

TiO₂ Nanowired Cerebrolysin enhances neuroprotective effects of mesenchymal stem cells following concussive head injury at hot environments

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ABSTRACT

Military personnel are often working in hot environment where concussive head injury (CHI) during combat operations is quite common. In this innovation we for the first time show that traumatic brain injury occurring in hot environment is exacerbated and this could be due to lack of neurotrophic factors. Thus, nanowired delivery of Cerebrolysin, a balanced composition of several neurotrophic factors and active peptide fragments could be useful for reducing the brain pathology either alone or together with mesenchymal stem cells (MSCs) in CHI during hot environments. Our observations show that TiO₂ nanowired delivered Cerebrolysin potentiates MSCs induced neurorepair and neuroregeneration. This research has potential clinical significance for our armed forces that are working hard in hot environment during combat operations.

Keywords: cerebrolysin, concussive head injury, brain pathology, TiO₂ nanowired drug delivery, blood-brain barrier, neuroprotection, mesenchymal stem cells

1 INTRODUCTION

Concussive head injury (CHI) could lead to either instant death or lifetime disabilities [1]. Our soldiers are highly vulnerable to CHI during combat operations [2]. Thus, to treat these soldiers effectively novel strategies using combination therapy is needed. Since stem cell therapy enhances neurorepair in brain or spinal cord injuries [3], this is quite likely that this may be effective in CHI as well. However, this is still unclear whether CHI occurring at high environmental temperature could adversely affect the outcome with regard to brain pathology or sensory motor disturbances. Since our soldiers are often engaged in

combat operations in desert environments under high heat conditions, this is likely that their outcomes following CHI require special treatment using combination therapy.

In this investigation we used nanowired delivery of mesenchymal stem cells (MSCs) intravenously following CHI [3] and also added a known neuroprotective multimodal drug Cerebrolysin [1,2] with or without nanowired delivery to see whether a synergistic better effects of this combination can result in good neurorepair following CHI at high environmental heat conditions.

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 Concussive head injury (CHI)

CHI was inflicted in rat model using a weight drop of 114.6 g on the parietal skull bone under Equithesin anesthesia from a 20 cm height using a guide tube. This arrangement induces an impact of 0.224 N on the right parietal skull surface causing serious brain edema and volume swelling particularly in the left hemisphere due to a “counter-coup” phenomenon [1,4,5]. The animals were allowed to survive 48 h after the insult.

2.2 Exposure to Hot environment

Separate group of rats were exposed to hot environment in a Biological Oxygen Demand (BOD) incubator maintained at 38° C for 1 h daily for 2 weeks. The relative humidity (45-47%) and wind velocity (20-25 cm/sec) was kept constant. After 2 weeks these heat-treated rats were subjected to CHI.

2.3 MSCs Treatment

Commercially available MSCs (1 million cells, Sigma Chemical Co., St. Louis, USA) were delivered intravenously in a group of rats with CHI either at room temperature or in heat treated animals immediately after CHI. In separate group of rats TiO₂ nanowired MSCs were also administered in identical conditions [3].

2.4 Cerebrolysin Treatment

Cerebrolysin (Ever NeuroPharma, Austria) was co-administered in a dose of 2.5 ml or 5 ml/kg, intravenously together MSCs. In separate group of CHI TiO₂-nanowired cerebrolysin was also co-administered with TiO₂-MSCs [1-3].

2.3 Parameters Measured

The following parameters were measured in animals that received TiO₂-nanowired MSCs either alone or together with TiO₂-nanowired cerebrolysin following CHI.

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 (¹³¹I-Iodine], 10 µCi/100g i.v.) extravasation in the brain. 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 [4,5].

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 [4,5].

2.3.3 Neuronal injury

Neuronal injury was evaluated using Nissl or Haematoxylin & Eosin (HE) staining using standard histopathological techniques [3-5]. For this purpose, animals were perfused in situ with 4 % buffered paraformaldehyde preceded by a brief saline rinse through cardiac puncture. Coronal 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 [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 CHI induces Neurotoxicity

When rats were exposed at 38°C for 1 h daily until 2 weeks and then the identical CHI was delivered, the magnitude and intensity of brain edema development and volume swelling was exacerbated by 2 to 3 fold as compared to the CHI delivered in rats kept at room temperature (21±1°C) [see Table 1].

