Nanowired delivery of mesenchymal stem cell reduces diabetes induced aggravation brain damage following heatstroke

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ABSTRACT

Our military personnel are often exposed to combat operations in hot environments in summer months (40 to 42°C) that often lead to heatstroke. Since endocrine disorders e.g., diabetes could result from posttraumatic stress disorders (PTSD) in military populations; these soldiers are highly vulnerable to additional heat stress. Previous reports from our lab suggest that diabetic rats showed greater brain pathology after heat stress. This suggests that military personnel with diabetes in heat stress have higher brain pathology during combat stress or other activities. Since stem cells are known to induce neuroprotection we examined whether nanodelivery of mesenchymal stem cells (MSCs) could enhance the potential neuroprotective effects of stem cells and reduce the aggravation of brain pathology in heat stress in diabetic rat model. Our observations clearly show that nanodelivery of MSCs markedly attenuated heat stress induced brain pathology in diabetic rats.

Keywords: heat stress, diabetes, brain patholoy, mesenchymal stem cells, nanodelivery, neuroprotection

1 INTRODUCTION

Exposure to military personnel to high environment heat in Middle East particularly during summer months often lead to heatstroke resulting in severe disability and eventually death [1]. Our military personnel are engaged in combat stress and often strenuous exercise in summer are also vulnerable to post-traumatic stress disorders (PTSD) causing serious physiological and endocrinological disturbances resulting in diabetes like syndromes [2,3]. Thus, it is quite likely that soldiers exposed to high summer heat with diabetes may have severe mental dysfunction or brain damage under such high heat conditions. Previous works from our laboratory demonstrated greater brain damage and brain pathology in diabetic rats exposed to whole body hyperthermia (WBH) as compared to healthy animals [4,5]. Thus, novel treatment strategies are needed to contain brain damage in diabetic group under high environmental heat conditions.

In recent years stem cells are shown to reduce brain damage in various neurological disease [6]. Furthermore, nanodelivery of drugs are also found more effective in enhancing the therapeutic effects of drugs in the central nervous system (CNS) [7,8]. Thus, it appears that nanodelivery of stem cells may also have better neuroprotective approaches in the CNS than stem cell treatment alone. Keeping these views in mind we have examined in these innovative experiments the effects of nanodelivery of mesenchymal stem cells (MSCs) in diabetic rats after WBH on brain pathology.

2 MATERIALS & METHODS

Experiments were carried out on Male Charles Foster Rats (200-300 g) that were made diabetic by administration of streptozotocine (75 mg/kg, i.p.) daily for 3 days and allowed to develop diabetes until 6 weeks (blood glucose 20±2 mM/L). Saline treated rats were used as controls. 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 Stress

Normal or diabetic rats were exposed to 4 h heat stress in a Biological Oxygen Demand Incubator (BOD) maintained at 38° C with relative humidity (45-47%) and wind velocity (20-25 cm/sec) were kept constant [4]. A

group of saline treated or diabetic rats were exposed in identical conditions at 21°C for comparison.

2.2 Treatment with Stem Cells

About 1 million commercially available MSCs from Sprague-Dawley Rat MSCs (Cyagen Biosciences Inc. Santa Clara, CA 95050, USA; Cat # RASMX-01001) were administered intravenously 1 week before the end of the experiment. The whole administration process of MSCs was performed slowly within 2 min [9].

2.3 Nanodelivery of Stem Cells

We used TiO2 nanowired MSCs for nanodelivery using identical conditions in diabetic or normal rats following heat stress. The viability of MSCs on nanowires was confirmed using thymidine and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MT) assays [7,8].

2.3 Parameters Measured

The following parameters to assess brain pathology were measured in diabetic and normal rats after heat stress and following treatment with MSCs.

2.3.1 Blood-brain barrier

The blood-brain barrier (BBB) leakage was measured using Evans blue albumin (EBA) and radioiodine ($^{[131]}$-Iodine] extravasation in the brain. For this purpose the EBA (2 % of 0.3 ml/100g body weight) was administered intravenously 5 min before termination of the experiment. After washing out if intravascular tracer with 0.9 % saline perfused through heart at 90 Torr, the brain were dissected out and examined for blue staining. The tissue pieces from selected brain areas were then dissected out weighed and radioactivity determined in a Gamma Counter (Packard, USA). Before saline perfusion about 1 ml whole blood was withdrawn from cardiac puncture to determine radioactivity or EBA concentration in the whole blood. 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 formation was determined using measurement of water content in the brain. For this purpose, small tissue pieces of brain were dissected out and weighed immediately 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 on paraffin sections using standard histopathological techniques [7,8]. For this purpose, animals were perfused in situ with 4 % buffered paraformaldehyde preceded with a brief saline rinse though cardiac puncture. After in situ fixation, the brain were removed and kept in the same fixative for 24 h. On the 2nd day coronals sections of the brain were cut passing through the hippocampus and the blocks were embedded in paraffin using standard procedures. About 3 µm thick sections were cut and stained with HE or Nissl using commercial protocol [7].

The sections were examined under an Inverted Carl-Zeiss Microscope and the images were recorded using a digital Olympus camera [8]. The number of damaged or distorted neurons in designated anatomical areas were counted manually and compared between controls; heat stressed healthy or diabetic rats with or without MSCs treatment.

