Research Article • DOI: 10.1515/tnsci-2018-0007 • Translational Neuroscience • 9 • 2018 • 33-37

Translational Neuroscience

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BENEFICIAL EFFECT OF B-ELEMENE ALONE AND IN COMBINATION WITH HYPERBARIC OXYGEN IN TRAUMATIC BRAIN INJURY BY INFLAMMATORY PATHWAY

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Abstract

Background: Present study evaluates the neuroprotective effect of β-elemene alone and in combination with hyperbaric oxygen (HO) in traumatic brain injury (TBI). Methodology: TBI was induced by dropping a weight from a specific height. All the animals were separated in to five groups (n=20) like control group; TBI group; β-elemene treated group which receives β-elemene (100 mg/kg, i.p.) half an hour after the injury; HO group which receives hyperbaric oxygen therapy and β-elemene + HO group which receives β-elemene (100 mg/kg, i.p.) half an hour after the injury and hyperbaric oxygen therapy. Neurological function was assessed to evaluate the effect of β-elemene in TBI rats. Thereafter level of inflammatory cytokines and expression of protein of inflammatory pathway was assessed in the brain tissues of TBI rats. In addition TUNEL assay was also done for the determination apoptosis in neuronal cells. Result: Data of the report reveals that β-elemene alone and in combination with hyperbaric oxygen (HO) significantly decreases the neurological score Compared to TBI group. Moreover level of inflammatory cytokines and expression of LTR4 and casepase 3 significantly decrease and increase in the expression of IkB in β-elemene alone and in combination with hyperbaric oxygen (HO) treated group compared to TBI group. Data of TUNEL assay also reveals that β-elemene treated group shows significant decrease in the TUNEL positive cells and apoptosis index compared to TBI group. Conclusion: Thus present study concludes the neuroprotective effect of β-elemene against TBI and it shows synergistic effect on TBI when treated with HO.

Keywords

• β-elemene • Hyperbaric oxygen • Traumatic brain injury • Inflammation pathway

Received 15 February 2018
accepted 09 March 2018

Introduction

Traumatic brain injury is one of the major causes of morbidity and mortality throughout the globe [1]. In TBI initially injury occurs due to violence or trauma and further several events occurs such as edema in brain, degeneration of neuron and impairment of cognitive function due to progression of secondary injury after few hrs of initial trauma [2]. Literature suggested that the progress of secondary injury can be prevented by targeting several pathways which involved in the pathogenesis of it [3]. Apoptosis in the neuronal cell in secondary brain injury stimulated due to overload of calcium, increase in oxidative stress and level of inflammatory mediators and other pathways are also involved for it [4].

Treatment with hyperbaric oxygen is reported to have beneficial effect management of secondary brain injury but there are several limitations of this therapy. Moreover drugs from natural origin gain importance for the management of several disorders including traumatic brain injury. β-elemene is isolated from the plant of ginger (*Curcuma zedoaria*) that is used traditionally as a medicine in China [5]. β-elemene is a novel anticancer agent that used in the treatment of multiple types of cancer like melanoma, glioblastoma, breast cancer, lung cancer, esophageal cancer and pancreatic cancer too [6-8]. β-elemene also reported to attenuates the motor disability in optic neuritis and also have strong anti inflammatory property [9]. Moreover it ameliorates the atherosclerosis on the basis of its strong anti inflammatory and antioxidant property [10]. Thus present investigation evaluates the effect of β-elemene alsone and synergistic effect with hyperbaric oxygen in the management of traumatic brain injury.

Material and methods

Animals

Male albino SD rats (120-150 g) were procured from Shanghai medical college, Shanghai, China. Animals were stored under the standard condition as per the guideline. All the animals were kept for the period of 7 day for the acclimatization to laboratory condition with free access to normal standard chow diet and tap water. Protocol of this study is approved by institutional animal ethical committee of Navy General Hospital (NGH/IAEC/2017/16) .

