Nanodelivery of cerebrolysin induces profound neuroprotection in heat stroke following chronic hypertension in combination with SiO2 and carbon nanoparticles induced exacerbation of brain damage

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ABSTRACT

Military personals are often exposed to combat stress induced hypertension in an environment rich in various nanoparticles (NPs) emanating from different sources e.g., gun powder explosion or blast injuries. Furthermore, combat or peacekeeping activity in the Middle East region also exposes them to high environmental heat and silica dust. Thus, it is quite likely that all these combinations of hypertension, silica dust (SiO2 NPs) and carbon NPs following missile explosion or blast injuries in desert high heat lead to exacerbation of brain damage following heat stroke. This suggests that hypertension and/or NPs exposure in high environmental heat is dangerous for our soldiers as a combination of these factors may induce brain dysfunction and damage along with functional disturbances. In this innovation we have shown that a combination of hypertension, SiO2 NPs and carbon nanoparticles (CNPs) at hot environment aggravates brain damage. In this complex situations TiO2-nanowired delivery of multimodal drugs e.g., cerebrolysin is needed to contain neuronal damage and restoring the functional disturbances effectively.

Keywords: TiO2 nanowired Cerebrolsyin, Heat Stroke, SiO2 NPs, Carbon NPs, hypertension, brain pathology, blood-brain barrier, brain edema, neuroprotection

1 INTRODUCTION

Our military personals are often exposed to combat stress induced hypertension in an environment rich in various nanoparticles emanating from different sources e.g., gun powder explosion or blast injuries [1,2]. Furthermore, combat or peacekeeping activity in the Middle East region also exposes them to high environmental heat and silica dust [3,4]. Thus, it is quite likely that all these combinations of hypertension, silica dust (SiO2 NPs) and carbon NPs following missile explosion or blast injuries in desert high heat lead to exacerbation of brain damage following heat stroke.

Previously, we have shown that hypertensive animals when exposed to heat stress exhibit massive brain damage and deterioration of their sensory motor dysfunctions [4,5]. In addition, rats exposed to engineered metal nanoparticles (NPs) i.e., Ag, Cu or Al also exacerbated brain pathology following heat stroke. This suggests that hypertension and/or NPs exposure in high environmental heat is dangerous for our soldiers as a combination of these factors may induce brain dysfunction and damage along with functional disturbances.

This hypothesis was tested in our rat model of heat stroke where renal hypertensive rats were intoxicated with SiO2 and single walled carbon nanotubes (SWCNT) before heat exposure. Furthermore, to find out novel therapeutic measures in these complex situations with several co-morbidity factors, we used a multimodal drug Cerebrolysin (Ever Neuro Pharma, Austria). Cerebrolysin is a well-balanced composition of multiple neurotrophic factors and active peptide fragments needed to enhance neurorepair and neuroregeneration [1-3]. Thus, nanodelivery of cerebrolysin could be useful in inducing neuroprotection following heat stroke associated with hypertension and NPS exposure more effectively [1-4].

2 MATERIALS & METHODS

Experiments were carried out on Male Wistar Rats (200-300 g) housed at controlled ambient temperature (21±1°C) with 12 h light and 12 h dark schedule. Food and water were provided ad libitum before 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 Exposure to heat

Rats were subjected to heat exposure in Biological Oxygen Demand Incubator (BOD) maintained at 34°C for 4 h per day for 8 days. The relative humimiduty 45-47 % and wind velocity 20-25 cm/Sec were kept constant. Control rats were either placed in identical incubator at 21°C for 4 h for 8 days or kept at room temperature (21±1°C).

On the 9th day rats from various grous were exposed ti heat stroke at 38°C in the BOD incubator for 4 h [6].

2.2 Chronic hypertension

Chronic hypertension was produced by using a silver clip to constrict one renal artery leaving both the kidneys intact (2K1C). After 4 to 6 weeks, rats develop hypertension similar to those seen in clinical situations (Mean Arterial Blood Pressure, MABP 180±8 torr, Controls 110±6) [4].

2.3 Exposure to nanoprticles

Intoxication of SiO2 NPs and SWCNT (50-60 nm) was done by administering 50 mg/kg, i.p. dose of each NPs once daily for one week either at room temperature (21°C) or at 34° C (wind velocity 20-25 cm/sec, relative Humidity 45-47 %) for 8 days [3].

2.3 Treatment with Cerebrolysin

Cerebrolysin (Ever NeuroPharma, Austria) 5 ml/kg or 10 ml/kg was used once daily for 3 days from day 5,6 and 7th of heat exposure either at 34°C or in control rats after NPs intoxication or saline administration in normal or hypertensive rats. On 9th day brain pathology was determined in these treated groups [1-3].