Leakage of Evans blue and radioiodine across the BBB was also much 2- to 4-fold higher in CHI group exposed to hot environments than identical injury at room temperature [Table 1].

Neural injury, neuronal loss, distorted neurons, perineuronal edema and sponginess of the neuropil were also exacerbated in animals with CHI performed at 38°C as compared to CHI inflicted on rats at 21°C [Table 1].

A general expansion of the neuropil supporting edema formation is also clearly evident in CHI at 21°C that was also exacerbated at 38°C. The uninjured left half became almost fluid in CHI group at hot environment, a feature that was seen only in a very mild form in rats subjected to CHI at room temperature treated animals.

These pathophysiological changes such as breakdown of the BBB, edema formation and neuronal injuries after CHI at normal or hot environments were progressive with time, from 12, 24 and 48 h periods examined (Result not shown).

Table 1. CHI 48 h induced neurotoxicity and neuroprotection in contralateral left hemisphere (LH) by co-administration of TiO₂ nanowired Cerebrolysin and MSCs 12 h after injury

Expt. Type	Control	CHI 21°C 48 h	CHI 38°C 48 h	CHI 48 h+ TiO ₂ -MSCs 38° C	CHI 48 h+ TiO ₂ CBL+MSCs 38°C
EBA mg%	0.24±0.06	2.38±0.14**	4.26±0.28**#	1.34±0.18*a	0.84±0.10*#b
^[131] Iodine %	0.30±0.08	2.96±0.19**	4.91±0.17**#	1.55±0.12*a	0.94±0.12*#b
Brain water %	74.76±0.18	79.84±0.14**	84.53±0.26**#	76.29±0.24a	75.16±0.23#b
Brain Swelling % f	0	+20**	+44**#	+8*a	+2*#b
Neuron Injury Nr	2±3	389±35**	659±46**#	108±34*a	86±24*#b

Values are Mean±SD of 5 to 6 rats, CHI = Concussive head injury; MSCs = mesenchymal stem cells; CBL = cerebrolysin; TiO₂-MSCs = TiO₂-nanowired; * P < 0.05, ** P < 0.01 from control, # P < 0.05 from CHI 21°C, a P < 0.05 from CHI 38°C, b P < 0.05 from CHI+MSCs. For details see text.

3.2 MSCs induced Neuroprotection

Commercially available MSCs (1 million cells) were delivered in a group of rats with CHI either at room temperature or in heat treated animals resulted in a mild but significant reduction in volume swelling and brain edema formation [Results not shown].

On the other hand, when TiO₂ nanowired MSCs are given under identical conditions CHI did not result in massive brain edema formation or volume swelling at room temperature. However, heat stressed rats did not show sufficient reduction in brain pathology [Table 1]. Thus, TiO₂ nanowired MSCs alone did not reduce neuronal injuries and edematous expansion of the neuropil when CHI was performed at hot environments.

3.3 Cerebrolysin induced Neuroprotection

Co-administration of Cerebrolysin 2.5 ml/kg, i.v. together with MSCs attenuated neurotoxicity in CHI at room temperature very effectively [Results not shown]. However, Cerebrolysin alone (5 ml/kg) or with MSCs did not reduce brain pathology in CHI rats at hot environments.

Interestingly when TiO₂ nanowired Cerebrolysin (2.5 ml/kg) was co-administered with nanowired MSCs either 8 or 12 h after CHI significant reduction in brain pathology and brain edema formation was seen in heat treated rats at 48 h [Fig. 1, Table 1]. Thus, these rats exhibited profound healthy neurons in the cortex (Fig. 1) in both the right and left halves. A significant reduction in EBA and radioiodine leakage as well as reduction in brain edema formation was also seen in this group (Table 1).