Induction of traumatic brain injury

Animals were separated in to five groups (n=20) like control group; TBI group; β-elemene treated group which receives β-elemene (100 mg/kg, i.p.) half an hour after the injury; HO group which receives hyperbaric oxygen therapy and β-elemene + HO group which

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receives β-elemene (100 mg/kg, i.p.) half an hour after the injury and hyperbaric oxygen therapy.

All animals were anesthetized with chlorpromazine (1 mg/kg) and ketamine (100 mg/kg) and a midline incision was made after shaving the scalp. Bone wax was used to fix a metallic disc in a central position. The rats were placed on a foam rubber platform and mild trauma was induced by dropping a 300-g steel weight from a height of 1 meter. At the end of the experiment, a neurological examination was performed and pathological changes were assessed in all animals.

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy was given to the respective groups 2 hr after the injury as per previously reported method. Animals were placed in a chamber and for the duration of 10 min pure oxygen was purged in the chamber to assure that oxygen fraction in the chamber was >95%. Thereafter for the period of one hr pressure was maintained upto 0.12 MPa and later pressure was reduced slowly for 20 min so that it reaches to normal. At an interval of 10 h hyperbaric oxygen therapy was given twice in the respective groups

Neurological examination

Behavior and motor changes were assessed using the 20-point neuro score. The behavior assessments included the response to and circling of nociceptive stimuli, postural and walking reflexes, extremity tonus, performance in a smooth climbing platform, and consciousness.

Measuring cytokine levels

The plasma interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) concentrations were measured using enzyme-linked immunosorbent assays, as per the manufacturer's instructions.

Western blot assay

All the animals were anesthetized after the one day of injury and infusion of saline (100 ml) was done via cardiac apex. Tissue present near the region of trauma was dissected out and till the use it was stored at - 80°C. Lysis solution was used to homogenize the cortical tissues and later centrifuge it for the duration of half an hour at 12000×. The supernatant was separated and bicinchoninic acid protein assay kit was used for the estimation of protein in the sample. An equal quality of protein (50 µg/lane) was separated by 10% sodium dodecylsulfate polyacrylamide gel electrophoresis polyvinylidene fluoride membran. Later blocking of membrane was done and incubated with cleaved caspase-3, NF-κ B, TLR4 and GAPDH like primary antibody for the period of overnight at 4°C. Thereafter incubate it with HRP-conjugated secondary antibody. Image J software was used to analyze the bands.

TUNEL assay

Isolated brain was fixed in the 4% paraformaldehyde solution and then brain tissues were seeded in the molten paraffin to prepare the wax cubes. Microtome was used to cut 4 µm thickness of tissue section. Later apoptosis of neuron was estimated by TUNEL assay and TUNEL assay kit was used for it as per the direction given by manufacturer of kit. Optical microscope was used at the magnification of 400× and the percentage of TUNEL positive cells were estimated in the TS of brain tissues.

Result

Effect of β-elemene, Ho alone and in combination on neurological functions

Effect of β-elemene on neurological function score in the traumatic brain injury rat model was shown in Fig. 1. There was significant increase in the neurological function score in TBI group compared to control group. Whereas treatment with β-elemene and HO alone and in combination significantly ($p<0.01$) decreases the neurological function score compared to TBI group. Data also reveals that β-elemene+HO in combination treated group significantly reduces the score of neurological functions than β-elemene and HO treated group.

Effect of β-elemene, Ho alone and in combination on the inflammatory cytokines

Effect of β-elemene, Ho alone and in combination on the concentration of inflammatory cytokines in the brain tissues of traumatic brain injury rat model was shown in Fig. 2. In TBI group level of inflammatory cytokines such as TNF-α and IL-1β was significantly enhanced ($p<0.01$) in the brain tissues compared to control group. However treatment with β-elemene and HO alone and in combination was attenuated in the altered

