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Intracranial venous injury, thrombosis and repair as hallmarks of mild blast traumatic brain injury in rats: Lessons from histological and immunohistochemical studies of decalcified sectioned heads and correlative microarray analysis☆

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ABSTRACT

Background: Many previous experimental studies of blast wave effects have reported vascular and parenchymal injury in brains extracted from the skulls prior to histopathological assessment. Brain removal disrupts vasculature and structural features of the meninges that may be sources of signs and symptoms of mild traumatic brain injury, particular at lower blast overpressures (<5 psi peak).
New method: Immunohistochemical and histopathological studies have been conducted in sections from decalcified, paraffin embedded, histologically sectioned whole rat heads. These sections preserve the entire cranial contents in situ, and permit evaluation of the inner ear, central nervous system and associated vasculature. The findings could also be correlated with mRNA expression patterns from whole brains subjected to similar treatment.
Results: Lower levels of blast wave exposure produce primarily vascular effects in rats. Messenger RNA profiles of the whole brains showed evidence of both blast intensity and time dependent effects on vascular wound healing markers. The rats exposed to 10–11 psi overpressure tended to show a similar pattern of mRNA expression changes in these vascular repair and inflammatory pathways as rats exposed to approximately 5 psi overpressure, but the changes were greater. The changes in mRNA expression after a 14–15 psi exposure were different and suggestive of more severe injury, particularly for DNA repair, lymphocyte activation and lymphocyte migration pathways. Histopathological examination of decalcified heads revealed that even 2.5–7.9 psi blast exposures produced a high prevalence of mild venous hemorrhage and thrombosis (accompanied by inflammatory markers) in the inner ear, vertebrobasilar circulation, hippocampal choroidal fissure and the veins associated with velum interpositum.
Comparison with existing method(s): The sites of vascular injury would not have been included in specimens extracted from the skull prior to processing.
Conclusions: The isolated regions of intravascular coagulation in small veins and the isolated, very small venous hemorrhages in the subarachnoid space are worthy of consideration as factors in both healing and chronic sequelae of mild blast concussion. Although small, remnants persisted in the subarachnoid space even 42 days after a single blast exposure. The high prevalence of very mild subdural and subarachnoid hemorrhage may be a target for clinical management.

1. Introduction

Blast related mild traumatic brain injury (mTBI) has been called a signature injury of modern warfare. Self-reports suggest that as many as 19.5% of military personnel deployed in Southwest Asia sustained a TBI (Rand Corporation, 2008). The Defense Centers of

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Excellence for Psychological Health and Traumatic Brain Injury (DCOE) estimates that over 30,000 military members last year were diagnosed with a new TBI (Defense Centers of Excellence for Psychological Health and Traumatic Brain Injury). Accidental explosions and terrorist improvised explosive devices also create mTBI casualties among civilians. Long-term effects of mTBI include pathological changes closely related to neurodegenerative disease, postconcussive syndrome, and long-term inflammatory changes throughout the brain (Morey et al., 2013; Yeh et al., 2013).

Although radiological findings are generally subclinical in mild TBI, there is increasing interest in more subtle vascular findings such as dilation of perivascular spaces (Inglese et al., 2005), fractional anisotropy changes and vascular changes that can be detected with susceptibility-weighted imaging (Van Boven et al., 2009). Subdural hematoma, cerebral contusion, and subarachnoid hematoma are among the pathological features associated classically with TBI (Greenfield, 1958). Effects may be acute or delayed; for example, subdural hematoma can emerge in acute, subacute or chronic stages after injury (Greenfield, 1958). Because in the brain (like lung, liver, kidney, heart and GI tract) the intracranial vascular contents are soft tissues, they are susceptible to similar blast injury effects, which include local plasma extravasation (local edema), local hemorrhage (including perivascular ring hemorrhage), disseminated intravascular coagulation (a term that includes microthrombi in small vessels) and introduction of air emboli (Sharpnack et al., 1991). Small injuries that are isolated or distributed diffusely could easily elude clinical detection with imaging protocols. In addition, localized hemorrhage is difficult to detect with imaging in extensions of the subdural space such as the velum interpositum and transverse cerebral fissure. However, these types of injuries can be detected in histological sections of decalcified whole heads of experimental animals.

Chemical decalcification of bony tissues prior to embedding and sectioning has long been used for histological studies of the structure of soft tissues encased in teeth and bone (Humason, 1962; Luna, 1968). In the neurosciences, decalcification has been used frequently in studies of inner ear structures within the temporal bone, including neural and non-neural tissues. For example, studies of paraffin or celloidin embedded, decalcified human temporal bones from autopsy have examined normal and pathologic structure with standard histological stains and immunohistochemistry for neuronal and inflammatory markers (Ganbo et al., 1997, 1999; Kamimura et al., 2000; Nguyen et al., 2014; Schuknecht, 1974; Ying and Balaban, 2009). Because these preparatory methods allow histological analysis in situ of fragile tissues such as intracerebral veins and meninges, it seems logical to section whole decalcified rodent heads for analysis of the effects of blast trauma to the intracranial contents.

