, 2, 7, and 14 days after CHI. Figure 3 depicts the percentage of mice failing to perform six of the tasks. Similar failure rates were observed for all tasks at 1 h after injury in both groups (day 0). However, already at 1 day post injury, there were significant differences (using the chi square test) in the percentage of hemiparetic animals and in those failing to perform beam balancing, round stick balancing, and 3-, 2-, and 1-cm-wide beam walks. The failure rate of easier motor tasks and reflexes (circle exiting, straight walk, startle reflex, and seeking behavior) decreased with time in both groups, at a similar rate.

NAP Reduces Brain Edema. Brain edema (percentage of water content) was measured in 11 injured control and nine NAP-treated mice and in four sham (noninjured) mice. All mice were evaluated for NSS at 1 h to validate similar severity of injury [7.0 ± 1.76 (control) and 6.3 ± 1.7 (NAP)] and sacrificed at 24 h post trauma. Edema was measured in the left (injured) and right (contralateral) hemisphere in samples of frontal brain tissue (Fig. 4, top). The water content in the sham mice was $78.4 \pm 0.2\%$. Head injury led to the accumulation of water, primarily in the contused (left) hemisphere, with a 4.74% increase in water content. NAP treatment reduced water accumulation (by >60%, ANOVA followed by Student-Newman-Keuls test, $P < 0.016$) to a level that was not different from that of the sham animals. It should be noted that only minor changes in water content were found in the contralateral hemisphere, and that these were not affected by NAP. The correlation between water content and NSS at 24 h for control ($n = 11$) and NAP-treated mice ($n = 9$) was evident with a highly significant coefficient constant ($r = 0.717$, Pearson test; Fig. 4, bottom). These two parameters are characteristically elevated in brain trauma (Chen et al., 1996).

MRI Evaluation of Trauma, Protection by NAP. T_2 weighted MRIs were previously used to assess edema formation and resolution in a rat model of CHI (Assaf et al., 1997). This technique was used to further evaluate the protective effect of NAP. Figure 5 shows four continuous coronal T_2 weighted MRIs of two representative mice (a, control; b, NAP-treated) acquired 24 h and 2 weeks after CHI. The damage at 24 h was clearly apparent and formation of edema and hemorrhage/brain fractures were evident. Two weeks after trauma, some areas of edema had resolved in the control mouse. However, areas of hyperintensity, which probably represented edema and/or cysts, were observed. Furthermore, tissue mechanical damage was evident. In contrast, in the NAP-treated mouse 2 weeks after injury, most of the
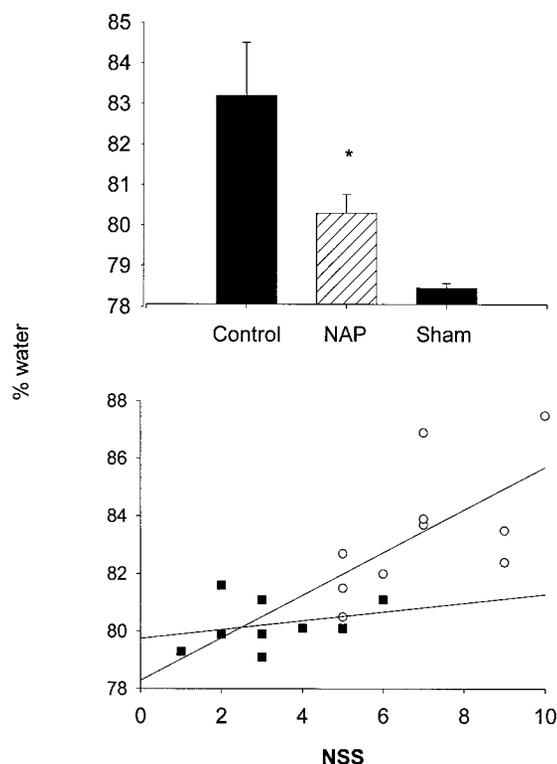

Fig. 4. NAP reduces brain edema. Top, brain water content was determined 24 h after injury in the contused hemisphere of sham-injured, control, and NAP-treated mice. Statistical tests using ANOVA with all pairwise multiple comparison procedures (Student-Newman-Keuls method) indicated differences between control-traumatized mice and both sham- and NAP-treated mice (*$P < 0.016$). NAP treatment reduced edema to levels that were not statistically different from those of the sham mice. Bottom, linear correlation between brain water content and NSS in NAP-treated mice (■) and control (○) mice. $r = 0.717$ by Pearson correlation test.

hyperintensity areas had resolved with only the mechanical tissue damage caused at the time of injury remaining. Because the T_2 weighted hyperintensity represents edema, it is likely that in the NAP-treated mice there was a much more pronounced resolution of the edema 2 weeks after trauma. A summary of the MRI results is given in Fig. 5c, which depicts the percentage of recovery in the T_2 abnormalities in both groups [$46 \pm 12\%$ and $73 \pm 13\%$ for control ($n = 5$) and NAP-treated ($n = 7$), respectively, $P < 0.01$ by Student's t test].

