# TiO2 nanowired delivery of cerebrolysin induces superior neuroprotection following exacerbation of blast brain injury pathophysiology in diabetes

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## ABSTRACT

Military personnel are quite vulnerable to blast brain injury (bBI) during combat operation. The bBI is multiple combination of pressure, rotation, penetration of sharp objects and chemical exposure to barin leading to massive cell and tissue injury. This is quite likely that combat stress alters endocrine regulation and induce diabetes like synptoms. In such cases bBI could further aggravte brain pathology and tissue injury. In this innovation, we demonstrate that a combination of bBI with diabetes (DB) adversely affect brain damage and exacerbates brain pathology. Treatment with cerebrolysin (a multimodal drug comprising neurotrophic factors and active peptide fragments) 30 min to 1 h after bBI (5 to 10 ml/kg, i.v.) significantly reduced brain pathology in normal animals. However, TiO2 nanodelivery of cerebrolysin (5 ml/kg, i.v.) is needed to induce neuroprotection in bBI in diabetic animals. These observations are the first to show that (i) bBI is exacerbated in diabetes, (ii) cerebrolysin has the potential to reduce brain pathology in bBI in healthy animals, whereas, TiO2-nanowired cerebrolysin is needed for neuroprotection in diabetic animals after bBI, not reported earlier.

***Keywords***: Brain blast injury, diabetes, brain pathology, blood-brain barrier, brain edema, TiO2 nanowired

## 1 INTRODUCTION

Military personnel are often exposed to blast brain injury (bBI) either during peacekeeping or combat operations [1,2]. In such situations, brain tissue injury is quite extensive. Due to severe work stress and irregular food habits they often develop hypertension and/or diabetes. However, bBI in diabetic situation is likely to furtehr aggravte brain damage and cell or tissue injury. The bBI is a combination of pressure, rotation, penetration of sharp objects and chemical exposure causing laceration, perforation and tissue loss in the brain. In present innovation, influence of diabetes on bBI was investigated in a rat model. In addition, attempts were made to induce neuroprotection with cerebrolsyin-a multimodal drug with a balanced composition of several neurotrophic factors and active peptide fragments in bBI either alone or its TiO2-nanowired delivery.

Previous studies in our laboratory show that TiO2 nanowired delivery of cerebroslyin induces superior neuroprotection in traumatic brain injury associated with diabetes. Thus, in this investigation we used TiO2-nanowroed delivery of cerebrolsyin to see whetehr brain pathology following bBI in diabetes could also be protected.

## 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 Blast brain injury

Animals were exposed to blast brain injury (bBI) in a rat model of shock tube. The shock tube blast devise comprise compressed air-and compressed helium-driven membrane rupture that induces pressure waves to simulate some aspects of bBI. The rats were anesthetized with Equithesin (3 ml/kg, i.p.) and their head was exposed to overpressure blast (100, 150 or 200 kPa) in the shock-tube with a shockwave velocity of ca. 400 to 450 m/sec). After the bBI the animals were allowed to survive 8,12 or 24h after trauma [1-4].

### 2.2 Induction of diabetes

Rats were made diabetic by injecting streptozotocine (50 mg/kg, i.p.) daily for 3 days. This treatment results in blood glucose elevation within a week by 20 to 40 mM/L [2-5].

Control group show blood glucose level 5-6 mM/L and MABP of 90-105 Torr.

Six weeks after development of clinical diabetes, the animals were subjected to bBI and allowed to survive 8,12 or 24 h aftre trauma.

### 2.3 Blood-brain barrier and brain edema

The BBB breakdown was examined using Evans blue (EB) and $^{[131]}$Iodine leakage across the brain microvessels 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. Brain edema was determined using regional water content by wet and dry weights of the brain samples [2-4].

### 2.4 Regional cerebral blood flow

Regional cerebral blood flow (rCBF) was measured using $^{125}$-Iodine labelled carbonized microspere (o.d. 15±0.6 µm) according to standard. About 1 million microsphere was injected into the internal carotid artery and blood samples were withdrawn from right femoral artery (0.8 ml/min) and radioactivity counted. The rCBF is calculated as ml/g/min [5].

### 2.5 Histopathology

In separate groups of animals, neuronal changes were examined using histopathological techniques for Nissl or Haematoxylin & Eosin (HE) staining [2,6]. Astrocytic activation was evaluated using glial fibrillary acidic protein (GFAP) immunoreactovity accroding to stabdard protocol as described earlier [4,5].

