Nanodelivery of Cerebrolysin as adjunct therapy with Functionalized magnetic iron oxide nanoparticles enhances neuroprotection following whole body hyperthermia

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ABSTRACT

The incidences of breast and lung cancer in military personnel are very high. Recently, functionalized magnetic iron oxide nanoparticles (FMIONPs) are used as safe adjuvant therapy in cancer patients with chemotherapy or heat treatment to destroy cancerous cells effectively. However, we found that FMIONPs following whole body hyperthermia (WBH) like cancer therapy resulted in exacerbation of brain pathology within 24 h. Thus, in this innovation we used Cerebrolysin (a balanced composition of neurotrophic factors and active peptide fragments) with or without TiO2 nanowired together with FMIONPs administration to reduce brain damage following WBH in the rat. The nanowired Cerebrolsyin significantly reduced brain pathology caused by FMIONPs following WBH. This innovation suggests that nanowired delivery of Cerebrolsyin, as an adjunct therapy is needed in cancer patients where FMIONPs are required to enhance the effects of chemotherapy or heat treatment.

Keywords: Cancer in military, iron oxide nanoparticles, cancer therapy, cerebrolysin, whole body hyperthermia, brain pathology

1 INTRODUCTION

The incidences of breast and lung cancer are significantly higher in our military personnel of both sexes than the civilian populations of the same age [1-3]. This is largely due to the fact that our soldiers are often exposed to environmental pollution where they are deployed for combat or peacekeeping operations [1,2]. In addition, during war-like situations, they are also exposed to toxic chemicals emanating from burned fuel, explosives, gunfire and rocket propulsion [1-3]. These toxic environmental and chemical exposure leads to development of lung and breast cancers in both female and male soldiers [2]. Thus, the need of the hour is to explore suitable therapy to treat cancer cases effectively with the help of multiple drug combinations.

Recently, in many cases of breast cancer, functionalized magnetic iron oxide nanoparticles (FMIONPs) are used as an adjuvant therapy together with chemotherapy and/or heat treatment for effectively killing these cancerous cells while healthy cells are not destroyed [4,5]. The magnetic particles could be relocated after intravenous injection to the effective areas where cancerous cells are present and then they could be heated to allow local cancerous cells destruction [4]. Available reports suggest that FMIONPs are rather safe to healthy cells [4,5].

However, this is a possibility that FMIONPs may affect brain cells and may induce cellular reactions especially in hot environment that is normally used for heat treatment of cancer patents, i.e. whole body hyperthermia (WBH).

Keeping tehse views in mind we evaluated the role of FMIONPs in a rat model of WBH similar to that used for heat treatment in cancer patients and evaluated brain pathology. In addition, we also evaluated the role of Cerebrolysin; a balanced composition of several neurotrophic factors and active peptide fragments on FMIONPs induced alteration in brain pathology after WBH.

2 MATERIALS & METHODS

Experiments were carried out on Male Wistar Rats (200-300 g) housed at controlled room temperature (21±1° C) with 12 h light and 12 h dark schedule. Food and water were supplied 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 Stress

Normal or FMIONPs treated 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 [5]. A group of saline treated rats were exposed in identical conditions at 21°C for comparison.

2.2 FMIONPs administration

FMIONPs administration (9-10 nm in diameter, Ocean Nano Tech, Springdale, AR, USA) was administeres intravenously in normal rats in a dose of 0.50 mg/mL in100 µl) and these animals were allowed to survive for 24 [4]. This dose and time schedule is quite comparable to those used in MRI studies or drug delivery to the brain in vivo situations [5].

2.3 Nanodelivery of Cerebrolysin

We used normal or TiO2 nanowired Cerebrolsyin (2.5 ml/kg) administered intravenously 30 min before subjection of rats to 4 h WBH in saline or FMIONPs treated groups under identical conditions [5-7].

2.3 Parameters Measured

The following parameters to assess brain pathology were measured and normal or SiO2 intoxicated rats after heat stress and following treatment with Cerebrolsyin.

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 [5-7].

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 [5,6].

2.3.3 Neuronal injury

Neuronal injury was evaluated using Nissl or Haematoxylin & Eosin (HE) staining on paraffin sections using standard histopathological techniques [5-7]. 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 [5].

The sections were examined under an Inverted Carl-Zeiss Microscope and the images were recorded using a digital Olympus camera [6,7]. 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 FMIONPs induce brain pathology

Administration of FMIONPs in normal rats kept at room temperature did not alter brain functions e.g., BBB disruption, brain edema formation or neuronal injuries as compared to saline treated control group within 24 h observations period (Table 1).

However, when identical doses of FMIONPs are administered and the rats were subjected to a 4 h WBH, mild to moderate disruption of the BBB to EBA and radioiodine was noted that was significantly higher than saline treated animals after WBH.