NAP Inhibits Increases in TNFα after CHI. TNFα levels were measured at 0 (noninjured, sham mice), 4, and 8 h after CHI in control and NAP-treated mice ($n = 5$ at each time point). Figure 6A shows the levels of TNFα in the contused hemisphere at various times after injury. At 4 and 8 h, TNFα levels increased to a significantly higher level in the controls ($P < 0.05$), whereas in the NAP-treated mice, the amounts of TNFα remained similar compared with the initial basal concentrations ($P < 0.05$ versus control at 4 h, Student's t test).

NAP Inhibits TNFα-Induced Toxicity in Vitro. To further explore the relationship between NAP-induced neuroprotection and inhibition of TNFα-mediated damage, an in vitro study was conducted in which PC12 cells were exposed to TNFα and its toxicity was quantified (Fig. 6B). When NAP (10^{-17}–10^{-14} M) was added to the culture medium along with the TNFα, the cells were protected and viability was not

a control

24 hours

2 weeks

c

b NAP treated

24 hours

2 weeks
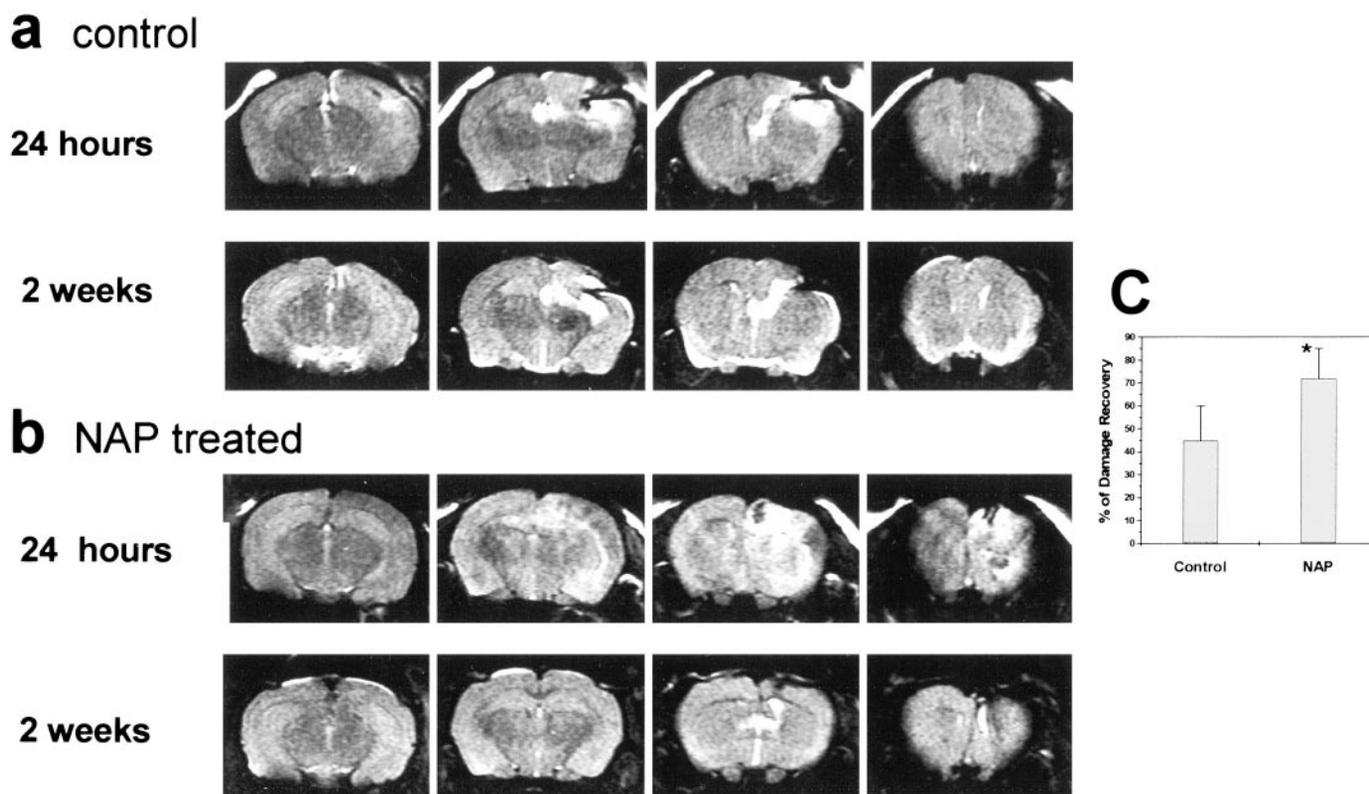
Fig. 5. MRI evaluation of brain damage. Areas of T_2 abnormalities were used to characterize brain damage. Areas of hyper- and hypointensity representing edema, hemorrhage, and tissue mechanical damage were evaluated (by an observer unaware of treatment) for each animal (a, control; b, NAP-treated) at 24 h and 2 weeks after trauma. From the two measurements for each animal, the percentage of reduction in the area of T_2 abnormality was calculated. This provided a measure for development/recovery, as seen by MRI in each animal. The procedure partially compensates for the variability in the severity of the damage, and provides a good and robust measure of the development of damage in each animal, and, hence, in each group. First, percentage recovery in the damaged area was calculated for each animal, and then their grouping (control and NAP-treated) was disclosed and the mean ± S.E.M. was calculated for each group (c). The statistical significance between the two groups ($P < 0.01$) was calculated by Student's t test.

