

# Nanodelivery of drugs for therapeutic strategies in CNS disorders. Current and Future perspectives

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## ABSTRACT

In this innovation, we examined sleep deprivation (SD) induced brain pathology and therapeutic strategies to induce neuroprotection using nanodelivery of cerebrolysin. SD is a serious problem in military and we have previously shown that SD of 12 to 48 h causes blood-brain barrier (BBB) disruption, brain edema formation and neuronal damages after 48 h SD. Measurement of BDNF showed 50 to 60 % decline in different brain areas in SD. Nanowired delivery of cerebrolysin 4 to 6 h after the onset of SD significantly reduced brain pathology and enhanced regional BDNF levels after 48 h SD. However, normal cerebrolysin given after the onset of SD has only minimal effects on regional BDNF level and brain pathology seen at 48 h SD. This indicates that nanodelivery of drugs have superior effects in achieving neuroprotection. Thus, we feel that our policy makers, researchers, clinicians and nanotechnologists should consider exploring the dose response relationship of nanoparticles from wide variety of nanocarriers on cellular toxicity both *in vivo* and *in vitro* situations urgently.

These studies will help create a database for suitable nanocarriers to use for drug delivery to the CNS as effective therapeutic tools for clinical practice. Only after these data, nanoneuropharmacology could be developed as a distinct discipline in the near future

**Keywords:** Sleep deprivation in Military, brain pathology, Brain derived neurotrophic factor, Cerebrolysin, nanodelivery

## 1 INTRODUCTION

Nanodelivery of drugs, diagnostic agents and other functionalized nanoparticles aiming for better treatment of diseases or diagnosis purposes are the new trends in medicine that appear promising [1,3,4]. However, nanoparticles or nanotechnology used to deliver these agents *in vivo* may have potential risks for cell and tissue damages [5-7]. Thus, before nanotechnology is widely accepted as a routine therapeutic tool for effective medical treatment or for diagnostic tools this is mandatory to study their potential or plausible neurotoxic effects in details. So far effects of nanoparticles or nanomaterials including biodegradable nanoparticles on toxicity in the central nervous system (CNS) is not very well documented in the literature. Also, there is an urgent need to find dose related studies on nanoparticles on cellular toxicity especially in *in vivo* situations. Without these details and systematic studies, the use of nanomedicine still remains questionable.

There are also reports that drugs delivered through different kinds of nanoparticles, nanowires or poly-lactic-co-glycolic acid (PLGA) nanoparticles even in identical doses have slightly but significantly different effects on cellular protection when administered in *in vivo* situations [1,3]. This suggests that drug effects could vary depending on the use of specific nanocarriers. In our hands, drugs i.e., cerebrolysin, DL-3-n-butylphthalide (DL-NBP) or H-290/51 tagged with TiO<sub>2</sub> nanowires or titanate nanospheres have superior neuroprotective effects in CNS injury than their delivery through PLGA-nanoparticles in identical manner [5]. Although, TiO<sub>2</sub> by itself has no cellular

toxicity effects within 48 h of its administration, data on other nanoparticles on neurotoxicity in vivo is still lacking.

In this investigation we examined TiO<sub>2</sub>-nanowired Cerebrolysin on sleep deprivation (SD) induced brain pathology and neuroprotection [1,2].

## 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 Sleep deprivation

Animals were subjected to SD using the well-established inverted flowerpot model that selectively deprives them from paradoxical sleep (PS) [1,2]. In this model each rat is placed on an inverted flower pot (diameter 6.5 cm) surrounded by a water-filled Plexiglas chamber up to the 1 cm below the surface of the flowerpot with free access to food and water. The water temperature was maintained at 30±1°C [1,2]. SD was induced in rats upto 48 h as described earlier [2]. Rats placed at room temperature were used as controls.

### 2.2 Brain derived neurotrophic factor

Brain derived neurotrophis factor (BDNF) was measured in control or SD rats using Rad BDNF ELISA Kit (ERBDNF, Thermo Scientific, Frederick, MD, USA). The BDNF levels (ng/g) was measured in parietal cerebral cortex, hippocampus and cerebellum.

### 2.3 TiO<sub>2</sub>-nanowired delivery of Cerebrolysin

Cerebrolysin (CBL, Ever NeuroPharma, Austria) was tagged with TiO<sub>2</sub> nanowires according to standard protocol [3-5]. The TiO<sub>2</sub> nanowired Cerebrolysin (2.5 ml/kg, i.v., NWCBL) was administered 4 to 6 h aftr SD. For comparison, normal CBL (2.5 or 5 ml/kg, i.v.) was also given in separate groups of SD [3,7,8]. The animals were allowed to survive 48 h.

### 2.4 Brain Pathology

In control and SD animals blood-brain barrier (BBB) breakdown to Evans blue albumin (EBA) and radioiodine (<sup>131</sup>Iodine) was examined after intravenous administration of these tracers (EBA 2 % solution 3 ml/kg, and radioiodine 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]. In separate groups of animals, neuronal changes were studied using histopathological examination of Nissl or Haematoxylin & Eosin (HE) staining [3,5,7].

