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Regional specific alterations in brain acetylcholinesterase activity after repeated blast exposures in mice☆

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ABSTRACT

Acetylcholinesterase (AChE) which catalyzes the hydrolysis of the neurotransmitter acetylcholine has been recognized as one of the major regulators of stress responses after traumatic brain injury (TBI). Repeated blast exposure induces TBI (blast TBI) with a variable neuropathology at different brain regions. Since AChE inhibitors are being used as a line of treatment for TBI, we sought to determine the time course of AChE activity in the blood and different brain regions after repeated blast exposures using modified Ellman assay. Our data showed that repeated blast exposures significantly reduced AChE activity in the whole-blood and erythrocytes by 3–6 h, while plasma AChE activity was significantly increased by 3 h post-blast. In the brain, significant increase in AChE activity was observed at 6 h in the frontal cortex, while hind cortex and hippocampus showed a significant decrease at 6 h post-blast, which returned to normal levels by 7 days. AChE activity in the cerebellum and mid brain showed a decrease at 6 h, followed by significant increase at 3 days and that was decreased significantly at 14 days post-blast. Medulla region showed decreased AChE activity at 24 h post-blast, which was significantly increased at 14 days. These results suggest that there are brain regional and time-related changes in AChE activity after tightly coupled repeated blast exposures in mice. In summary, acute and chronic regional specific changes in the AChE activity after repeated blast exposures warrant systematic evaluation of the possibility of AChE inhibitor therapeutics against blast TBI.

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1. Introduction

Blast-induced closed head injuries can lead to mild, moderate or severe traumatic brain injuries (TBIs), which are considered as major cause of permanent neurological disabilities or death in active service members [21,25]. Cholinergic system has been recognized as a major regulator of stress responses due to closed head injuries and is reported to function as an essential route by which neurons can talk to immune cells [22,28,34]. Acetylcholinesterase (AChE) is the central part of the cholinergic system which catalyzes the hydrolysis of major neurotransmitter acetylcholine. Disruption of cholinergic system results in an imbalance in synaptic transmission and neuroimmunomodulation leading to acute or chronic changes to the brain [22,31]. The stress generated from closed head TBI has been reported to show changes in the activity of AChE at different regions of the brain in humans and experimental rats [1,11,27,31]. Also, centrally acting AChE inhibitors such as rivastigmine, donepezil or galantamine were classified as group of second prominent potential therapeutic candidates against TBI for long-term treatment [6,16,33,36,38,40].

Blast TBI is reported to be different from other forms of TBI including penetrating TBI, with unique changes in duration of unconsciousness, polytrauma, cerebral vasospasm, and higher incidence of visual, auditory and vestibular deficits [2,14,21,25]. Modulation of AChE activity in the blood and brain after blast TBI is largely unknown. Recently, we established a tightly coupled repeated blast exposure TBI mouse model which showed significant impairment in neuropathology and neurobehavioral parameters [39]. Neuropathological analysis of repeated blast exposed mice showed significant changes in specific locations (cerebellum and cerebral cortex) after the blast exposure indicating regional specific alteration after blast exposure [39]. We utilized this established mouse model of repeated blast exposures to

Abbreviations: AChE, acetylcholinesterase; TBI, traumatic brain injury; ACh, acetylcholine; ChAT, choline acetyl transferase; iso-OMPA, tetra monoisopropyl pyrophosphortetramide; DTP, 4,4′-dipyridyl disulfide, 4,4′-dithiodipyridine; BCA, bicinchoninic acid.

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evaluate the modulation of AChE activity in systemic and central nervous system after blast exposure. AChE activity in the whole blood, plasma, erythrocytes and various regions of brain (frontal cortex, hind cortex, hippocampus, cerebellum, mid brain and medulla) was analyzed at different time points after the blast exposure to assess the acute and chronic effects of blast exposures on AChE activity.

