

Nanodelivery of Cerebrolysin in combination with neprilysin induces neuroprotection in Alzheimer's Disease pathology following brain injury

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ABSTRACT

Our military personnel are often victims of traumatic brain injury (TBI) for which no suitable therapeutic measures are available till now. Since neprilysin (NPL) and endogenous enzyme is known to downregulate in TBI and also in Alzheimer's disease (AD), in this innovation we found that nanodelivery of Cerebrolysin using TiO₂-nanowired biotechnology together with NPL markedly attenuated brain pathology following TBI. Interestingly, when AD like symptoms were induced by intracerebroventricular (i.c.v.) administration of amyloid beta peptide (A β P 1-40, 250 ng/10 μ l) following TBI for 4 weeks exacerbation of brain pathology occurs. In this situation, co-administration of TiO₂ nanowired NPL (1 μ g in 10 μ l) and Cerebrolysin (25 μ l) significantly reduced brain pathology and achieved remarkable neuroprotection. These observations are the first to demonstrate that nanodelivery of cerebrolysin together with NPL is capable to induce AD pathology exacerbated by TBI, not reported earlier.

Keywords: Alzheimer's Disease, brain pathology, traumatic brain injury, Neprilysin, Cerebrolysin, nanodelivery

1 INTRODUCTION

Neprilysin (NPL) is an endogenous enzyme that functions as rate-limiting step in amyloid-beta peptide (A β P) degradation [1,2]. There are reasons to believe that

an imbalance between production and clearance of A β P results in its accumulation leading to development of Alzheimer's Disease (AD) [3-6]. In several cases of AD the metalloprotease NPL brain concentration is decreased [6]. Also NPL knocked out mice exhibited AD like brain pathology and behavioural dysfunctions [4-6]. This suggests that enhancing the NPL concentrations by therapeutic means may reduce brain pathology in AD. Recently some evidences suggest that focal brain injury or traumatic head injury could also induce alterations in NPL activity in the brain and in the CSF [1,3,4]. Although brain injury alone could result in deposition of A β P in the brain indicating that AD may result following brain trauma [7,8]. Thus, it is interesting to find out whether traumatic brain injury (TBI) could further exacerbate A β P infusion induced brain pathology. Furthermore, in such situation whether NPL has any protective role if administered exogenously either alone or with other neuroprotective agents e.g., cerebrolysin.

2 MATERIALS & METHODS

Experiments were carried out on Male Wistar rats (200-250 g body weight) housed at controlled room temperature (21 \pm 1°C) with 12 h light and 12 h dark schedule. Food and tap water were supplied *ad libitum* before the experiment. All the experiments were carried out according to the Guidelines & Care for Laboratory Animals as described by National Institute of Health and approved by Local Institutional Ethics Committee.

2.1 Alzheimer's Disease Pathology

AD like brain pathology was produced by A β P (1-40) infusion intracerebroventricularly (i.c.v.) in the left lateral ventricle 250 ng/10 μ l once daily for 4 weeks using standard procedures in normal and TBI animals [3]. After 30 days of infusion, various brain pathological symptoms were measured as described earlier [2,3,6].

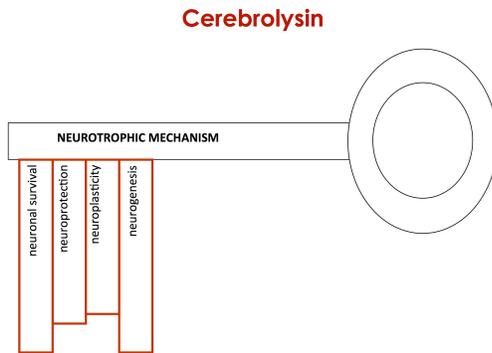


Fig. 1. Cerebrolysin is a multimodal drug and powerful neuroprotective agent.

2.2 Traumatic Brain Injury

Traumatic brain injury (TBI) was produced in separate group of rats under Equithesin Anesthesia over the right parietal cerebral cortex by making a longitudinal incision (2 mm deep and 4 mm long) (Fig. 2). In these rats A β P infusion was made in the left lateral cerebral ventricle as mentioned above [3].

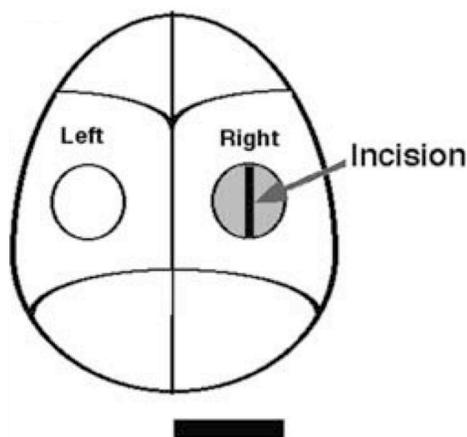


Fig. 2. Traumatic brain injury (TBI) model showing longitudinal incision (2 mm deep and 4 mm long) over the right parietal cerebral cortex. A β P was infused in the left lateral cerebral ventricle (i.c.v.). Bar = 5 mm.

