Nanodelivery of Chinese traditional medicine extract of Gingko Biloba (EGb-761) induces superior neuroprotection following traumatic brain injury in heat stroke

Aruna Sharma[1,3-5], Dafin F Muresanu[2,3], José Vicente Lafuente[4,9], Zhi-Qiang Zhang[5], Cong Li[5], Ranjana Patnaik[6], Z Ryan Tian[7], Asya Ozikzilcik[8], Hari S Sharma*[1,3-5]

[1]Int. Expt. CNS Injury & Repair (IECNSIR), Dept. of Surgical Sciences, Anesthesiology & Intensive Care Medicine, Uppsala University Hospital, Uppsala University, SE-75185 Uppsala, Sweden Email: Aruna.Sharma@surgsci.uu.se
[2]Dept. Clinical Neurosciences, University of Medicine & Pharmacy, Cluj-Napoca, Romania
[3]''RoNeuro'' Institute for Neurological Research and Diagnostic, 37 Mircea Eliade Street, 400364, Cluj-Napoca, Romania
[4]LaNCE, Dept. Neuroscience, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain
[5] Department of Neurosurgery, Chinese Medicine Hospital of Guangdong Province; The Second Affiliated Hospital, Guangzhou University of Chinese Medicine, Dade road No.111, Yuexiu District, Guangzhou, China
[6]School of Biomedical Engineering, Dept. of Biomaterials, Indian Institute of Technology, Banaras Hindu University, Varanasi, India
[7]Dept. Chemistry & Biochemistry, [8]Dept. of Biomedical Engineering, University of Arkansas, Fayetteville, AR, USA
[9]Nanoneurosurgery Group, BioCruces Health Research Institute, 48903 Barakaldo, Bizkaia, Spain

ABSTRACT

Heat stroke (HS) affects millions of people worldwide especially during summer months. Military personnel working in hot environment are often highly vulnerable to HS. Morepver when these military personnel during combat operation are inflicted with traumatic brain injury (TBI)at hot envireonment, the pathophysiology of brain dmage is far more serious than the identical brain trauma at thermoneutral ambient temperature. Thus, efforts are needed to find out superior neuroprotective therapy in situations of TBI at hot environment. The traditional Chinese mediicne extract of Gingko Biloba (EGb-761) is used for mental health and other disease as antioxidant. Previosuly we reported marked neuroproetction following heat exposure induced brain pathology by EGb-761. Thus, apossibility exists that nanodelivery of EGb-761 may have additional suprior neuroproetctive effects on TBI induced brainpathology at hot environment. Our results showed that TiO2-nanowired delivery of EGb-761 (50 mg/kg,p.o.) or BN-52021 (2 mg/kg, p.o.) for 5 days induced significant neuroprotection following TBI in HS, not reported earlier.

Keywords: Heat Stroke in Military, brain pathology, Brain edema, Blood-brain barrier, EGB-761, BN-52021 nanodelivery, Brain pathology

1 INTRODUCTION

Heat stroke (HS) associated wth heat stress is a serious medical condition resulting in instant death of more that 60 % of the victims. Those who survive exhibit long term brain dysfunction and disability [1-3]. Military personnel working in hot environment are highly susceptible to HS. Moreover additional injury to brain or spinal cord at hot enviroenment results in exacerbation of brain pathology. Thus, efforts should be needed to find better therapeutic strategies to reduce mortality and morbidity follwing TBI in HS.

Previous studies fromour laboratory showed breakdown of the blood-brain barrier (BBB) to protein tracers resulting edema and cellular injuries in HS. Also, TBI done at high ambient temperature (30°C) exacerbates brain pathology in naïve rats [4]. Thus, a possibility exists that TBI inflicted in HS condition will further aggravate brain damage and behavioural dysfunction.

EGb-761 is a teaditional Chinese medicine used to treat several neurological ailments in clinical settings. We have shown earlier that treate ment with EGb-761 and BN-52021 given separately daily for 5 days reduces brain pathology in HS. Since nanodelivery of neuroproettive drugs induces superior beneficial effects in this innovation we used TiO2 nanowired EGb-761 or BN-52021 in HS following TBI. Our observations showed superior neuroprotection with TiO2-nanowired EGb-761 than BN-52021 in TBI with HS.