2. Materials and methods

2.1. Materials

Acetylthiocholine, tetra monoisopropyl pyrophosphortetramide (iso-OMPA), and 4,4′-dipyridyl disulfide, 4,4′-dithiodipyridine (DTP) were purchased from Sigma–Aldrich (St. Louis, MO). Tissue protein extraction reagent and bicinchoninic acid (BCA) protein assay kit were purchased from Pierce Chemical Co. (Rockford, IL).

2.2. Animals and repeated blast injury model

All animal procedures were performed at Walter Reed Army Institute of Research (WRAIR) in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council Publication 1996 edition) with an approved Institutional Animal Care and Use Committee protocol. Male C57BL/6J mice (8–10 weeks old, 22–26 g purchased from Jackson Laboratory, Bar Harbor, ME) were used in this study. A compressed air-driven shock tube available at the institute was used for blast overpressure exposure of mice [3,24,39]. Groups of animals (n = 6–10 per time point) were anesthetized with 4% isoflurane gas (O_2 flow rate 1.5 L/min) for 8 min and quickly placed on a holder in prone position (perpendicular to the direction of blast shock waves) and exposed to 20.6 psi blast overpressure for three times with 1–30 min intervals as described earlier [39]. The animals were returned to the cage after blast exposure. At indicated time points (3, 6, and 24 h, and 3, 7, and 14 days) after the last blast exposure, blood and brain were collected from sham and blast exposed animals. Heparinized blood was separated into plasma and erythrocytes for AChE analysis. Brain tissue from sham and blast exposed animals was dissected into different regions (frontal cortex, hind cortex, hippocampus, cerebellum, mid brain and medulla) under the guidance of a board certified pathologist and used for AChE analysis. The whole blood, plasma, erythrocytes and different regions of brain samples were kept at −20 °C until use.

2.3. Acetylcholinesterase enzyme activity assay

Whole blood and erythrocyte samples collected at various time points were diluted with de-ionized water. Different parts of brain (frontal cortex, hind cortex, hippocampus, cerebellum, mid brain and medulla) were homogenized with 1:7 tissue protein extraction reagent (25 mM bicine, 150 mM NaCl, pH 7.6 containing mild detergent like 0.1% TX-100) at 4 °C using a tissue homogenizer and clarified by centrifugation. Aliquots of diluted blood or tissue samples were pre-incubated with 4 μM of iso-OMPA for 30 min at room temperature to inhibit the associated butyrylcholinesterase activity. AChE activity was measured by using modified Ellman assay with 1 mM of acetylthiocholine substrate and 0.2 mM DTP as chromogen [4,12,13]. The change in activity was measured at 412 nm for 5 min in a kinetic mode using a SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA). Total protein content in brain tissue extracts was determined by BCA protein assay kit. The activity of AChE enzyme in the brain and blood was expressed as milliunits/mg protein or milliunits/ml, respectively. One unit of AChE activity was defined as the number of μmol of acetylthiocholine (substrate) hydrolyzed per minute at room temperature.

2.4. Statistical analysis

Statistical analysis was performed by using GraphPad Prism software. The AChE enzyme activity data of blood and brain were analyzed by using Mann–Whitney test. Probability (p) values less than or equal to 0.05 are considered as significant.

3. Results

3.1. AChE activity in different regions of brain after repeated blast exposure

The spectrum of changes in AChE activity of various regions of brain of mice exposed to repeated blast overpressure is shown in Fig. 1. Compared to sham controls, the AChE activity in the frontal cortex of blast exposed animals showed a significant acute increase