2.3 Brain Pathology

After 30 days of the 1st A β P infusion in normal or TBI rats, The BBB breakdown was examined using Evans blue (EB) and [¹³¹I]Iodine leakage across the brain microvessels after intravenous administration of these tracers (EB 2 % solution 3 ml/kg, and radioiodine 100 μ Ci/kg) 5 min before the end of the experiment. Brain edema was determined using regional water content by wet and dry weights of the brain samples [3]. In separate groups of animals, immunohistochemistry of albumin, and A β P was examined using standard procedures. Neuronal changes were studied using histopathological examination of Nissl or Haematoxylin & Eosin (HE) staining [3,7,8].

2.4 TiO₂-nanowired delivery of Cerebrolysin

Cerebrolysin (CBL, Ever NeuroPharma, Austria) was tagged with TiO₂ nanowires according to standard protocol [3]. The TiO₂ nanowired Cerebrolysin (25 μ l, NWCBL) was infused into the left cerebral ventricles daily starting from 1 week after the onset of A β P infusion and terminated 1 week before the last infusion. For comparison, normal CBL was administered in identical doses instead of NWCBL [3,7,8].

2.5 TiO₂-nanowired delivery of Neprilysin

Recombinant soluble neprilysin (NPL, BML-SE532-0010, Enzo Life Sciences, Inc. Farmingdale, NY, USA) was tagged with TiO₂ nanowires using standard procedures. Nanowired NPL (NWNPL) was co-administered (i.c.v., 01 μ g in 10 μ l) with NWCBL in both healthy and TBI animals as mentioned above (see Table 1).

2.6 Statistical Analyses

ANOVA followed by Dunnett's test for multiple group comparison with one control was used to analyze statistical significance of the data obtained. A p-value less than 0.05 was considered significant.

3 RESULTS

3.1 AD Brain Pathology in TBI

After 30 days of the 1st A β P infusion, normal rats exhibited profound breakdown of the BBB as evident with extravasation of endogenous serum albumin, Evans blue and radioiodine in the cerebral cortex, hippocampus thalamus, hypothalamus and the cerebellum that was 2- to 4-fold exacerbated in TBI animals (Table 1, Fig. 3). The brain edema formation and neuronal damages were also exacerbated by several folds in TBI group as compared to normal animals after A β P infusion. The deposition of A β P was also enhanced in the TBI group as compared to intact rats (Table 1).

Table 1. Effect of co-administration of TiO₂ nanowired Cerebrolysin (25 µl, NWCBL) and TiO₂ nanowired NPL (1 µg in 10 µl, NWNPL) in Alzheimer's Disease (AD) pathology in normal and in traumatic brain injured (TBI) rats induced by AβP (1-40) infusion intraventricularly (i.c.v.) in the left lateral ventricle 250 ng/10 µl once daily for 4 weeks.

Expt. Type	saline Control		AβP infusion		NWCBL+AβP infusion		NWCBL+NWNPL+AβP infusion	
	Intact	TBI	Intact	TBI	intact	TBI	Intact	TBI
A. Blood-brain barrier dysfunction								
1. Evans Blue mg %	0.25±0.05	1.34±0.23*	1.34±0.08*	2.78±0.10*#	0.32±0.12*	0.65±0.11*#§	0.20±0.08	0.34±0.08°
2. ¹³¹ Iodine %	0.34±0.08	2.45±0.22*	3.56±0.14*	4.23±0.14*#	0.45±0.11*	1.48±0.13*#§	0.44±0.08	0.67±0.11°
B. Brain Edema Formation								
1. Brain water content %	74.34±0.14	76.49±0.21*	78.67±0.14*	80.32±0.21*#	74.87±0.08*	77.65±0.21*#§	75.01±0.18*	76.21±0.32°
2. Volume Swelling % f	nil	+8	+16	+24	+1	+12	+3	+6
C. Brain Pathology								
1. AβP deposits nr/area	1±1	6±2*	23±8*	56±12*#	8±4*	12±4*#§	10±4*	14±2°
2. Albumin deposit nr/area	2±3	23±6*	78±14*	156±23*#	12±6*	86±8*#§	12±8*	24±10°
3. Neuronal injury nr/area	2±1	56±12*	88±23*	201±34*#	23±8*	89±6*#§	23±12°	34±18°

Data represents parietal cerebral cortex of 6 to 8 rats at each point. * P <0.05 from saline control, # P <0.05 from TBI control, ° P <0.05 from NWCBL treated, § P <0.05 from NWCBL+NWNPL treatment. 1 % increase in brain water = ca. 4 % increase in % f, nil = absent. ANOVA followed by Dunnett's test for multiple group comparison from one control.

3.2 AβP and Albumin in AD Brain

Our immunohistochemistry investigation showed several fold increase in the AβP deposition along with albumin immunoreaction following TBI in in AβP infused group in several brain areas [Table 1, Fig. 3]. In these AD brains AβP deposits, albumin leakage (Fig. 3), gliosis and axonal damages (results not shown) were also most prominent.

