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

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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. Moreover when these military personnel during combat operation are inflicted with traumatic brain injury (TBI) at hot environment, the pathophysiology of brain damage 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 medicine extract of Gingko Biloba (EGB-761) is used for mental health and other disease as antioxidant. Previously we reported marked neuroprotection following heat exposure induced brain pathology by EGB-761. Thus, a possibility exists that nanodelivery of EGB-761 may have additional superior neuroprotective effects on TBI induced brain pathology 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 with 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 environment results in exacerbation of brain pathology. Thus, efforts should be needed to find better therapeutic strategies to reduce mortality and morbidity following TBI in HS.

Previous studies from our 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 traditional Chinese medicine used to treat several neurological ailments in clinical settings. We have shown earlier that treatment with EGB-761 and BN-52021 given separately daily for 5 days reduces brain pathology in HS. Since nanodelivery of neuroprotective 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 described 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 (4mm²). The wound was covered with 0.9 % saline to prevent direct exposure to air [4]. The animals were allowed to survive 8 h after 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 mentioned above.

2.4 TiO2-nanowired delivery of Gignko 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 identical doses by gavage. The 1st dose was administered 24 h after 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 electron microscopy (TEM) to analyse ultrastructural changes 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-52021 treatment was able to reduce brain pathology, BBB disruption and brain edema formation in HS rats. However, their effects on TBI induced greater brain pathology and BBB disturbances were only slightly 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).

3.5 TiO2 EGB-761 on BBB and brain edema

Massive disruption of the BBB to protein tracers in TBI of HS rats was significantly reduced by the TiO2 nanowired EGB-761 and BN-52021, a feature 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

Ultrastuctural 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 effects 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 tri lactones (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 neuroprotection [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 for 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 nanowired delivery 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 after 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 neuroprotection. This is a subject that is currently being investigated in our laboratory.

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7 REFERENCES


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