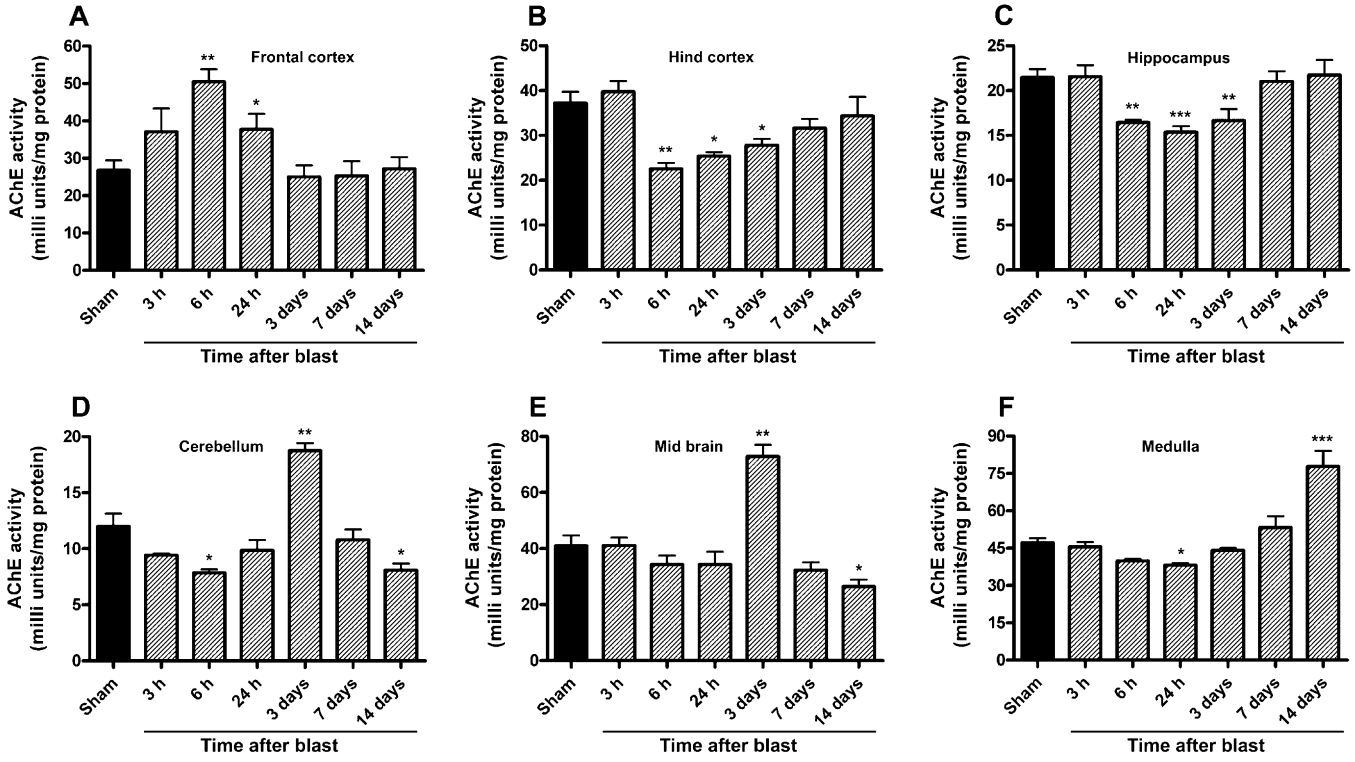

Fig. 1. Brain AChE activity of mice exposed to repeated blast overpressure. Isoflurane anesthetized C57BL/6J mice were exposed to three repeated blast overpressure (20.6 psi) with duration of 1–30 min, followed by collection of brain samples at indicated time points as described in Section 2. Anesthetized control mice without blast exposure served as sham. AChE activity in the brain extracts was analyzed by modified Ellman assay and the activity of enzyme was expressed as milliunits/mg protein. Shown are the mean ± SEM values for AChE activity from frontal cortex (A), hind cortex (B), hippocampus (C), cerebellum (D), mid brain (E), and medulla (F) (n = 10 for sham; n = 6 for 3, 6, and 24 h post-blast; n = 7 for 3, 7, and 14 days post-blast; (*) $0.05 < p < 0.01$; (**) $0.01 < p < 0.001$; (***) $p < 0.001$).