## 2.5 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 SD and Brain Pathology

After 48 h of SD, normal rats exhibited profound breakdown of the BBB as evident with extravasation of endogenous Evans blue albumin (EBA) and radioiodine in the cerebral cortex, hippocampus and the cerebellum (Table 1). The brain edema formation and neuronal damages were also exacerbated by several folds SD group as compared to normal animals ket at their home cage. Neuronal distortion and damage are more frequesnt in the brain areas showing edema fornation or sponginess of the neuropil. In general hippocampus showed grater neurak damages in the CA-3 and 4 areas along with dentate gyrus as compared to CA-1 and CA-2 areas of the hippocampus (Results not shown). Cerebellar granule cells and Purkinge cells both showed cellular swelling, distortion and damage in the vermis as well as the lateral cerebellar cortices in a selective and specific manner (results noy shown).

At transmission electron microscopy (TEM), membrane vacuolation, synaptic damage and edema are freuquent in neuropil from the above brain regions (Fig. 1).

### 3.2 SD and regional brain BDNF Levels

BDNF measurmnt using ELISA showed a significant decrease in this neurotrophic factor content following 48 h SD in all brain regions examined (Table 1). Thus, there was a significant decrease of BDNF content by 75 % in hipppcamnpus followed by 50 % in parietal cerebral cortex and about 40 % in the cerebellum (see Table 1) following SD 48 h as compared to control group.

### 3.3 TiO<sub>2</sub> Cerebrolysin and Brain pathology

Treatment with TiO<sub>2</sub> nanowird Cerebrolysin (NWCBL) 4 to 6 h after the onset of SD resulted in profound neuroprotection in terms of restoration of the BBB function and reduction in brain edema and volume swelling along with protection of nerve cells against damage casued by SD (Table 1).

Table 1. Sleep deprivation (SD) induced brain pathology, alterations in brain derived neurotrophic factor (BDNF) content and their modification with nanowired cerebrolysin (CBL).

Expt. Type	Control			Sleep Deprivation (SD) 48 h			Sleep deprivation (SD) 48 h+TiO2 CBL		
	Parietal-Cortex	Hippo-campus	Cerebellum	Parietal-Cortex	Hippo-campus	Cerebellum	Parietal-Cortex	Hippo-campus	Cerebellum
<b>A. Blood-brain barrier breakdown</b>									
EBA mg %	0.20±0.04	0.28±0.08	0.12±0.03	1.23±0.12**	0.98±0.08**	1.07±0.12**	0.34±0.10*#	0.40±0.10#	0.21±0.11*#
[131]-Iodine %	0.32±0.09	0.43±0.09	0.24±0.08	1.89±0.10**	1.78±0.14**	1.67±0.09**	0.56±0.21*#	0.54±0.21*#	0.33±0.08*#
<b>B. Brain Edema</b>									
Brain water %	74.45±0.13	78.43±0.55	75.56±0.16	75.67±0.32**	80.34±0.33**	76.89±0.76**	75.03±0.13#	78.98±0.11#	76.04±0.10#
Volume Swelling (% f)	nil	nil	nil	+5	+8	+6	+2	+1	+1
<b>C. Brain derived Neurotrophic factor</b>									
BDNF ng/g brain tissue	2.82±0.23	4.34±0.25	3.48±0.45	0.98±0.23**	1.36±0.43**	1.05±0.12**	2.44±0.23#	3.87±0.43#	2.89±0.45#
<b>D. Neuronal injury</b>									
Neuronal distortion or Damage (nr of cells/area)	2±1	1±3	1±2	84±8**	124±23**	154±34**	12±7*#	34±18*#	36±14*#

Values are Mean±SD of 5 to 7 rats at each time point, \* P < 0.05, \*\* P < 0.01 from control, # P < 0.05 from SD, EBA = Evans blue albumin, % f 1 % increase in brain water = ca. 4 % increase in volume swelling. Data from Sharma A et al., (unpublished observations).

### 3.4 TiO2 Cerebrolysin and BDNF level

Our results further show that NWCBL treatment also partially but significantly restored the BDNF levels in all the brain areas examined after 48 h SD (Table 1). Thus, in NWCBL treated animals BDNF level was significantly elevated after 48 h SD than the untreated group. This restoration of BDNF level by NWCBL in SD rats was almost 80 to 90 % of the control group (Table 1).

### 3.5 TiO2 Cerebrolysin and Neuronal Injury

The NWCBL was able to reduce neuronal injuries in all brain areas examined as seen either at light or electron microscopy (Table 1, Fig. 1). Thus, in NWCBL treated SD rats did not show membrane vacuolation, edematous expansion or synaptic damage as compared to untreated SD rats as seen by TEM analysis (Fig. 1). At light microscopy also, several neurons were healthy with a distinct nucleus and clear nucleolus in NWCBL treated SD rats in different brain areas as compared to untreated rats that exhibited marked neuronal damages, distortion and loss of nucleus and nucleolus in many brain areas after 48 h SD (results not shown).