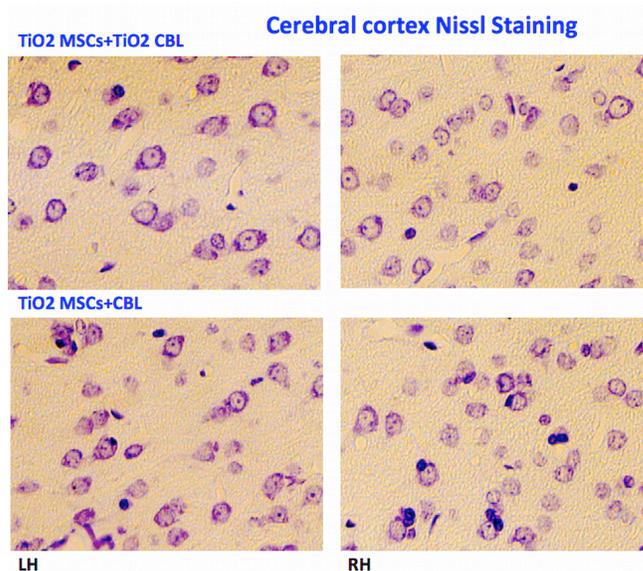


Fig. 1. Shows neuroprotection by co-administration of TiO₂-nanowired mesenchymal stem cells (MSCs) and TiO₂-nanowired cerebrolysin (2.5 ml/kg) 12 h after concussive head injury (CHI) in rats at hot environment (38°C, Upper panel). Most marked neuroprotection in terms of healthy neurons are seen in the left half (LH) after CHI. Whereas, TiO₂ MSCs with cerebrolysin alone (lower panel) reduced neuronal damages in LH but some neurons in RH still show damage. Nissl staining on 3-µm paraffin section x 40.

4 DISCUSSION

The salient novel finding in this innovation suggests that Cerebrolysin if co-administered with MSCs 8 to 12 h after CHI is able to induce profound neuroprotection than any one of the drugs given alone. However, when the same combination was given in rats in which CHI was inflicted at hot environment, the magnitude of neuroprotection was much less evident. This suggests that efficacy of any drug also depends on the magnitude and intensity of CHI, not reported earlier. Since hot environment exacerbated brain pathology caused by CHI this is necessary to effectively reduce these additional burden on neurotrauma by effective and prolonged administration of drugs in question. To that end when TiO₂ nanowired delivery of both MSCs and cerebrolysin in combination was done, most marked neuroprotection was seen in CHI at hot environment. This opens up new possibilities to deliver drugs using nanowired technologies in clinics for better healthcare.

The possible mechanisms behind a combination of nanowired MSCs and cerebrolysin is unclear from this investigation. However, available evidences point out that nanodelivery of drugs could be more effective than parent compounds [1,2]. This is due to the fact that binding with nanoparticles makes these drugs rapidly penetrable into the CNS and also make them less vulnerable to biodegradation [1-3]. As a result MSCs could survive for longer duration and prolonged continuous exposure of cerebrolysin that is a balanced composition of neurotrophic factors and active peptide fragments is able to induce neurorepair and enhance neuroregeneration [see 1-3].

Obviously, a combination of these agents using nanodelivery could synergistically enhance neurorepair in CHI. This would result in much better neuroprotection than any drug given alone. The biological mechanisms of improved neuroprotection by the combined administration of cerebrolysin and MSCs are not well known. However, it appears that the combination of these agents may reduce exacerbation of oxidative stress that could be caused by heat treatment and CHI combination more effectively [2-4].

5 CONCLUSION

In conclusion, our results show that CHI at hot environment exacerbated brain pathology indicating that military personnel when injured at hot environment their treatment regimen should be modified than standard therapy to achieve good results. Furthermore, our observations are the first to point out that a combination of MSCs with a multimodal drug, Cerebrolysin if given using nanotechnology is far more effective in CHI at hot environment than normal compounds. This suggests that nanodelivery of therapeutic agents in combination with stem cells will enhance therapeutic efficacy in CHI at hot environments, not reported earlier.

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