Timed release of cerebrolysin using titanate nanospheres induces neuroprotection in Parkinson's Disease

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ABSTRACT

In this innovation, we report the potential neuroproetctive effects of slow release of Cerebrolysin, a multimodal drug comprising a balance composition of several neurotrophic factors and active pepride fragments on brain pathology induced by Parkinson's Disease (PD). PD like symptoms was induced in the mice by intraperitoneal injections of or 1-metyl-4-fenyl-1,2,3,6-tetrahydropyridin (MPTP, 20 mg/kg) daily within 2-h intervals for 5 days. A significant decrease in dopamine (DA) and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) along with the number of tyrosine hydroxylase (TH) positive cells in the Substantia Nigra Pars Compacta (SNpc) and striatum (STr) confirmed the validity of our model

Our observations showed that timed release of CBL using titanate nanospheres (TiNS) (in a dose of 3 ml/kg, i.v.) when given after 2-days of MPTP administration for 5 days resulted in a marked increase in TH-positive cells in the SNpc and STr as compared to normal CBL. Also TiNS-CBL resulted in significantly higher levels of DA, DOPAC and HVA in SNpc and STr on the 8th day as compared to normal CBL. Tehse observations are the first to point out that timed release of TiNS-CBL has far more superior neuroprotective effects in PD than normal CBL, not reported earlier.

Keywords: Parkinson's Disease, Brain Pathology, MPTP neurotoxicity, Cerebrolysin, nanodelivery, Titanate nanospheres, Neuroprotection

1 INTRODUCTION

Military personnel during combat operations are highly susceptible to traumatic brain and spinal cord injury. There are reasons to believe that a focal injury to the central nervous system (CNS) causes multiple biochemical and pathological changes in the fluid microenvironment in which neurons and glial cells are suspended. This would lead to a process of cascade leading to neurodegenerative diseases e.g., Parkinson's Disease (PD) and/or Alzheimer's Diseases (AD) [1,2]. So far no suitable therapeutic strategies are available to reduce or contain the pathophysiology of PD/AD in clinics. Thus, there is an urgent need to explore new therapeutic strategies to reduce pathophysiological consequences of AD/PD. Since Cerebrolsyin (CBL) is a balanced composition of several neurotrophic factors and active peptide fragments, the drug could be more beneficial in treating PD [3,4]. Thus, in present investigation we examined nanodelivery of CBL using titanate nanospheres in an animal model of PD and evaluated the pathophysiological responses in the brain.

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 MPTP model of Parkinson's Disease

PD like symptoms was induced in the mice by intraperitoneal injections of or 1-metyl-4-fenyl-1,2,3,6-tetrahydropyridin (MPTP, 20 mg/kg) daily within 2-h intervals for 5 days [1,2]. This treatment results in PD like symptoms after 1 week. This was confirmed on the 8th day by observing a significant decrease in dopamine (DA) and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Furthermore, marked decrease in the number of tyrosine hydroxylase (TH) positive cells in the Substantia Nigra Pars Compacta (SNpc) and striatum (STr) further confirmed the validity of our model (see Fig. 1) [1,4].

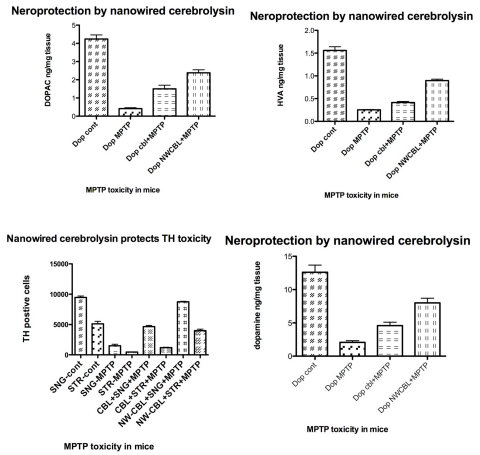

Fig. 1. Slow release of cerebrloysin (TiNS-CBL) reuces pathophysiology of PD in MPTP mice.

2.2 Timed release of Cerebrolsyin

In separate group of rats, timed release of cerebrolysin (Fig. 1) (CBL) using titanate nanospheres (TiNS) (in a dose of 3 ml/kg, i.v.) (Fig. 2) was given after 2-days of MPTP administration for 5 days.

