Blast exposure modulates genes involved in auditory processing: Possible role in tinnitus

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Abstract

Blast and impulse noise exposure in the battlefield is the major cause of hearing impairment and tinnitus, the most widespread dysfunctions associated with traumatic brain injury (TBI) in service members. The symptoms of hearing impairment and tinnitus are difficult to diagnose accurately and present particular challenges to the blast TBI field due to their overlap with post traumatic stress disorder, mental illness and cognitive deficits, where apparent auditory dysfunction may arise from different underlying psychotraumatic mechanisms. Calcium binding proteins in the cochlear nucleus play a major role in central auditory processing and elevated levels of intracellular calcium can lead to hypersensitivity of auditory neurons leading to tinnitus. Modulation of calretinin and parvalbumin, the two major calcium binding proteins in the auditory neurons in cochlear nucleus, was found to be associated with hearing impairment. Using a mouse model of repeated blast exposures, we observed up-regulation of both calretinin and parvalbumin in the brain by proteomic and Western blot analyses after blast exposure indicating their possible roles in the pathogenesis or repair of blast-induced auditory impairment and tinnitus. Pharmacological intervention to regulate calcium signaling may be an effective countermeasure against blast-induced auditory impairment and tinnitus. Microarray analysis showed decreased expression of microRNA-181a which is involved in hair cell regeneration in the cochlea after injury suggesting an attenuated hair cell regenerative process leading to auditory impairment and tinnitus and intracochlear administration of microRNA-181a as an effective countermeasure. Messenger RNA analysis showed significant increase in otoancorin, which is involved in attachment of non-cellular gel to hair cell and causes neurosensory deafness when defective. On the other hand, otoferlin, which plays a role in glutamate vesicle release in the auditory nerve signaling and mutation of which is involved in deafness, was decreased significantly after blast exposures. Up-regulation of otoancorin and down-regulation of otoferlin and subsequent hypersensitization of auditory signaling process may underlie long-term hearing impairment and development of tinnitus following repeated blast exposures.

Background

Auditory system involves external auditory canal, tympanic membrane, malleus incus and stapes, tympanic cavity, hair cells, cochlea and cochlear nerve (Fig. 1). Sound waves are sensed in the brain though cochlear nerve pathways. Blast exposure, high intensity noise from explosion, firing weapons, aviation, machineries or ototoxic medicines can cause auditory dysfunction in military. Hearing impairment and tinnitus are the most common dysfunctions associated with bTBI. The mechanism of hearing impairment is not well known and precise diagnosis of TBI induced auditory dysfunction is challenging due to confusion with many overlapping symptoms of post traumatic stress disorder, mental illness and cognitive deficits

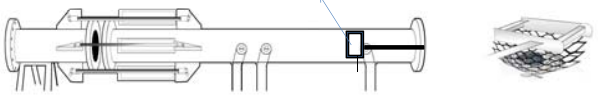
Fig. 1. Auditory system

Methods

Animals: Male C57BL/6J mice, 8-10 week old that weighed between 21-26g (Jackson Lab)

Blast exposure: WRAIR shock tube (Fig. 2a), 8 min anesthesia (isoflurane, 4%). Animal were kept in prone position in the shock tube (Fig 2b)

Fig. 2a. Shock tube — Animal position — Fig. 2b. Animal holder



Wang et al., J. Neurotrauma, 2011

Proteomic analysis: Proteomic analysis was carried out in the brain cerebellum by 2D-DIGE method at ITSI Biosciences, Johnstown, PA. Protein showed more than 2-fold changes in the 2D-gel analysis were selected for mass spectroscopy.

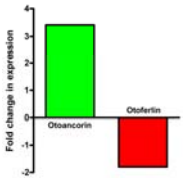
Western blotting: Proteins specific antibodies and chemiluminescence detection system were used for Western blotting.

MicroRNA analysis: MicroRNA analysis was carried out in the brain cerebellum using µParaflo™ MicroRNA microarray at LC Sciences, Houston, TX.

cDNA microarray: Total RNA was isolated from the brain hippocampus. Fluorescent cRNA was generated by using Agilent's Quick Amp Labeling Kit and hybridized to Agilent gene chips (mouse cDNA) and processed as described by the manufacturer. The slides were scanned using GenePix 4000A scanner and the data was extracted by using Agilent Feature Extraction Software.

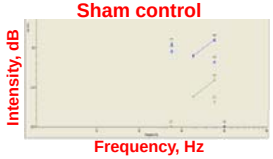
Results

cDNA microarray

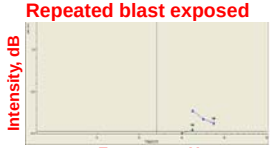


cDNA microarray analysis of hippocampus after blast exposure showed 3.4-fold increase in the expression of otoancorin and 1.8-fold decreased expression of otoferlin

Sham control



Repeated blast exposed

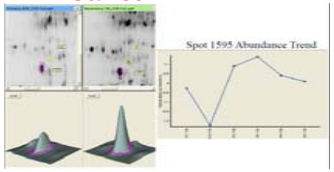


Grason-Stadler Model Audera DPOAE system

DPOAE analysis:

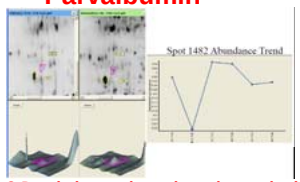
DPOAE analysis showed significant hearing impairment at 24 h after repeated blast exposures

Calretinin



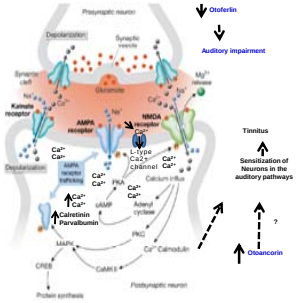
2-D gel electrophoresis and quantitation of the expression calretinin in the brain of control and blast exposed (20.6 psi, 3 repeats) animals

Parvalbumin

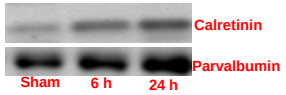


2-D gel electrophoresis and quantitation of the expression parvalbumin in the brain of control and blast exposed (20.6 psi, 3 repeats) animals

Proposed pathway of auditory injury



Western blotting



MicroRNA microarray

Micro RNA	Sham	Blast	Known Function
miRNA-181a*	2675±473	1824±78*	Hair cell regeneration in auditory system
miRNA-762	491±228	2268±721*	Unknown
miRNA-2861	1494±523	4681±830*	Promotes osteoblast differentiation
miRNA-23a	3575±713	1653±101*	Promotes apoptosis
miRNA-132	4243±385	1766±422*	Regulates dendritic growth and inflammation
miRNA-128	15797±1428	6561±905*	Inhibits glioma cell proliferation

Summary/Discussion

- This is the first report showing that repeated blast exposures results in alteration in the expression of multiple proteins, mRNAs and microRNAs reportedly associated with hearing loss and tinnitus.
- Since hearing loss and tinnitus are prevalent dysfunctions after blast exposure, these data provide a molecular basis of changes in auditory system leading to hearing impairment and tinnitus after blast exposure.
- Probably, blast pressure wave induced injury to auditory system releases glutamate that leads to influx of calcium and damages the auditory processing.
- Pharmacological intervention of the modulation of these proteins may help to prevent/cure hearing impairment and tinnitus due to blast exposure.
- Up-regulation of otoancorin and down-regulation of otoferlin and subsequent hypersensitization of auditory signaling process may underlie long-term hearing impairment and development of tinnitus following repeated blast exposures.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army, the Navy, or the Department of Defense.
The research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals. It adhered to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NRC Publication 1996 edition.