Interestingly, in these FMIONPs treated rats after WBH brain edema formation and neuronal injuries were slightly but significantly exacerbated than saline treated rats following WBH (Table 1).

Table 1. Superior neuroprotective effects of TiO2 Cerebrolsyin on FMIONPs induced and brain pathology in WBH

Expt. Type	Control	Heat Stress 4h	FMIONPs+HS	CBL+HS+ FMIONPs	TiO2-CBL+HS+ FMIONPs
EBA mg%	0.24±0.08	1.84±0.12**	2.17±0.08**#	0.98±0.08*a	0.58±0.10*#b
$^{[131]}$Iodine %	0.34±0.08	2.03±0.10**	2.43±0.06**#	1.16±0.06*a	0.68±0.11*#b
Brain Water %	75.36±0.12	80.14±0.14**	81.28±0.18**#	78.44±0.17'a	76.08±0.13*#b
Neuronal Injury Nr	1±2	234±45**	285±21**#	82±8'a	34±8*#b

*Values are Mean±SD of 5 to 6 rats. CBL = Cerebrolysin, * P <0.05, ** P <0.01 from control, # P <0.05 from Heat stress, a P <0.05 from FMIONPs, b P <0.05 from CBL+FMIONPs;TiO2-CBL = nanodelivered Cerebrolysin. For details see text.*

3.2 Neuroproetction by TiO2 Cerebrolsyin

Treatment with Cerebrolysin (2.5 ml/kg, i.v.) resulted in marked neuroprotection in saline treated rats after 4 h WBH (Table 1). Thus, in these rats BBB breakdown, brain edema formation and neuronal injuries were significantly reduced as compared to saline treated heat stressed rats (Table 1). However, this dose of Cerebrolsyin was not that affective in reducing brain pathology in animals that received FMIONPs before WBH (Table 1, Fig. 1).

However, when TiO2-nanowired Cerebrolsyin (2.5 ml/kg, i.v.) was administered in FMIONPs treated heat stressed rats, significant neuroprotection was observed in this group. Thus, marked reduction in the BBB breakdown, brain edema formation and neuronal injuries were seen that were even further lowered than the saline treated WBH group (Table 1, Fig. 1).

4 DISCUSSION

Our novel findings demonstrated that Cerebrolysin has the capability to thwart FMIONPs adverse effects leading to brain pathology in WBH. This innovation suggests that Cerebrolsyin could be used as an adjunct therapy in cancer patients in which FMIONPs are administered with chemotherapy or heat treatment for effective destruction of localized cancerous tissues.

Another important finding of this investigation clearly show that FMIONPs are innocuous to brain function under normal conditions. This suggests that these nanoparticles when used in disease situations may induce brain pathology. Thus, to reduce or attenuate brain damage by FMIONPs in disease conditions, suitable neuroprotective agents e.g., Cerebrolsyin may be co-administered. We further observed that TiO2 nanowired Cerebrolsyin has superior effects in reducing FMIONPs induced brain pathology in WBH than normal Cerebrolsyin in identical doses. This indicates that effective drug delivery to then brain of Cerebrolysin is needed to attenuate FMIONPs induced adverse cellular reactions in the brain. Obviously, TiO2 nanowired Cerebrolsyin reached to the brain in high concentrations without being metabolized for long time due to its binding with TiO2 nanowires. Alternatively, high doses of Cerebrolsyin may be used to achieve better neuroprotection. However, this is a feature currently being analyzed in our laboratory.

What could be the basic reasons for enhancing brain damage in WBH by FMIONPs is still unclear. However, there are reasons to believe that oxidative stress that could be generated by WBH alone could be more intense after administration of FMIONPs [5,7]. Obviously, intense oxidative stress and generation of free radicals by a combination of FMIONPs and WBH could lead to an exacerbation of brain damage. Cerebrolsyin with its balanced components of neurotrophic factors and active peptide fragments induced neuroprotection in present innovation as well as its ability to reduce the magnitude and intensity of oxidative stress is in line with this idea [5-7]. An effective delivery of Cerebrolsyin using TiO2 nanowires lead to further enhancement of its neuroprotective efficacy in FMIONPs induced brain damage following WBH. This observations also supports the idea that in high doses or effective concentration Cerebrolsyin reaching brain cells following TiO2.naowored delivery could also lead to more effective reduction in the oxidative stress in this model [7]. However, further studies

are needed to find out the levels of oxidative stress parameters in a combination of FMIONPs and WBH as compared to WBH alone. Since FMIONPs alone did not induce brain damage in normal rats it appears that a combination of FMIONPs and WBH is more dangerous. It may be that FMIONPs at high ambient or body temperature induces greater oxidative stress than at normal room or body temperatures [4]. Thus, this is likely that FMIONPs when used in cancer therapy with heat treatment it could affect some normal cells more adversely.