This study assessed the intracranial injury in rats after shock tube delivery of single, low level Friedlander waves (2.9 psi [20 kPa] to 17.5 psi [120.7 kPa] peak overpressure). The histological analyses spanned acute (2 h post-exposure) to chronic (42 days post-exposure); changes in whole brain mRNA expression were assessed in separate groups at only the shorter survival times (2–72 h). The analyses revealed previously undescribed vascular injury patterns in the inner ear and within the subarachnoid space and evidence of small subarchnoid hemorrhages and thrombosis in small veins on the brain surface and subarachnoid space.

2. Methods

2.1. Blast overpressure delivery

A 30 in. cast aluminum tube (10 in. inner and 8 in. outer diameter) compression chamber was used to generate the blast pressure waves. The tube houses an adjustable reservoir chamber that releases a compressed air volume ranging from 1570 to 7850 cm^3 through a PVP film diaphragm. A second 15-inch tubular portion attaches to the wave generation chamber and serves as the animal exposure and holding area (Figure 1, left panel). A 1.6 hp compressor (Sears Inc) produces up to 175 psi in the compression chamber. Between the reservoir chamber and the animal holding area is a variable aperture that holds the PVP film diaphragm. Three, four or five-inch diameter apertures can be interchangeably placed in this area to adjust the wave intensity from a mild, moderate or more severe BOP exposures. The input to peak output pressures are linear over the range of 0–100 psi, and the velocity of the BOP wave was measured at 1221 feet/sec at six inches from the compression chamber aperture. The exposure peak intensity levels for this study ranged from 4.7 to 20 psi.

Adult female Sprague-Dawley rats were anesthetized intramuscularly with a ketamine (80 mg/kg)–xylazine (5 mg/kg) cocktail, placed horizontally in the holding/exposure area and tethered to a removable wire rack either six or 12 inches from the film diaphragm. The head faced the blast source. The blast overpressure (BOP) wave is measured with Tucker Davis Technologies (TDT) System 3 and Endevco pressure transducers (model 8510B-200) rated for 200 psi and electronic conditioners interfaced with a computer. Fig. 1 shows examples of overpressure/underpressure Friedlander waves generated by this system. Brain and lung tissues were harvested at 2 h, 24 h and 72 h after single BOP exposure. Behavioral assessments were conducted daily for Roto-rod performance and at least one week post-BOP for open field activity.

All animals were monitored for respiratory rate, heart rate and blood oxygen saturation using a SurgiVet Pulse oximeter prior to, during the exposure and for the entire recovery period after the exposure. Once functional and cognitive measures are complete on surviving blast-exposed animals, animals were euthanized for collection of tissues, body fluids and blood.

2.2. Tissue preparation

Brain tissues from one set of rats were harvested at NMCSD at 2, 24 and 72 h after blast exposure, frozen on dry ice, and shipped to University of Pittsburgh for RT-PCR analysis. The tissues were homogenized and the mRNA was extracted by standard methods with commercially available reagents (Quiagen RNAlater reagent, RNeasy kits). RT-PCR microarray analyses with standard commercially available products and qRT-PCR on individual mRNAs were used to examine changes in specific response pathways (Applied Biosystems 7300 Real Time PCR System). The arrays include SuperArray Biosciences RT^2 Profiler™ PCR Arrays PARN-024A (rat angiogenesis microarray), PARN-003A (rat stress and toxicity pathway microarray), PARN-060A (rat neurotransmitter array) and PARN-011A (rat inflammatory cytokine/chemokine and receptor array) for assays on each blast overpressure exposure group. The microarrays were performed in at least duplicate on pooled samples of equal amounts of cDNA from 3 to 5 animals in each exposure or control group, to yield an estimate of the mean mRNA response patterns. The means of the cycle threshold (C_t) values for mRNAs for ribosomal protein large P1 (Rplp1), lactic acid dehydrogenase A (Ldha), beta actin (Actb), hypoxanthine guanine phosphoribosyltransferase (Hprt) and ribosomal protein L13A (Rpl13a) was used for housekeeping gene normalization; it was subtracted from each C_t value to obtain ΔC_t. The difference between the average ΔC_t in each shock wave intensity group and the average ΔC_t in the control group yielded the cycle threshold change, $\Delta\Delta C_t$. The fold change in mRNA expression is reported as $2^{-\Delta\Delta}C_t$. Data were expressed as $-\Delta\Delta C_t$ for statistical analyses.

After survival times ranging from 2 h to 42 days, sham and blast exposed rats were anesthetized with sodium pentobarbital