### 2.6 TiO2-nanowired Cerebrolsyin

Cerebrolysin (CBL, Ever NeuroPharma, Austria) was tagged with TiO2 nanowires according to standard protocol [2,3]. The TiO2 nanowired Cerebrolysin (5 ml/kg, i.v. NWCBL) was administered either 30 min or 1 h after bBI. For comparison, normal cerebrolysin was administered in identical doses instead of NWCBL [2,4,6,7].

### 2.7 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 Blood-brain barrier and brain edema

A progressive increase in the BBB permeability to EBA and radioiodine in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus and brain stem was seen that correlates well with the blast overpressure strength. Brain edema formation as measured using water content exhibited a 2- to 4 % increase (ca. 8 to 16 % volume swelling). These pathological changes were much more aggravated in diabetic animals aftre bBI as compared to identical trauma in normal rats (results not shown).

### 3.2 Regional cerebral blood flow

The regional brain areas exhibiting BBB breakdown also showed severe reductions in the rCBF. Thus, in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus and brain stem the rCBF was reduced by -30 to -58 %. This reduction in rCBF is well correlated with the blast overpressure. Diabetic animals exhibited greater reductions in the rCBF at all points as compared to normal rats after identical bBI (results not shown).

### 3.3 Brain pathology

Expansion of neuropil, sponginess and neuronal, glial and myelin damages are quite frequent in normal animals after

bBI (Figs. 1 & 2). These pathophysiological changes were 2-to 5-fold exacerbated in diabetic animals after bBI. Thus, loss of neurons in the cerebral cortex, hippocampus and cerebellum was the most prominent in normal healthy rats after bBI. The magnitude and intensity of these pathological changes were progressive in nature with time and blast overpressure strength. The number of damaged and distorted neurons increased significantly by 2- to 4-fold in diabetic rats as compared to normal animals after identical bBI. The occurrences of damaged cells are seen in the areas showing marked upregulation of GFAP immunoreactivity (Figs. 1 & 2).

## 3.4 Effect of Cerebrolysin Treatment

Treatment with TiO2 nanowired Cerebrolysin (5 ml/kg, i.v., NWCBL) 30 min or 1 h after the bBI in normal or diabetic animals resulted in a significant reduction in GFAP immunoreaction in several parts of the brain (Fig. 2).

In these brain areas, brain pathology was also significantly reduced. Thus, neuronal dmages and distortion was markedly reduced by NWCBL in normal or diabetic rats aftre bBI (Fig. 1). On the other hand normal cerebrolysin when administered in higher doses (10 mg/kg, i.v.) under identical conditions was able to achieve comparable reduction in GFAP immunoreaction and pathological neuronal changes in the above brain areas (results not shown).

At the ultratsructural level also, TiO2-nanowired cerebrolsyin (5 ml/kg, i.v.) was also able to reduce edema, membrane disruption and sponginess in normal or diabetic animals aftre bBI most effectively as compared to normal cerebrolysin administration (10 ml/kg, i.v.) (results not shown).

TiO2 nanowired cerebrolysin resulted in signifcant reduction in the BBB breakdown to EBA and radioiodine tracers in several brain areas in normal and diabetic rats aftre bBI. A close parallesim was observed between the magnitude of reduction in the BBB breadown, enhancement of the rCBF and the intensity of edema formation following NWCBL teratment of diabetic or normal animals aftre bBI (unpublished observation).

# 4 DISCUSSION

Our novel findings clearly show that TiO2 nanowired cerebrolysin is capable to attenuate bBI induced brain pathology in normal and diabetic rats. Furthermore our observations are the first to point out that TiO2-nanowired cerebrolysin has superior effects in inducing neuroprotection in diabetic rats after bBI as compared to normal cerebrolysin in higher doses. These observations suggest that TiO2-nanowired cerebrolysin could be used in clinics in bBI cases complicated with co-morbidity factors, e.g., diabetes not reported earlier.