Our innovation thus clearly suggests that Cerebrolsyin given together with FMIONPs under heat stress remarkably protect the brain damage. This indicates that FMIONPs could be administered with Cerebrolysin in suitable doses in cancer therapy for the benefit of the patients.

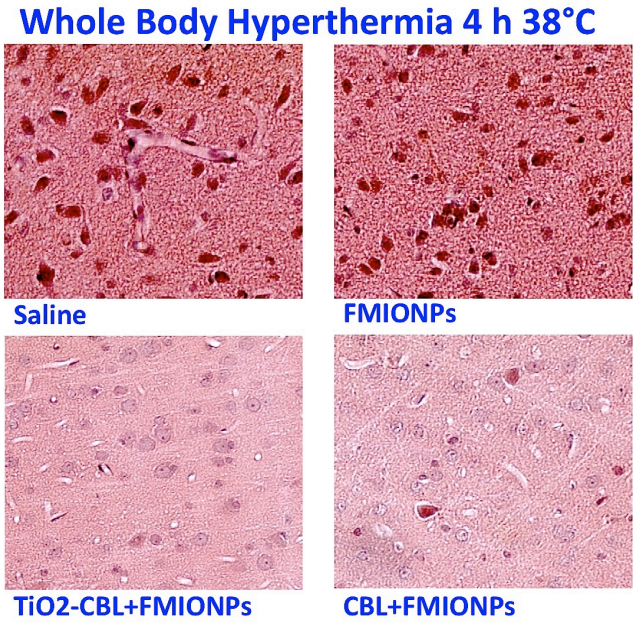

Fig. 1. TiO2-Nanowired Cerebrolysin induces superior neuroprotection in FMIONPs treated rats after WBH. Bar = 35 µm.

5 CONCLUSION

Taken together our innovation demonstrated novel use of Cerebrolsyin as an adjunct therapy in combination with chemotherapy and/or heat treatment for cancer patients to reduce healthy cell damage. Furthermore, when cancer therapy requires FMIONPs administration for concentrating heat-induced damage of cancerous cells, co-administration of TiO2-nanowired Cerebrolsyin is needed to attenuate nanoparticles induced toxicity. In addition, when FMIONPs induced drug delivery is required co-administration of Cerebrolsyin could also be beneficial in enhancing the drug effects by reducing cellular toxicity. However, additional investigation is needed in this direction for further clinical advancements in cancer therapy using Cerebrolsyin.

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7 REFERENCES

[1] Anderson JR, Alarming breast cancer rates among troops reported. Military Times 12:26 p.m. EDT October 2, 2012,*http://www.usatoday.com/story/news/nation/2012/10/02/breast-cancer-troops/1608293/* accessed on April 14, 2014

[2] Rennix CP, Quinn MM, Amoroso PJ, Eisen EA, Wegman DH. Risk of breast cancer among enlisted Army women occupationally exposed to volatile organic compounds. Am J Ind Med. 2005 Sep;48(3):157-67.

[3] Harris RE, Hebert JR, Wynder EL. Cancer risk in male veterans utilizing the Veterans Administration medical system. Cancer 1989;64:1160-8

[4] Ahmed M, Douek M. The role of magnetic nanoparticles in the localization and treatment of breast cancer. Biomed Res Int. 2013;2013:281230. doi: 10.1155/2013/281230. Epub 2013 Jul 9. Review.

[5] Sharma HS, Menon PK, Lafuente JV, Aguilar ZP, Wang YA, Muresanu DF, Mössler H, Patnaik R, Sharma A. The role of functionalized magnetic iron oxide nanoparticles in the central nervous system injury and repair: new potentials for neuroprotection with Cerebrolysin therapy. J Nanosci Nanotechnol. 2014 Jan;14(1):577-95.

[6] Menon PK, Muresanu DF, Sharma A, Mössler H, Sharma HS. Cerebrolysin, a mixture of neurotrophic factors induces marked neuroprotection in spinal cord injury following intoxication of engineered nanoparticles from metals. CNS Neurol Disord Drug Targets. 2012 Feb;11(1):40-9. Review.

[7] Sharma HS, Muresanu DF, Patnaik R, Stan AD, Vacaras V, Perju-Dumbrav L, Alexandru B, Buzoianu A, Opincariu I, Menon PK, Sharma A. Superior neuroprotective effects of cerebrolysin in heat stroke following chronic intoxication of Cu or Ag engineered nanoparticles. A comparative study with other neuroprotective agents using biochemical and morphological approaches in the rat. J Nanosci Nanotechnol. 2011 Sep;11(9):7549-69.

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