Nanodelivery of cerebrolysin reduces depressive stress induced exacerbation of Alzheimer’s disease brain pathology following amyloid-beta peptide infusion

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ABSTRACT

Previous studies show that stressful situations alone induce breakdown of the blood-brain barrier (BBB) and neuronal damages that is long lasting. Thus, a possibility arises that breakdown of the BBB in stress could play critical roles in development of AD. In present innovation we demonstrated that AD induced brain pathology caused by amyloid beta peptide (AβP) infusion is exacerbated in rats subjected to chronic hypertension either induced by repeated immobilization for 2 h for 1 week or renal hypertension produced by 2 Kidney 1 clip (2K1C) method. Chronic hypertension (CHR) induced marked BBB breakdown to Evans blue albumin and radioiodine tracers in the cerebral cortex, hippocampus, thalamus, hypothalamus, caudate nucleus, cerebellum and brainstem from the naive rats. Infusion of AβP in these CHR rats further enhanced the BBB breakdown to protein tracers by several-folds and aggravation of neuronal damages, astrocytic activation and brain swelling. It appears that TiO2-nanowired delivery of cerebrolysin has superior neuroprotective effects in this AD model as compared to PLGA-delivery of identical doses of cerebrolysin. Taken together our observations are the first to demonstrate that CHR exacerbates AD brain pathology and nanodelivery of cerebrolysin has superior neuroprotective effects, not reported earlier.

Keywords: Alzheimer’s Disease, Brain Pathology, chronic hypertension, immobilization stress, Cerebrolysin, TiO2 nanowired delivery, PLGA-nanoparticles, Neuroprotection

1 INTRODUCTION

Military personnel are highly vulnerable to Alzheimer’s disease (AD) [1]. This is because of the fact that severe stress of trauma, sleep deprivation or combat stress causes increased deposition of amyloid beta peptide in the cerebrospinal fluid (CSF) and in brain parenchyma [1-3]. Continued stress in military is one of the main causes of development of hypertension and mental abnormalities [4-6]. Thus, exploration of novel therapeutic strategies are needed to reduce brain damage in military following stress and thwarting development of AD like brain pathology.

Previous studies show that stressful situations alone induce breakdown of the blood-brain barrier (BBB) and neuronal damages that is long lasting. Thus, a possibility arises that breakdown of the BBB in stress could play critical roles in development of AD. In present investigation we explored whether AD induced brain pathology caused by amyloid beta peptide (AβP) infusion is exacerbated in rats subjected to chronic hypertension (CHR) either by repeated immobilization that induces mild hypertension or by employing 2 Kidney and 1 Clip method to produce renal hypertension on AbP infusion induced modulation of AD brain pathology.
Furthermore, in order to induce neuroprotection in AD models, we used cerebrolysin, a balanced composition of several neurotrophic factors and active peptide fragments on BBB breakdown and brain pathology. We also examined two different modes of nanodelivery i.e., TiO2 nanowired delivery or PLGA-loaded nanoparticles containing cerebrolysin to compared the efficacy of neuroprotection in AD model.

2 MATERIALS & METHODS

Experiments were carried out on Male Wistar rats (200-250 g body weight) housed at controlled room temperature (21±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 AβP model of Alzheimer’s Disease

AD like brain pathology was induced by AβP (1-40) administration intraventricularly (i.c.v.) in the left lateral ventricle 250 ng/10 µl once daily for 4 weeks using standard procedures [3]. After 30 days of infusion, various biochemical and pathological parameters were measures as described earlier [1,3]. (see Fig. 1) [1,4].

2.2 Chronic hypertension

Chronic hypertension (CHR) was induced in naïve rats either by subjecting them to 2 h immobilization stress in a tube for 1 week daily, or constricting the renal artery flow by inserting a silver clip to compress the left renal artery (0.5 mm) for 4 weeks. In both the situations the mean arterial blood pressure (MABP) was elevated to 120 to 140 mmHg in stressed rats as compared to control group (MABP 90 to 105 mmHg).

![Fig. 2. Regional blood-brain barrier (rBBB) breakdown in AβP infusion model of AD in normal and CHR rats as compared to controls. ** P<0.01 from control, # P <0.05 from AβP infusion.](image)

![Fig. 3. Effect of TiO2 nanowired cerebrolysin vs. PLGA nanoparticles induced delivery on regional blood-brain barrier (rBBB) breakdown in AβP infusion model of AD in normal and CHR rats as compared to controls.](image)

2.3 Brain Pathology

In control, AβP infused rats or PLGA-cerebrolysin or TiO2 nanowired-cerebrolysin (NWCBCL) treated animals blood-brain barrier (BBB) breakdown to radioiodine ([131]Iodine) was examined after intravenous administration (100 μCi/kg) 5 min before the end of the experiment [1-5]. Brain edema was determined using regional water content by wet and dry weights of the brain samples [3,6]. In separate groups of animals, neuronal changes were studied using histopathological examination of Nissl staining. Furthermore, deposition of AβP in the brain and leakage of...
serum albumin in the brain was evaluated using immunohistochemistry by employing Anti-beta Amyloid antibody (ab2539) and Anti-Albumin antibody [EPSISR1] (ab137885), respectively.