different from that of controls. This protective effect was dose-dependent, and reached statistical significance at NAP concentration of 10^{-14} M ($P < 0.05$, ANOVA followed by Student-Newman-Keuls test). Two independent experiments were performed, each with three repeats.

Discussion

In the present study, NAP treatment produced a dramatic neuroprotection in head-injured mice. This was demonstrated by a reduction in overall mortality rate and facilitation of the functional recovery of the survivors of traumatic brain injury. The correlation between water content and neurological status at 24 h post injury indicated that NAP had beneficial effects on these two parameters, which are not necessarily interdependent.

Neuroprotection can be provided by various strategies aimed at reducing cell death. Nerve growth factor, which maintains target-neuron interactions (Levi-Montalcini et al., 1969), was the first described neurotrophin. Other neurotrophins and their receptors, cytokines, antioxidants, protease inhibitors, glial cell-line-derived neurotrophic factor, and related proteins were discovered. The field of neuroprotection subsequently expanded rapidly with enormous interest in developmental regulation and the potential of these molecules as therapeutic agents (Dragunow et al., 1997; Lapchak, 1998; Zhao and Schwartz, 1998).

The rationale for choosing NAP as a potential protective agent in pathologies associated with CHI is based on previous demonstration of its in vitro and in vivo neuroprotective properties (Bassan et al., 1999). Femtomolar concentrations of NAP rescued rat cerebral cortical neurons from death associated with a wide range of neurotoxic agents, including β-amyloid peptide and NMDA (Bassan et al., 1999). Overstimulation of the NMDA receptors is a leading cause of brain damage and NMDA antagonists are considered as neuroprotective agents against post-traumatic brain damage (Okiyama et al., 1998), implicating NAP as a general neuroprotectant against excitotoxicity. The β-amyloid peptide precursor (the amyloid precursor protein, APP) has been shown to accumulate in traumatically injured axons 1 h after injury. This accumulation may be due to interruption of fast axoplasmic transport and/or up-regulation of APP synthesis. APP immunostaining has been shown to be a reliable method for detecting the damage caused to axons associated with fatal head injury (Gentleman et al., 1995; Oehmichen et al., 1998; Van Den Heuvel et al., 1998). Increases in APP may lead to enhanced β-amyloid production, resulting in a surge in toxic free radicals (Mattson, 1994), a major cause for the progression of traumatic brain injury (Beit-Yannai et al., 1996, 1997), which may be protected by NAP (Bassan et al., 1999). Indeed, in a previous study, NAP protected neuronal cells against decreases in reduced glutathione, a potent endogenous antioxidant (Offen et al., 2000). Daily injection of
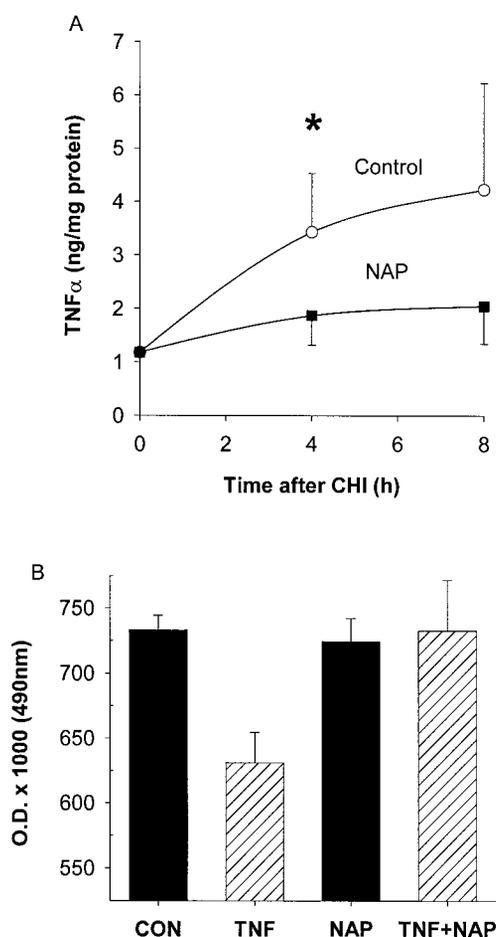
Fig. 6. NAP inhibits TNFα production (A) and toxicity (B). A, mice were subjected to CHI and 15 min later were treated with saline (control) or NAP (0.25–0.3 μg/g b.wt.). They were sacrificed after sham operation (time 0), 4 or 8 h after CHI (n = 5 at each time), their brains were removed, the contused hemisphere separated, and the cytokine extracted and assayed using enzyme-linked immunosorbent assay kit (Genezyme Diagnostics). *P < 0.05 compared with ANP-treated at 4 h, and with sham (0 h). B, PC12 cells were exposed to TNFα (100 ng/ml) with or without NAP (10^{-14} M) for 48 h. Cell viability was measured by the MTS assay, a colorimetric assay for mitochondrial function of living cells. Reduced optical density reflects higher cell death. Two independent experiments were performed, each with three repeats. *P < 0.05 compared with all other groups (ANOVA, followed by Student-Newman-Keuls).