### 3.6 Effect of PLGA-Cerebrolysin

Interestingly, when PLGA-labelled Cerebrolysin was administered in identical doses the effects on neuroprotection, BBB disruption, brain edema formation and/or BDNF levels were much less effective than those seen by NWCBL administration in SD (results not shown). However, PLGA-labelled cerebrolysin also showed significant neuroprotection, restoration of BBB and brain edema formation together with a higher level of BDNF in SD brains (unpublished observations). PLGA alone did not induce any brain pathology in normal or SD rats.

### 3.7 Effect of Immobilization stress on SD

To further test the hypothesis that soldiers confined to narrow space on the battle ground with sleep deprivation, we exposed rats to immobilization (IMBZ) for 4 h daily for 1 week. After that we subjected them to 48 h SD. Our observations showed greater brain damage in IMBZ rats after SD as compared to normal rats (Fig. 1, results not shown).

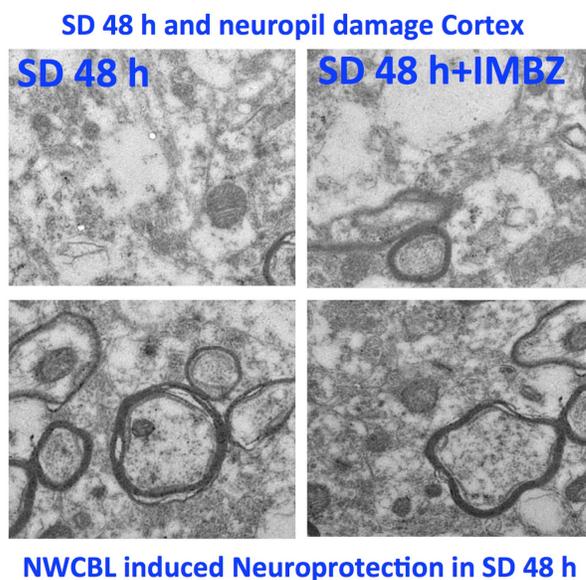


Fig. 1. Low power transmission electron micrograph showing TiO2 Nanowired cerebrolysin (NWCBL) significantly reduced 48 h sleep deprivation (SD) induced brain pathology including synaptic damage and membrane vacuolation (lower panel) as compared to SD alone (upper panel with or without immobilization, IMBZ stress) x 6000.

## 4 DISCUSSION

The salient findings in this investigation clearly show that SD is capable to induce brain pathology at 48 h. This indicates that SD in soldiers for long time could be dangerous for their mental functions. However, our present results did not shed any light whether the SD induced changes in the brain pathology are reversible or permanent. To find out this, new experiments are needed in which SD rats will be allowed to survive several weeks after the episode and to investigate brain pathology using identical protocol.

The other interesting finding came out from this study is that prior emotional stress e.g., immobilization further aggravates the SD induced brain pathology. This suggests that stress and SD together potentiate brain damage [5-7].

Our observations for the first time show that SD was able to reduce BDNF levels in the brain that correlates well with the brain pathology. This indicates that a decrease in BDNF levels is instrumental in brain damage in SD.

This idea is further supported by TiO<sub>2</sub> nanowired cerebrolysin therapy. Cerebrolysin is a balanced composition of several neurotrophic factors including BDNF and active peptide fragments [3]. Nanodelivery of cerebrolysin is thus able to restore the BDNF content in rats following SD. This could be one of the mechanisms by which NWCBL reduced the brain pathology in SD. However, PLGA-labeled cerebrolysin or normal cerebrolysin given in identical conditions are not equally effective in enhancing BDNF levels in SD. This suggests that TiO<sub>2</sub> nanowired delivery of cerebrolysin is required to attenuate brain pathology in SD and to restore BDNF levels.

The reasons for NWCBL effectiveness in SD are unclear. It appears that TiO<sub>2</sub> tagged Cerebrolysin effectively penetrates deeper into the brain or establish cellular communication better than other forms of cerebrolysin delivery [3,6]. Obviously, restoration of BBB function and reduction in brain edema are instrumental in neuronal survival [3]. A slow degradation or metabolism of NWCBL within the brain may also be responsible for maintaining high level of BDNF in the brain of SD rats resulting in neuroprotection.

## 5 CONCLUSION

In conclusion, our observations are the first to show that SD pathology is related with a decrease in BDNF levels in the brain and NWCBL restores the level of BDNF in SD in the most efficient way as compared to PLGA-labeled cerebrolysin or normal drug. This suggests that NWCBL is the most efficient in inducing neuroprotection in SD, not reported earlier.

It remains to be seen whether NWCBL given after longer duration of SD i.e., 12 h after could still be able to restore BDNF levels and induce neuroprotection, a subject that is currently being examined in our laboratory.

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