In separate group of rats TiO2-nanowired cerebrolysin was also administered in a dose of 5 ml/kg under identical conditions. Cerebrolysin was tagged with TiO2- nanowires (Fig. 1) using standard procedures as described earlier [2].

In control groups, TiO2 nanowires alone was administered to examine any neurotoxicity of nanowires alone. Our observations show that TiO2 nanowires do not induce any brain pathology (results not shown).

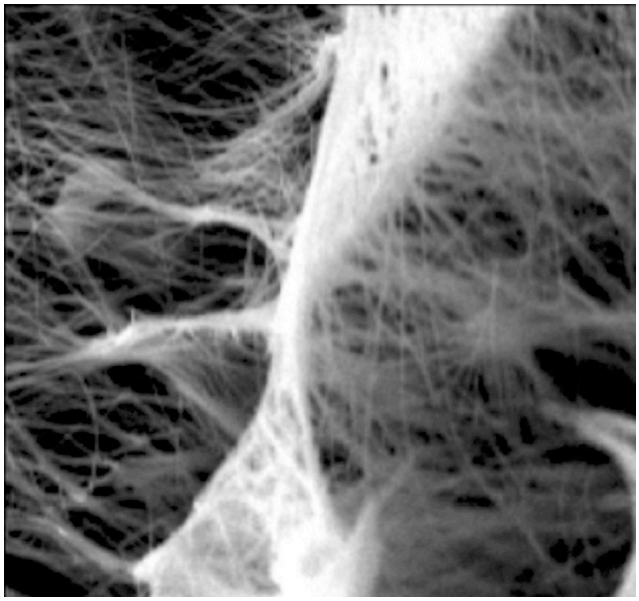

Fig. 2. TiO2 nanowired for Nanodelivery of Cerebrolysin

2.3 Parameters measured

The following parameters were measured in normal or hypertensive rats exposed to hot environments intoxicated with SiO2 NPs and SWCNT.

2.3.1 Blood-brain barrier

The blood-brain barrier (BBB) leakage was measured using Evans blue albumin (EBA, 2% of 0.3 ml/100g i.v.) and radioiodine ($^{[131]}$-Iodine], 10 µCi/100g i.v.) extravasation in the brain [2,3]. After washing out of intravascular tracer with 0.9 % saline through heart the brain were dissected out and examined for blue staining. After that tissue pieces from selected brain areas were then dissected and radioactivity determined in a Gamma Counter (Packard, USA). Leakage of these tracers was expressed as percentage increase in the brain over blood concentration [2,3].

2.3.2 Brain Edema formation

The brain edema was measured using brain water content. Desired tissue pieces from brain were dissected out and weighed to determine their wet weight. After that these tissue pieces were kept in an oven maintained at 90° C for 72 h to obtain their dry weight. The percentage water content was calculated from the differences between wet and dry weight of the samples [2,3].

2.3.3 Neuronal injury

Neuronal injury was evaluated using Nissl or Haematoxylin & Eosin (HE) staining using standard histopathological techniques [3]. For this purpose, animals were perfused in situ with 4 % buffered paraformaldehyde preceded with a brief saline rinse though cardiac puncture. Coronals sections of the brain were the cut passing through the hippocampus and embedded in paraffin. About 3 µm thick sections were cut and stained with HE or Nissl using commercial protocol [1-3]. The number of damaged or distorted neurons in specific anatomical brain areas were counted manually.

2.4 Statistical analyses

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

3 RESULTS

3.1 Brain pathology in heat exposure

Hypertensive rats exposed to SiO2 NPs and SWCNT in combination at high environmental temperature exhibited 3- to 5-fold higher increase in BBB permeability in 10 brain regions to EBA and radioiodine aftre heat challenge (38°C for 4 h) as compared to those rats placed at room temperature.

The brain edema in 8 regions showed a 6- to 8-fold increase in NPs treated hypertensive rats at 34°C and neuronal and glial damages were enhanced by 4- to 6-fold that their counterparts placed at 21° C (results not shown). The heat exposed hypertensive rats following NPs exposure also showed severe functional disturbances in behavioral tests as compared to those placed at 21°C [Teble 1].

Interestingly, a combination of SiO2 NPs and SWCNT in hypertensive animals exhibited about 2-fold increase in BBB disruption, edema formation and brain pathology as compared to normotensive rats under identical conditions. [Table 1].

3.2 Cerebrolysin treatment

Cerebrolysin treatment (5 ml or 10 ml/kg, i.v.) did not reduce brain pathology or behavioral disturbances in hypertensive rats with NPs exposure following heat stress. However, mild to moderate reduction in behavioral functions and brain pathology was seen in this group of rats at 21°C.