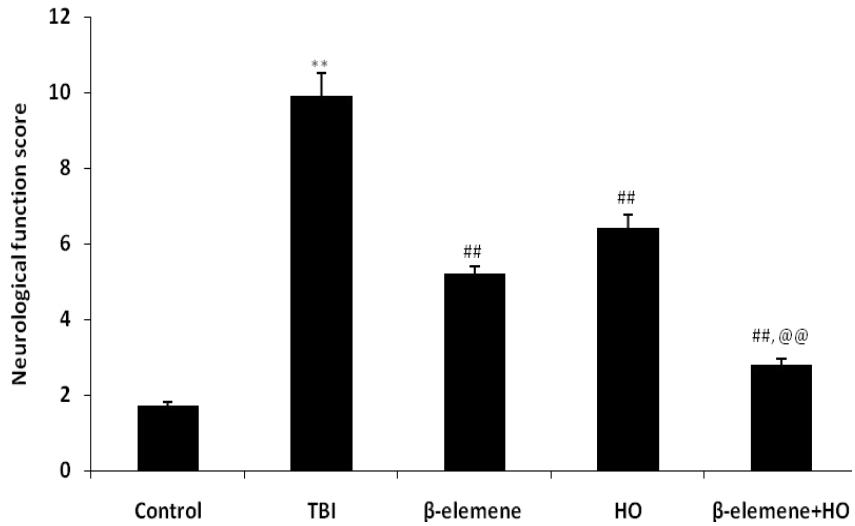

Fig. 1. Effect of β-elemene, Ho alone and in combination on neurological function score in the traumatic brain injury rat model Data mean±SD (n=10), **p<0.01 compared to control group; ##p<0.01 compared to TBI group, @@p<0.01 compared to HO group

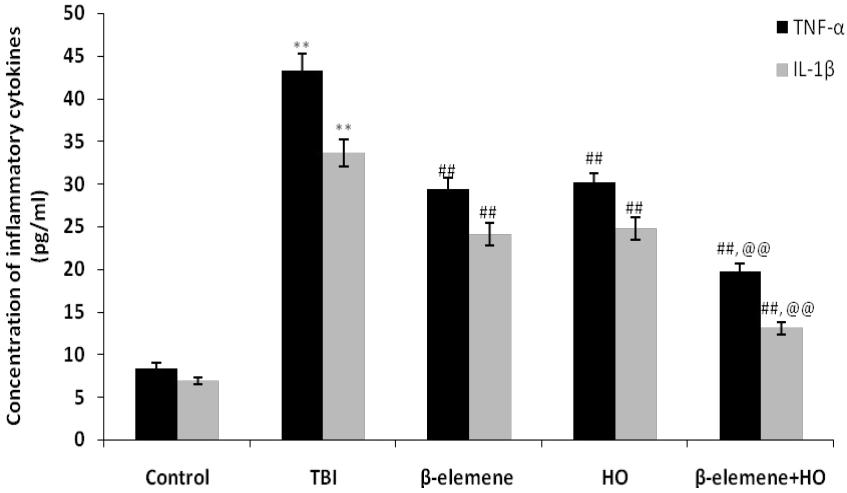

Fig. 2. Effect of β-elemene, Ho alone and in combination on the concentration of inflammatory cytokines in the brain tissues of traumatic brain injury rat model Data mean±SD (n=10), **p<0.01 compared to control group; ##p<0.01 compared to TBI group, @@p<0.01 compared to HO group