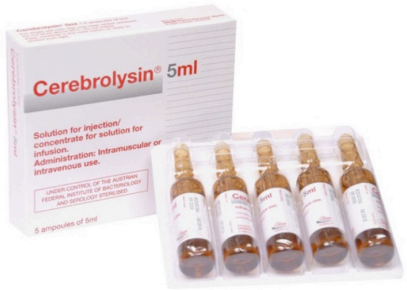

Fig. 2. Cerebrolysin (Ever Neuro Pharma, Austria) is a powerful multimodal neuroproetctive drug in central nervous system diseases.

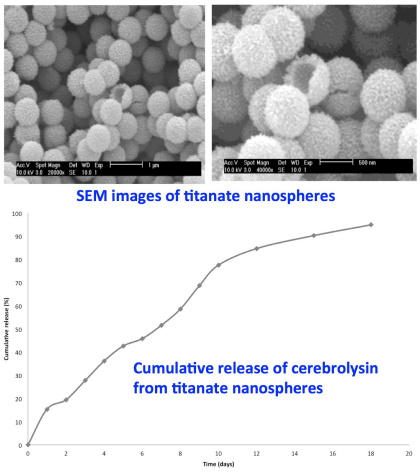

Fig. 3. Slow release of Cerebrolsyin from Titanate nanospheres (TiNS).

2.3 Brain Pathology

In control, PD untreated or cerebrolysin alone or nanowired-cerebrolysin (NWCBL) 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 [1,3]. Brain edema was determined using regional water content by wet and dry weights of the brain samples [3]. Before measuring these pathological changes, motor function was evaluated on Rota-Rod treadmill (see Table 1) using standard procedures. In separate groups of animals, neuronal changes were studied using histopathological examination of Nissl staining and astrocytic activity was evaluated using glial fibrillary acidic protein (GFAP) immunoreactivity [3,4].

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 PD and Brain Pathology

In our hands, MPTP (20 mg/kg) daily within 2-h intervals for 5 days resulted in PD like symptoms on the 8th day (see Fig. 1). Thus, biochemical measurements exhibited significant decrease in dopamine (DA) and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the PD brains as compared to controls (Fig. 1). Also, immunohistochemical studies showed marked decrease in the number of tyrosine hydroxylase (TH) positive cells in the Substantia Nigra Pars Compacta (SNpc) and striatum (STr) (Fig. 1).

Furthermore, SNpc, STr and brain stem exhibited significant extravasation of radioiodine in PD brains indicating breakdown of the BBB (Table 1). Interestingly, these brain areas also showed prefund edema formation as evident with significant increase in tissue water content of as compared to controls (Table 1).

Morphological studies showed neuronal damages in various brain areas in PD brain including cerebrla cortex (Fig 4), SNpc, STr, thalamus, hypothalamus and brain stem (results not shown). These neuronal damages are located in the edematous areas of the brain where activation of astrocytes as evidence with increased immunoreactivity of GFAP is seen (Fig. 4).

3.2 TiO2 Cerebrolysin and PD Biochemistry

Treatment of animals in PD group by TiO2-nanodelivery of Cerebrolsyin (NWCBL) in form of timed release of CBL using titanate nanospheres (TiNS) (in a dose of 3 ml/kg, i.v.) starting after 2-days of MPTP administration for 5 days resulted in a marked increase in TH-positive cells in the SNpc and STr as compared to normal CBL (Fig. 1). Also TiNS-CBL resulted in significantly higher levels of DA, DOPAC and HVA in SNpc and STr on the 8th day as compared to normal CBL. (Fig. 1).

Table 1. Neuroprotective effects of TiO2-Cerebrolysin in MPTP Induced Parkinson's Disease in Mouse

Type of Expt.	Control	MPTP-PD	CBL+MPTP-PD	NWCBL+MPTP-PD
		A. Blood-brain barrier disturbances $^{[131]}$-Iodine leakage %		
SNpc	0.21±0.02	1.21±0.11**	0.68±0.08*#	0.34±0.11*#§
Striatum	0.18±0.06	1.32±0.09**	0.74±0.10*#	0.43±0.08*#§
Brain Stem	0.12±0.04	0.89±0.06**	0.56±0.09*#	0.23±0.06*#§
		B. Brain Edema formation % brain water		
SNpc	64.34±0.21	67.34±0.21**	66.23±0.26*#	65.08±0.10*#§
Striatum	65.32±0.18	68.28±0.16**	67.17±0.22*#	66.08±0.76*#§
Brain Stem	63.43±0.15	66.67±0.25**	65.03±0.14*#	63.87±0.08*#§
		C. Rota-rod performances 16 rpm staying duration (sec)		
Staying Time	120±0	60±6**	82±8*#	108±6*#§