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 Environmental Heat Stress

Animals were subjected to HS in a biological oxygen demand (BOD) incubator maintained at 38±0.5°C with relative humidity 45-47 % and wind velocity 22-25 cm/sec as describerd earlier [1,2]. This model simulates clinical conditions of HS as seen with behavioral symptoms and brain pathology after 4 h exposure [2]. Thus, rats were exposed to 2 h HS daily for 8 days in the BOD incubator that does not induce brain pathology. Rats placed at room temperature 21±1° C were used as controls.

2.2 Traumatic brain injury

On the 8th day, the animals were inflicted brain injury under Equithesin anesthesia in naïve or HS group by making a longitudinal lesion into the parietal cerebral cortex (2.5 mm deep and 4 mm long) after opening of the parietal bone (4mm^2). The would was covered with 0.9 % saline to prevent direct exposure to air [4]. The animals were allowed to survive 8 h aftre traumatic brain injury (TBI).

2.3 Treatment with extracts of Gingko biloba

Extracts of Gingko biloba EGb-761 (50 mg/kg, p.o.) with or without bilobalide BN-52021 (2 mg/kg, p.o.) were administered daily for 5 days starting from 24 h after initiation of HS in rats with or without TBI.

Saline treated group served as controls.

The drugs were in water suspension and given in control or experimental groups by gavage in the dose mentione above..

2.4 TiO2-nanowired delivery of Gigko biloba

The drugs EGb-761 and BN-52021 were tagged with TiO2 nanowires according to standard protocol [4-6]. The TiO2 nanowired EGb-761 oe BN-52021 were administered inidentical doses by gavage. The 1st dose was administered 24 h aftre the onset of HS and continued daily for 5 days in HS alone or HS with TBI group as above. Naïve rats were also administered nanowired EGb-76 and BN-52021 in identical manner.

2.5 Blood-Brain Barrier and brain edema

The blood-brain barrier (BBB) breakdown to Evans blue albumin (EBA) and radioiodine ($^{[131]}$Iodine) was examined 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 [1,4]. Brain edema was determined using regional water content by wet and dry weights of the brain samples [5].

2.6 Brain Pathology

In separate groups of animals, neuronal changes were studied using histopathological examination of Nissl or Haematoxylin & Eosin (HE) staining on 3-µm thick paraffin sections [4,5]. Cortical brain tissues were processed for transmission electrom microscopy (TEM) to analyse ultrastructuralc hanges in the brain as well [5].

2.7 Behavioral parameters

Rota-rod performance, inclined plane angle test and walking on a mesh grid was used to evaluate behavioural functions in HS group in rats with or without TBI or drug treatments as described earlier [4,5].

2.8 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 HS exacerbates TBI induced BBB and brain edema

TBI inflicted on chronically exposed HS showed an exacerbation of the BBB breakdown as evident with extravasation of endogenous Evans blue albumin (EBA) and radioiodine in the cerebral cortex, hippocampus and the cerebellum. The brain edema formation and neuronal damages were also much more aggravated by prior HS in TBI group as compared to normal animals (results not shown). Eight hours after TBI in HS exhibited 4- to 6 times greater BBB leakage to Evans blue and $^{[131]}$Iodine, 5-to 6 fold increase in brain edema in the injured side and 4- to 6-fold higher neuronal damages in the remote cortical tissues in the injured hemisphere as compared to identical TBI in naïve rats.

3.2 TBI exacerbates brain pathology in HS

Neuronal distortion and damages are more frequent in the brain areas showing edema formation or sponginess of the neuropil. In general hippocampus showed grater

neuronal damages in the CA-3 and 4 areas along with dentate gyrus as compared to CA-1 and CA-2 areas of the hippocampus following TBI in HS. Cerebellar granule cells and Purkinje cells both showed cellular swelling, distortion and damage in the vermis as well as the lateral cerebellar cortices in a selective and specific manner in HS with TBI.

A representative example of neuronal damage following chronic HS and its exacerbation is shown in Fig. 2. As evident with the Fig. 2, loss of neurons, perineuronal edema and sponginess is much more marked in 8 h TBI rats following HS as compared to HS alone (See Fig. 2).