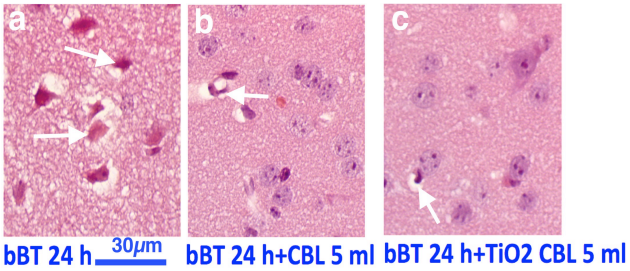


**Fig. 1. TiO2 NWCBL reduces neuronal damages in the parietal cerebral cortex (c) as compared to normal CBL (b) following 24 h bBI in normal rats. The bBI induces greater damage in the neuropil as evidenced with severe perineuronal edema (arrows), sponginess and expansion of the neuropil. H&E stain on 3-µm thick paraffin sections. Bar = 30 µm, bBT = Blast brain trauma.**

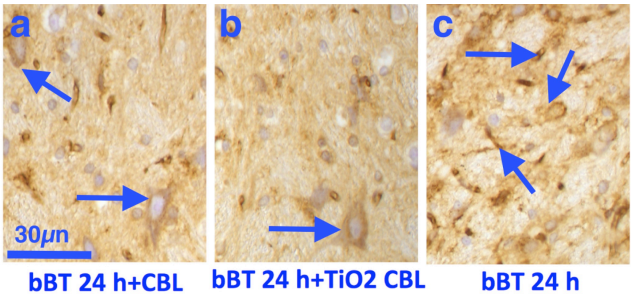


**Fig. 2. TiO2 NWCBL reduces activation of astrocytes in the III layer of parietal cerebral cortex (b) as compared to normal CBL (a) following 24 h bBI in normal rats. The bBI induces severe damage to astrocytes as evidenced with intense immunostaining of astrocytes with glial fibrillary acidic protein (GFAP) activity (arrows c). GFAP immunostaining on 3-µm thick paraffin sections. Bar = 30 µm, bBT = Blast brain trauma.**

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The possible mechanisms by which TiO2 nanowired cerebrolysin is capable to attenuate brain pathology in bBI complicated with diabetes is unclear. However, it appears that a reduction in oxidative stress triggered by bBI and diabetes by TiO2-nanowired cerebrolysin could play important roles [1-4]. Since cerebrolysin is a potent antioxidant with neuroregenerative capabilities due to several neurotrophins, strengthening the cell membrane of endothelial cells be another important factor for reducing brain pathology [2-5,7]. A significant reduction in BBB breakdown and brain edema formation in TiO2-nanowired cerebrolysin treated groups in normal or diebetic rats in bBI further support the idea.

Diabetes alone could induce oxidative stress and free radical formation [2-5]. This effect could be exacerbated by additional exposure to bBI. This could be one of the main reasons for neuronal and glial cells injury as seen with GFAP expression and neuonal damages (Figs. 1 & 2). Thus,

it is quite likely that oxidative stress and lipid peroxidation could alter membrane permeability resulting in greater BBB leakage to proteins [8-10]. Obviously, extravasation of proteins into the brain fluid compartment will lead to vasogenic edema formation. Volume swelling of the brain in a close cranial compartment could compress the brain cells and also expose unwanted substances from the vascular compartment causing brain injury [4-7]. Accordingly, neuronal, glial and axonal injuries could aggravate in diabetic rats following identical bBI as compared to healthy rats. Alternatively, a direct effect of cerebrolysin on endothelial membrane stability may also be responsible for neuroprotection, a feature that requires additional investigation.

Potentiation of cerebrolysin induced neuroprotection caused by TiO2 nanowired delivery may either be due to an enhanced penetration of the drug within the brain or to a slow degradation or metabolism of the compound within the CNS [2- 6]. Higher doses of normal cerebrolysin having quite remarkable effects on neuroprotection in diabetic rats after identical bBI furtehr supports this hypothesis.

## 5 CONCLUSION

In conclusion, our observations indicate that TiO2 nanodelivery of cerebrolysin has superior effects in diabetes induced exacerbation of brain pathology following bBI. These results suggest tour military personnel who are often exposed to bBI with or without additional co-morbidity factors i.e. diabetes and/or hypertension may get better therapeutic strategies using cerebrolysin in the future.

It remains to be seen whether nanodelivery of cerebrolysin using other technology e.g., Poly (L-lactide-co-glycolide) could also be equally effective to contain brain pathology in bBI with diabetes. This is a feature that is currently being investigated in our laboratory.

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