Data are Mean±SD of 6 to 8 mice at each time point. SNpc = Substantia niagra pars compacta, CBL = Cerebrolysin, ** P <0.01, * P<0.05 from saline control, # P <0.05 from MPTP-PD group, § P <0.05 from CBL-MPTP-PD group

3.3 TiO2 Cerebrolysin and Brain pathology

NWCBL in form of TiNS significantly reduced the BBB disruption and edema formation as compared to normal CBL therapy (Table 1).

The NWCBL was also able to able to reduce neuronal and glial cell injuries in all brain areas examined as compared to normal CBL (results not shown). An example of neurorestoration in cerebral cortex by NWCBL is shown in Fig. 4. Also in this Fig. activation of GFAP was reduced in NWCBL treated PD group (Fig, 4). (results not shown).

3.4 TiO2 Cerebrolsyin on Motor function

NWCBL treatment resulted in far superior performances in PD group on Rota-rod treadmill as compared to normal CBL given in identical conditions (See Table 1).

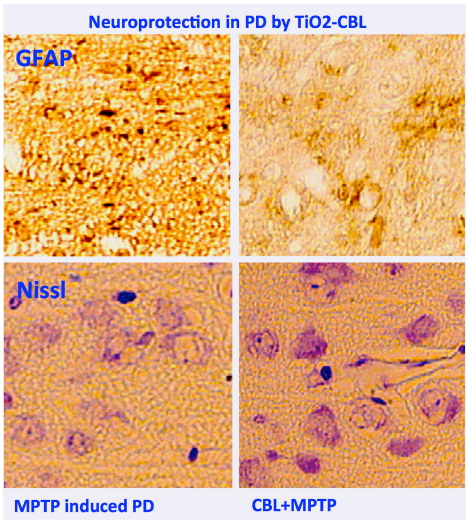

Fig. 4. Slow release of Cerebrolsyin from Titanate Nanosphere (TiNS-CBL) markedly reduces MPTP induced pathological damages in neurons (lower panel) as well as glial cells (upper panel) in mice cortex (x 40).

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 PD. Furthermore our innovation showed that nanodelivery of cerebrolysin tagged with TiO2 nanospheres (TiNS) potentiated the neuroprotective efficacy of cerebrolysin in our PD model. This indicates that TiO2-cerebrolysin could be novel therapeutic advances in treating future PD cases in clinics.

Our observations further show that MPTP when administered in mice repeatedly induces profound PD like symptoms as evidenced with biochemical measurement of neurochemicals or loss of TH immunoreactivity in several brain areas. This suggests that our PD model could be used to evaluate drug efficacy for exploring new therapeutic strategies.

Another important observation has come out from our PD model showing that in PD cases profound breakdown of BBB to proteins also occurs, not reported earlier. Extravasation of proetins 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 injuries [3,4]. This is clearly evident in these investigations in our PD model that showed profound leakage of radioiodine in several brain areas exhibiting neuronal and glial cell injuries. Also in these brain areas expansion of neuropil and sponginess is evident (Fig. 4).

Interestingly, TiO2 cerebrolysin therapy significantly attenuated BBB breakdown resulting in neuroprotection as evident in PD group that did not show edema formation or neuronal or glial cell injuries. When cerebrolysin is given alone higher dose of the drug (10 ml (kg) is needed to induced identical neuroprotection as compared to TiO2-cerebrolsyin (3 ml/kg). This suggests that TiO2 delivery of cerebrolysin is required to attenuate brain pathology in PD and to restore TH, DA, DOPAC and HVA levels in the brain.

The reasons for NWCBL 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 [3,4]. A slow degradation or metabolism of NWCBL within the brain may also be responsible for maintaining high level of CBL in PD resulting in superior neuroprotection.

5 CONCLUSION

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

6 ACKNOWLEDGEMENTS

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