3.3 EGb-761 treatment in HS

EGb-761 or BN-52021treatment was able to reduce brain pathology, BBB dusruption and braun edema formation in HS rats. However, theor effects on TBI induced greater brain pathology and BBB disturbances were only slighly but significantly reduced. Thus, treatment with EGb-761 with BN-52021 in identical disease reduced brain damage only by 20 to 30 % following TBI in HS (results not shown).

3.4 TiO2 EGb-761 and Brain pathology

However, when TiO2 nanowired EGb-761 or TiO2 BN-52021 were administered in identical doses, more than 80 % reduction in brain pathology was observed following TBI in HS (Fig. 2).

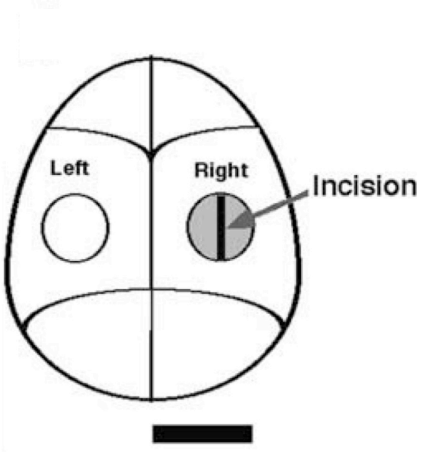

Fig. 1. Traumatic brain injury (TBI) model in the rat. A longitudinal incision into the right parietal cerebral cortex was perfromed under sterotaxic guidance (3 mm deep and 5 mm long) to induce cortical injury aftre opening the skull (4 mm^2) on both side. Neuronal changes were examined on the right parietal cortex aftre 8 h TBI in HS (for details see text). Bar = 5 mm.

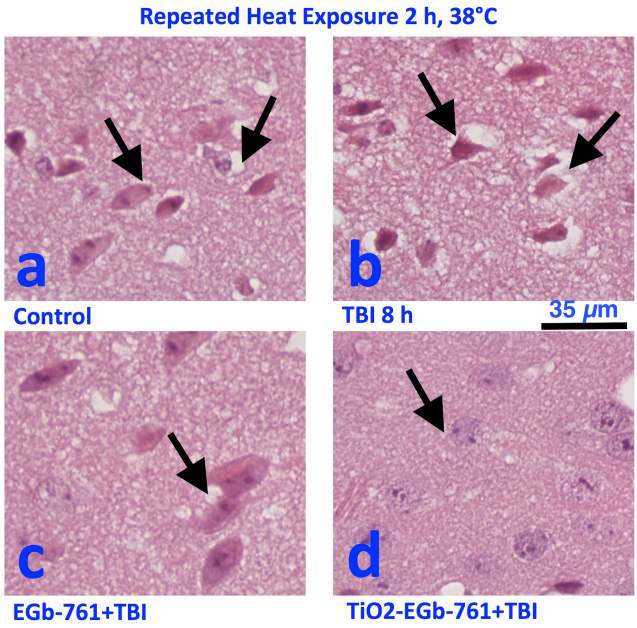

Fig. 2. Shows loss of neurons and sponginess with edema in the parietal cerebral cortex in HS (a) and its exacerbation by traumatic brain injury (b). Treatment with TiO2-naowired EGb-761 signifcicantly reduced the neuronal dmages, edema and sponginess in HS with TBI (d) as compared to EGb-761 alone (c). H&E stain on 3-μm thick paraffin sections. Bar: 35 μm.

3.5 TiO2 EGb-761 on BBB and brain edema

Massive disruption of the BBB to proetin tracers in TBI of HS rats was significantly reduced by the TiO2 nanowored EGb-761 and BN-52021, a deature not observed by the normal drugs given in the identical doses. Likewise, TiO2 nanowired EGb-761 or nanowired BN-52021 was able to reduce brain edema and volume swelling in TBI rats with HS by 80 to 90%. In this respect EGb-761 appears to be more potent than BN-52021 (results not shown).

3.6 TiO2 EGB-761 and ultrastructural changes

Ultratsructural studies show that the TiO2 nanowired EGb-761 or nanowired BN-52021 was able to reduce neuronal injuries in the cortex of TBI rats with HS confirming our light microscopic observations (see Fig. 1). Our TEM studies showed less vacuolation, edematous expansion or synaptic damage in TiO2 nanowired EGb-761 treated TBI rats with HS. Nanowired BN-52021 was also effective in reducing ultrastructural damages following TBI in HS. However, the neuroprotective efects of nanowired EGB-761 was superior than TiO2-nanowired BN-52021 treated identical group (results not shown).