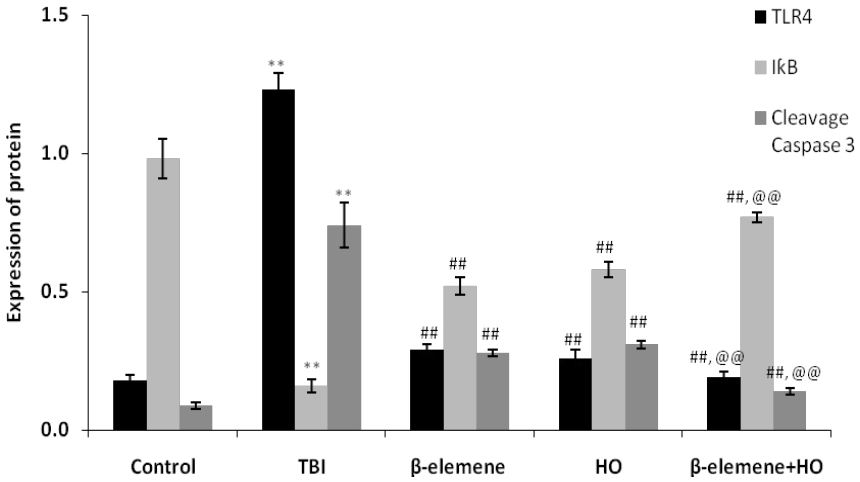

Fig. 3. Effect of β-elemene, Ho alone and in combination on the expressions of Cleavage caspase-3, IkB and LTR4 protein in the brain tissues of traumatic brain injury rat model Data mean±SD (n=10), **p<0.01 compared to control group; ##p<0.01 compared to TBI group, @@p<0.01 compared to HO group

level of inflammatory cytokines in the brain tissues of traumatic brain injury rat model. It was observed that β-elemene and HO in combination shows synergistic effect on the level of inflammatory cytokines in the brain tissues of traumatic brain injury rat model.

Effect of β-elemene, Ho alone and in combination on the expression of cleaved caspase-3, IκB and LTR4

Effect of β-elemene, Ho alone and in combination on the expressions of Clevage caspase-3, I_kB and LTR4 protein in the brain tissues of traumatic brain injury rat model was shown in Fig. 3. It was observed that expressions of cleaved caspase-3 and LTR4 was significantly enhanced and decrease in the expression of IκB in the brain tissues of β-elemene, Ho alone and in combination treated group compared to TBI group. There was synergistic effect of β-elemene was found when treated in combination with HO therapy compared to of β-elemene and HO alone treated groups.

Effect of β-elemene, Ho alone and in co mbination on the apoptosis of neuronal cells

Effect of β-elemene, Ho alone and in combination on the apoptosis of neuronal cells in the brain tissues of traumatic brain injury rat model was assessed by TUNEL assay as shown in Fig. 4. TUNEL staining was done to identify the apoptosis in neuronal cells. It was observed that the number of TUNEL stained cell quantity enhanced in TBI group compared to control group of rats. However in β-elemene and Ho alone and in combination treated group number of TUNEL positive cells significantly reduced compared to TBI group (Fig. 4.A). In addition apoptosis index was also significantly reduced in β-elemene and Ho alone and in combination treated group compared to TBI group (Fig. 4.B).

Discussion

Present investigation evaluates the neuroprotective effect of β-Elemene alone and in combination with hyperbaric oxygen in traumatic brain injury rats. Data of the study reveals that β-Elemene alone and in combination attenuates altered neurological score in TBI rats. In TBI neurological behavior changes due to neuronal degeneration and the drugs used in the management of TBI attenuates the neuro behavioral changes including in hyperbaric oxygen therapy.

Hyperbaric oxygen therapy used for the management of TBI and it reported to posses neuroprotective effect by acting on inflammatory pathway [11]. There is several limitations of this therapy that precise the use of HO in TBI. Moreover β-Elemene also has the anti-inflammatory potential [12]. Data of report reveals that β-Elemene alone and in combination with hyperbaric oxygen attenuates the level of inflammatory cytokines in the brain tissues of TBI rats. Moreover β-Elemene treatment with HO potentiating effect by decreasing the level of inflammatory cytokines in the brain tissues compared to β-Elemene alone treated group. Previously reported study also supports the findings of this investigation [13].

LTR4 is a protein of LTR family and reported studies reveal that expressions of LTR4 reported to be high in the brain injury condition [14]. Moreover it is also responsible for the enhancement of secondary injury in TBI and thus LTR4 inhibitors shows the promising effect for the amelioration of brain injury. Result of

the study also reveals that expression of LTR4, I_kB and cleavage caspase-3 also attenuated by treating the TBI injured rats with β-Elemene alone and in combination with hyperbaric oxygen. Apoptosis in neuronal cell is enhanced by due to the stimulation of inflammatory pathways [15]. Result of the study also shows that treatment with β-Elemene alone and in combination with hyperbaric oxygen significantly decreases the activity of caspase-3 enzyme and thus it also alleviates the neuronal apoptosis.