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 MSCs induces Neuroproetction

Our results showed that MSCs infusion resulted in significant reduction in the leakage of EBA and radioiodine in various brain areas after heat stress in healthy rats. Also the development of brain edema was also thwarted in these rats following MSCs infusion (Table 1). Neuronal injuries were also much less evident in MSCs treated heat stressed healthy animals.

However, the diabetic rats showed much more pronounced breakdown of the BBB, brain edema formation and neuronal injuries as compared to healthy animals after identical heat stress.

Interestingly, MSCs administration in diabetic rats after heat stress was not that effective in reducing the BBB disruption, brain edema formation or neuronal injuries. Thus, only a partial reduction in these parameters was observed in diabetic rats after heat stress by MSCs administration.

Table 1. Superior neuroprotective effects of nanodelivered MSCs in Heat Stroke

Expt. Type	Control	Heat Stress 4h	Diabetes+HS	MSCs+HS+DB	TiO2-MSCs+HS+DB
EBA mg%	0.20±0.05	1.89±0.12**	2.67±0.14**#	1.06±0.04*a	0.54±0.12*#b
Brain Edema	75.36±0.12	80.34±0.19**	82.43±0.98**#	77.34±0.17*a	76.89±0.23*#b
Neuron Injury	1±2	234±45**	465±81**#	120±23*a	24±12*#b

*Values are Mean±SD of 5 to 6 rats. DB = Diabetes, * P <0.05, ** P <0.01 from control, # P <0.05 from Heat stress, a P <0.05 from DB, b P <0.05 from MSCs+HS. For details see text.*

3.2 Nanowired delivery of MSCs is superior

Nanodelivery of MSCs with TiO2 under identical conditions resulted in much superior neuroprotective effects in diabetic rats subjected to 4 h heat stress. Thus, in TiO2 nanowired MSCs treated animals the BBB leakage to EBA and radioiodine was significantly reduced in diabetic group after heat stress. In the animals the brain edema was also thwarted near normal levels after heat exposure. Neuronal injuries were considerably reduced by nanodelivered MSCs following heat stress in diabetic rats (Fig. 1, Table 1).

Interestingly, nanodelivered MSCs prevented brain pathology, BBB leakage and brain edema in healthy animals as well after HS (Table 1).

4 DISCUSSION

The salient novel finding in this innovation suggests that nanodelivery of MSCs are far superior in inducing neuroprotection in heat stress induced brain damage. Furthermore, another novel observation in this investigation shows that exacerbation of brain damage in diabetic rats after heat stress was markedly attenuated by nanodelivered MSCs, a feature that was not seen suing normal MSCs administration. This suggests that a combination of diabetes and heat stress induced much more profound brain damage that requires nanodelivery of drugs or MSCs to have beneficial effects.

These observations are in line with the idea that new therapeutic approaches are necessary i.e., nanodelivery to treat our soldiers who are suffering from PTSD with endocrine dysfunction are exposed to high environmental heat or other adverse situations.

The possible mechanisms of superior effects of nanodelivered MSCs in heat stress are nit clear from this investigation. However, it appears that nanodelivery of MSCs could induce more longevity of stem cells for longer periods as compared to normal MSCs in vivo situations [7-9]. Further study is needed to clarify these points.

How MSCs are protecting brain function or recovery? To that end our knowledge is still unclear. However, available evidences suggests that these MSCs could induce more better cell to cell communication, release of endogenous neurotrophic factors and other chemical and immunologic profiles that may have membrane strengthening properties [9,10]. As a result neuronal, vascular and glial cells appears to be repaired or less injured after any noxious insult to the CNS.

A reduction in BBB breakdown in diabetic rats after heat stress in MSCs treated rats is in line with this idea. This is possible that nanodelivered MSCs could repair or strengthens endothelial ells of the cerebral microvessels causing less damage to the BBB and thus reducing edema formation. When edema formation is reduced neuronal injuries are lessened causing neuroprotection [4,5,7,8,10].

It remains to be seen whether nanowired MSCs could also enhance several neurotrophic factors e.g., brain derived neurotrophic factor (BDNF), glia derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF) and other growth factors in the brain or plasma.

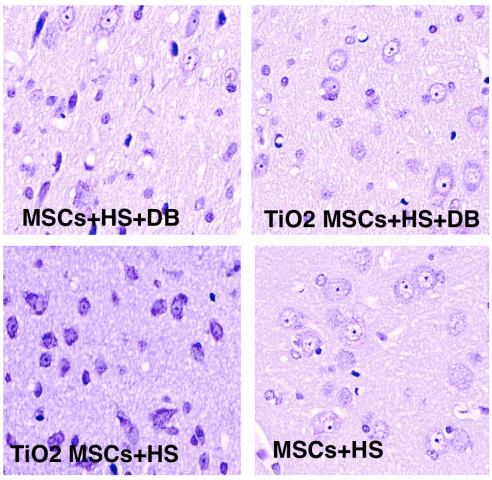

Fig. 1. Nanowired MSCs induces superior neurorprotection in diabetes induced brain damage in heat stress.

5 CONCLUSION

Taken together our innovation clearly shows that nanowired MSCs are needed to effectively reduce brain pathology in diabetic rats after heat stroke. It remains to be seen whether TiO2 nanowired MSCs are more superior to other models of nanodelivery, e.g., encapsulation of MSCs using soft nanoparticles. This is a feature currently being investigated in our laboratory.

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