3.7 TiO2 EGb-761 and behavioural functions

The functional outcome e.g., walking on a tilted mesh grid (45°), staying on a Rota Rod treadmill (16 r.p.m.) and finding placing of forepaw on a wire mesh were significantly reduced by TiO2 nanowired EGb-761 and BN-52021 treated group after TBI in HS. When EGb-761 and BN-52021 were given separately using nanowired delivery, the effects of EGB-761 were more superior than BN-52021 alone (results not shown).

4 DISCUSSION

The novel findings in this investigation clearly show that repeated HS is capable to induce brain pathology and addition trauma e.g., TBI could further aggravates HS induced brain pathology. This suggests that HS potentiates TBI induced brain damage [5-6].

Our observations are the first to show that TiO2 nanodelivery of EGb-761 or BN-52021 is capable of neuroprotection in HS with TBI. This indicates that oxidative stress plays a paramount role in exacerbation of brain pathology in HS. EGb-761 contains flavonol glycosides (ca. 25 %), terpene trilactones (ca. 3 %), ginkgolides A, B, C (3 %) and ginkgolic acid (ca. 5ppm). These characteristics of EGb-761 makes it very powerful antioxidant or free radical scavengering ability to induce neuroproetction [6]. This could be one of the reasons that EGb-761 has far more superior effects than BN-52021 in our present or previous studies [2-4].

HS alone induces profound oxidative stress causing BBB disruption and brain edema formation. TBI also is capable to disrupt BBB function and leads to edema formation and cellular injuries. Thus, it is quite likely that TBI in HS group leads to exacerbation of oxidative stress or free radical induced aggravation of brain pathology [7].

The idea gets additional support from nanowired delivery fo these compounds in TBI with HS. Nanodelivery of compounds are effective in penetrating brain much faster and could be widely distributed within the the CNS [4-6]. Obviously, restoration of BBB function and reduction in brain edema could be much better with TiO2 nanowred deleivery of EGB-761 [4]. A slow degradation or metabolism of nanowired EGb-761 or BN-52021 within the brain may also be responsible for maintaining high level of these compounds in the brain aftre TBI in HS resulting in neuroprotection.

5 CONCLUSION

Taken together these observations suggests that nanodelivery of EGb-761 in TBI following HS has a potential therapeutic value in TBI in HS that require further investigation, not reported earlier.

It remains to be seen whether nanowired EGb-761 or BN-52021 if given together at different time periods after TBI in HS could still be able to induce marked neuroproetction. This is a subject that is currently being einvestigated in our laboratory.

6 ACKNOWLEDGEMENTS

Supported by grants from Ministry of Science & Technology, People Republic of China, the Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065; Swedish Medical Research Council (Nr 2710-HSS), Göran Gustafsson Foundation, Stockholm, Sweden (HSS), Astra Zeneca, Mölndal, Sweden (HSS/AS), The University Grants Commission, New Delhi, India (HSS/AS), Ministry of Science & Technology, Govt. of India (HSS/AS), Indian Medical Research Council, New Delhi, India (HSS/AS) and India-EU Co-operation Program (RP/AS/HSS) and IT 794/13 (JVL), Government of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain, & Society for Neuroprotection and Neuroplasticity (SSNN), Romania. We thank Suraj Sharma, Uppsala, Sweden for computer and graphic support. The U.S. Government is authorized to reproduce and distribute reprints for Government purpose notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Air Force Office of Scientific Research or the U.S. Government. The views and conclusions are exclusively those of the authors and should not reflect the official policies or endorsements of the Air Force Office of Scientific Research or the U.S. Government or any of the granting organizations or collaborating entities mentioned above

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[1]***Hari S Sharma**, Dr. Med. Sci. (UU), Director Int. Expt. CNS Injury & Repair (IECNSIR), University Hospital, Uppsala University, Prof. Neurobiology (MRC); Docent Neuroanatomy (UU); Frödingsgatan 12:28, SE-75421 Uppsala, Sweden, Phone & Fax: +46 18 243899, Cell Phone: +46 70 2011 801; Email: Sharma@surgsci.uu.se, harishanker_sharma55@icloud.com