On the other hand TiO2 nanowired cerebrolysin (5 ml/kg) was able to reduce brain pathology and behavioral dysfunction in heat exposed hypertensive rats treated with SiO2 and SWCNT. [See Fig. 2, Table 1].

Neural injury, neuronal loss, distorted neurons; perineuronal edema and sponginess of the neuropil were also markedly reduced by TiO2-nanowired cerebrolysin in heat-exposed animals after NPs intoxication [See Fig. 3].

In these animals treated with TiO2 nanowired cerebrolysin, glial and myelin reactions ware also reduced (Result not shown).

Table 1. Nanoparticles intoxication in hypertension exacerbates hyperthermia induced brain pathology.

Expt. Type	BBB [131]-Iodine %	Brain Edema Water Content %	Neural Injury Nr./section	Astrocytic Reaction GFAP+ cells Nr
Control	0.23±0.02	74.34±0.12	2±2	6±2
4 h Heat Stress 38°C	2.34±0.32**	80.56±0.12**	180±24**	204±28**
Hypertension+HS	2.98±0.12*#	81.78±0.11*#	240±14*#	267±21*#
HS+SiO2	2.89±0.12*#	81.45±0.23*#	220±18*#	256±12*#
HS+CNT	2.96±0.23*#	81.98±0.34*#	234±19*#	289±19*#
HY+HS+SiO2	3.34±0.12*#	82.34±0.56*#	298±12*#	304±21*#
HY+HS+CNT	3.67±0.22*#	82.98±0.32*#	356±32*#	402±29*#

* P < 0.01. ** P <0.01 from control, # P <0.05 from Hypertensive (HY) group. Values±SD (5 to 8 rats at each point)

4 DISCUSSION

Our observations are the first to show that nanowired cerebrolysin is the most powerful agent in reducing brain pathology in hypertensive rats reared at hot environments following their exposure to a combination of factors e.g., SiO2 and SWCNT after heat stroke. This indicates that military personnel that are exposed to various kinds of external (NPs) or internal insults (hypertension) at battlefield when gets additional heat stress they could develop greater brain pathology and abnormal behavioral dysfunction. In such situation, normal drugs are unable to treat them effectively [1-3]. Thus, the multimodal drug Cerebrolsyin that is a balanced composition of several

neurotrophic factors and active peptide fragments could be of immense help [see 1-3]. In addition, our observation further show that nanodelivery of cerebrolysin could offer even much superior neuroprotection in multiple combinations of insults during heat stroke.

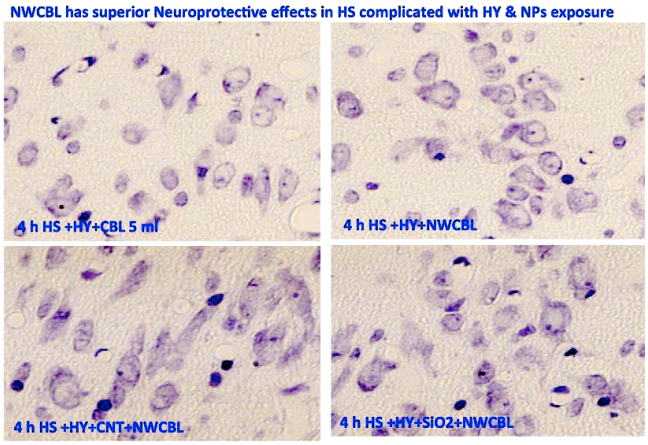

Fig. 2. Shows marked neuroprotection by TiO2-nanowired cerebrolysin (NWCBL) in hypertensive rats after NPs intoxication at hot environment. Nissl Stain x 40

The mechanisms by which a superior neuroproetction even in lower dose by nanodelivered cerebrolysin is seen in heat stroke is not well understood. However, it appears that TiO2 nanowired could reach the deeper brain tissues faster due to breakdown of the BBB [2]. Obviously, a greater BBB breakdown in hypertensive rats exposed to NPs following heat stress is in line with this idea [2,3,6]. Another possibility for superior effects of TiO2-nanodelivery of cerebrolsyin could be due to the fact that the drug could resist biological degradation because of its tight binding with TiO2 nanowires. This would results in gradual release of the drug from nanowires over a long period of time [2,3].

However, it remains to be seen whether TiO2 nanodelivery of cerebrolsyin following heat stroke in hypertensive group intoxicated with several NPs could also reduce brain pathology if given after loger therapeutic window, a feature that is currently being investigated in our laboratory.

5 CONCLUSION

In conclusion, our observations point out a new role of TiO2 nanowired cerebrolsyin in terateing complicated heat stroke cases in clinical situations. Since TiO2-nanodelivery of cerebrolsyin appears to be superior in nature, new studies are needed to evaluate safety and relaiability of TiO2 nanowires over longer time periods for its clinical usage.

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