3.3 Neuronal injuries in AD brain

AβP infusion in TBI also exacerbated neuronal injuries in several brain regions e.g., cerebral cortex, hippocampus, thalamus, hypothalamus and cerebellum (results not shown). An example of cortical nerve cell damage is shown in Table 1 in AD brain in intact and in TBI groups.

3.4 Effect of Cerebrolysin Treatment

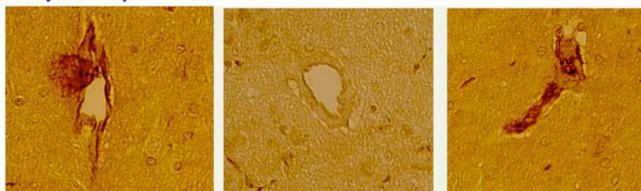
When TiO₂ nanowired Cerebrolysin (25 µl, NWCBL) was infused into the left cerebral ventricles starting from 1 week after the onset of AβP infusion and terminated 1 week before the last infusion, significant reduction of AβP and albumin immunoreactivity was seen in intact animals (Fig. 3) as compared to normal cerebrolysin infusion (see Fig. 3). However, NWCBL alone has minimal neuroprotective effects following AβP infusion in TBI group (Table 1).

3.5 Effect of Neprilysin co-administration

The NPL alone was able to reduce some of the brain pathology in healthy animals after AβP infusion (results not

shown). On the other hand a combination of NWCBL and NWNPL resulted in profound neuroprotection in TBI on brain pathology following AβP infusion (Table 1).

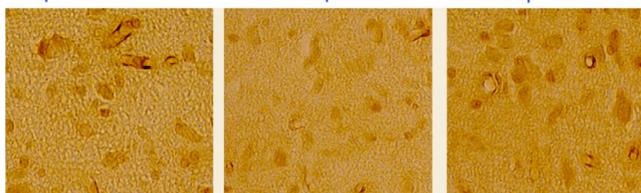
Amyloid deposit around the blood vessels in cortex



AβP infusion

NW CBL+AβP infusion

CBL+AβP infusion



Albumin immunoreactivity in cortex

Fig. 3. TiO₂ Nanowired cerebrolysin (NWCBL) significantly reduced AD pathology as seen AβP deposits (upper panel) and albumin leakage (lower panel) in the rat after AβP infusion (x 40).

4 DISCUSSION

Our novel findings demonstrate that TiO₂ nanowired cerebrolysin in combination with TiO₂ NPL potentiates neuroprotection following AβP induced brain pathology in both normal and in TBI animals. Our observations are the first to point out that TiO₂-nanowired NPL significantly thwarted AβP deposition along with albumin immunoreactivity in AD brain. Another salient new

findings of our investigation showed that a focal TBI exacerbates A β P induced brain pathology associated with greater deposits A β P in various brain areas. This indicates that our military personnel with TBI are more vulnerable to AD like symptoms with advancing age.

The reasons for exacerbation of brain pathology in AD following TBI are not well known. However, it appears that TBI induced breakdown of the BBB and A β P deposit could further aggravate pathological, immunological and neurochemical reactions leading to greater brain damage [3,7,8]. Since NPL is known to clear A β P deposition faster in AD models, obviously a combination of NWCBL and NWNPL is more effective in inducing neuroprotection in our AD model with or without TBI. Interestingly, NWCBL alone has minimal neuroprotective effects in AD model in TBI co-administration of NWNPL and NWCBL is a powerful tool to achieve greater neuroprotection in AD following TBI.

The basic mechanisms by which cerebrolysin is able to thwart brain pathology in AD could be due to strengthening of the BBB function and thereby reducing vasogenic brain edema formation [2-6]. Restoration of BBB function and reduction in brain edema are instrumental in neuronal survival [3]. Enhancement of neuroprotection by NWCBL may either be due to an enhanced penetration of the drug within the brain or to a slow degradation or metabolism of cerebrolysin within the brain [3,5,6]. A combination of NWNPL and NWCBL could be more powerful in neutralizing A β P deposition that is neurotoxic in the brain microenvironment [7,8]. Thus, co-administration of these neuroprotective agents has superior neuroprotective activity in AD models in TBI.

5 CONCLUSION

In conclusion, our observations are the first to show that AD pathology is exacerbated in TBI following identical A β P infusion. This indicates that TBI exacerbates AD symptoms. Furthermore our investigation showed that co-administration of nanowired cerebrolysin and neprilysin is far more effective in reducing AD brain pathology in TBI. This suggests that NPL and cerebrolysin are complimentary to each other and cerebrolysin may potentiate the effect of NPL in downregulating A β P metabolism in the brain, not reported earlier.

It remains to be seen whether NWCBL and NWNPL when co-administered at various time intervals after A β P infusion in TBI could also be able to reduce brain pathology in our AD models. This is a feature that is currently being investigated in our laboratory.

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