2.4 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 and Brain Pathology

In our hands, after 30 days of 1st AβP infusion, the rats exhibited profound breakdown of the BBB as evident with extravasation of endogenous serum albumin as well as exogenous protein tracers, e.g., EB and radi iodine in the cerebral cortex, hippocampus and the cerebellum [Figs. 1-4]. In these brain areas, significant increase in brain water content was also observed indicating brain edema formation. Immunohistochemical studies showed deposition of AβP around microvessels in the cortex, hippocampus and cerebellum.

Interestingly, BBB [Fig. 1] or rBBB [Fig. 2] or extravasation of endogenous serum albumin or AβP depositions were much more pronounced in chronic hypertensive rats (CHR) as compared to normal healthy animals [Fig. 4]. Morphological studies showed neuronal damages in various brain areas showing albumin leakage or AβP depositions. These neuronal damages are located in the edematous areas of the brain where activation BBB breakdown is clearly evident [Fig. 4]. Exacerbation of brain pathology in CHR groups following AβP infusion was not much different whether hypertension was produced either by immobilization stress or 2Kidney 1Clip method.

3.2 TiO2 Cerebrolysin and Brain pathology in AD

Treatment of animals in AD group by TiO2-nanodelivery of Cerebrolysin (NWCBL) in a dose of 3 ml/kg, i.v.) starting after 2-days of AβP administration resulted in a marked decrease in the BBB breakdown, edema formation, AβP depositions and serum albumin leakage [Fig. 1-4]. Interestingly, CHR group after AβP infusion also exhibited marked neuroprotection following TiO2 nanowired delivery of cerebrolysin [Figs. 1-4].

3.3 PLGA Cerebrolysin and Brain pathology in AD

The neuroprotective effect of PLGA-nanoparticles labelled cerebrolysin was also effective in reducing BBB breakdown, brain edema formation, neuronal injuries, AβP deposits and serum albumin leakage [Figs. 1-4]. However, it appears that the magnitude and intensity of neuroprotection was more pronounced following TiO2 nanowired delivery of cerebrolysin in our AD model in both normal and in CHR group [Fig. 1-4]. The NWCBL was also able to able to reduce neuronal injuries in all brain areas examined as compared to normal CBL (results not shown).

Fig. 4. Deposition of AbP (upper panel) and leakage of serum albumin in the cerebral cortex of AD rats after AbP infusion in CHR group and theirreduction in TiO2 nanowired cerebrolysin (CBL) or PLGA-labelled cerebrolysin (CBL). It appears that NWCBL has some superior effects on reducing AbP deposition or albumin leakage (x 40).

4 DISCUSSION

The salient findings in this investigation clearly demonstrate that Cerebrolysin that is a multimodal drug composed of several neurotrophic factors and active peptide fragments is capable to induce significant neuroprotection in AD [3,7]. Furthermore our innovation showed that nanodelivery of cerebrolysin tagged with TiO2 nanowires potentiated the neuroprotective efficacy of cerebrolysin in our AD model in chronic hypertensive (CHR) group. This indicates that TiO2-cerebrolysin could be one of the novel therapeutic advances in treating future AD cases accompanied with co-morbidity factors e.g., hypertension.

Our observations further show that hypertension either produced by 2Kidney 1Clip method or by repeated immobilization stress did not differ in augmenting AD induced brain pathology following AbP infusion. This suggests that the possible adverse mechanism of renal hypertension or emotional stress induced hypertension could be similar with regard to brain pathology in AD.
Hypertension either produced by drugs, renal artery compression or stress induced mechanisms could exert powerful mechanical load over the cerebral microvessels making them more vulnerable to any additional insults [1,7]. This could be one of the reason that AbP infucon induced AD may have much more aggravated brain pathology in CHR group, not reported earlier. This suggests that our AD model could be used to evaluate drug efficacy for exploring new therapeutic strategies or effects of other co-morbidity factors e.g., diabetes or drugs of abuse on brain pathology.

Another important observation from our AD model shows that AD cases could induce breakdown of BBB to proteins. Extravasation of protiens into the cerebral microenvironment leads to brain edema formation. Obviously, vasogenic edema formation and accumulation of edema fluid within the extra- or intra-cellular microenvironment could results in neuronal, glial and axonal pathologies [3-4].

Interestingly, TiO2 cerebrolysin therapy significantly attenuated BBB breakdown resulting in neuroprotection as evident in AD group including CHR. This suggests that TiO2 delivery of cerebrolysin is required to attenuate brain pathology in AD with CHR [5,6].

The reasons for NWCBBL effectiveness in PD could be due to quick and deeper penetration of CBL as well as slow and sustained release of the compound for long time that is needed for effective neuroprotection [3-8]. A slow degradation or metabolism of NWCBBL within the brain may also be responsible for maintaining high level of CBL in PD resulting in superior neuroprotection [8]. Although PLGA labelled CBL was also quite effective in reducing brain pathology in AD with or without CHR, some minor differences in neurorpeotecton could be due to penetration of CBL into the brain when used PLGA-nanodelivery as compared to TiO2 nanowired administration of the compound. However, this is a feature that requires additional investigations.

5 CONCLUSION

In conclusion, our observations are the first to point out that timed TiO2 nanowired delivery of CBL has far more superior neuroprotective effects in AD than PLGA laoded nanoparticles, not reported earlier. Further research using prolonged therapeutic time window is currently being investigated in this model to find out a suitable role of TiO2-nanowired delivery of CBL in clinical situations for the benefit of AD victims in Military or civilian populations.

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7 REFERENCES


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