Conclusion

Present investigation reveals that β-Elemene protects the neuronal damage in TBI rats by attenuating the alteration in the inflammatory pathway. Data also suggest that β-Elemene shows the synergistic effect when treated with hyperbaric oxygen in TBI. Thus β-Elemene could be a promising molecule required to be use clinically for the management of TBI.

Conflict of interest: No

Acknowledgement

All the authors of this manuscript are thankful to Navy General Hospital, China for providing necessary facility for the presented work.

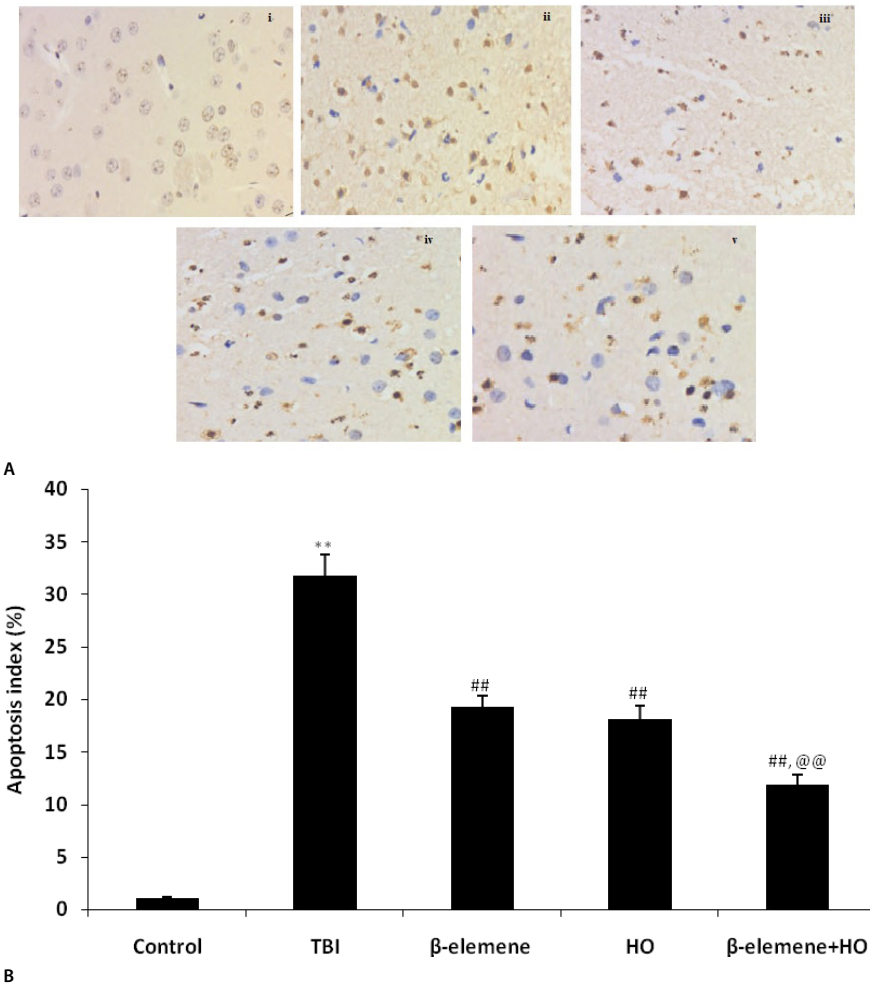

Fig. 3. Effect of β-elemene, Ho alone and in combination on the apoptosis of neuronal cells in the brain tissues of traumatic brain injury rat model A: TS of brain Tissue by TUNEL assay (i: Control, ii: TBI, iii: β-elemene, iv: HO, v: β-elemene+HO) ; B: Apoptosis Index (%) Data mean±SD (n=10), $^{**}p<0.01$ compared to control group; $^{\#\#}p<0.01$ compared to TBI group, $^{@@}p<0.01$ compared to HO group

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