microgram amounts of NAP to newborn apolipoprotein E-deficient mice for the first 2 weeks of life, resulted in accelerated acquisition of developmental milestones of behavior, increased cholinergic activity, and amelioration of cognitive deficits. Closed head injury was earlier shown to further exacerbate cognitive impairments in apolipoprotein E-deficient mice (Chen et al., 1997). Based on these observations, NAP was chosen to be evaluated as an agent against head injury-associated damage.

In the present study, the most pronounced effect of NAP was protection against the mortality and morbidity associated with head trauma. This protection may be reflected, in part, by the dramatic reduction in brain edema, one of the most common and destructive consequences of head injury. The focus was functional recovery in vivo. Earlier in vitro studies had shown the NAP protected primary neurons and neuronal-like cell lines (Bassan et al., 1999; Offen et al., 2000). The protection against brain edema was reflected in both the direct measurement of water content (at 24 h post

injury) and in MRI evaluations, suggesting endothelial cells as additional potential cell targets for NAP's protective effect. The MRI was assessed in the same animal over a period of 2 weeks. In comparison with other protective agents (e.g., HU-211, a novel noncompetitive NMDA antagonist and Tempol, a stable nitroxide radical) tested in the same paradigm (Shohami et al., 1995, 1996; Beit-Yannai et al., 1996), NAP protection was among the best. Although the efficacy of NAP treatment reported here is encouraging, further optimization is required before clinical application.

TNFα is a member of a family of signaling molecules that exert their biological activity by interacting with high-affinity receptors (for review, see Shohami et al., 1999). This proinflammatory cytokine is produced upon stimulation by monocytes, macrophages, T and B lymphocytes, neutrophils, and mast cells. In addition, ischemic and traumatic brain injury induces the release of soluble TNFα from neurons and astrocytes into the extracellular space. TNFα is suggested as one of the mediators of delayed brain damage (Shohami et al., 1999). We recently suggested that in the early hours after trauma, the presence of reactive oxygen species in the injured tissue aggravates its toxicity (Trembovler et al., 1999). It has been recently shown that VIP inhibits the production of TNFα in injured spinal cord and in activated microglia (Kim et al., 2000), while increasing the synthesis of the NAP-containing protein ADNP (Bassan et al., 1999). Therefore, the levels of TNFα were measured in the brains of injured controls and NAP-treated mice at times shown previously for maximal TNFα production (Shohami et al., 1994). Our results showed that NAP prevented the trauma-induced accumulation of TNFα (Fig. 6A) and suggested that the protective effect of NAP might be, at least in part, mediated by inhibiting TNFα toxicity (as demonstrated in PC12 cells; Fig. 6B). Taken together, CHI induces the release of TNFα, which acts as neurotoxic mediator, and the correlation reported here between inhibiting this cytokine and the facilitated neurobehavioral recovery after CHI support TNFα inhibition as one of the protective mechanisms of NAP.

Long-term accumulation of TNFα has been associated with neurodegeneration in AIDS dementia, Alzheimer's, and Parkinson's disease (Bjugstad et al., 1998), and head trauma has been suggested as a major risk factor for Alzheimer's disease (Schofield at al., 1997). The administration of NAP, a novel, very short, and highly efficacious peptide, should thus be further evaluated as a potential drug for amelioration of delayed brain damage after traumatic injury and as a preventive measure against progressive neurodegenerative diseases (Gozes et al., 2000).

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