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Research paper

Acute decrease in alkaline phosphatase after brain injury: A potential mechanism for tauopathy

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HIGHLIGHTS

- Brain injuries due to blast or weight drop decreased the level/activity of TNAP.
- Decreased level/activity of brain TNAP is associated with accumulation of *pTau*.
- Brain injury decreased plasma alkaline phosphatase activity.
- APP accumulation in the brain after injury did not correlate with *pTau* deposition.

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ABSTRACT

Dephosphorylation of phosphorylated *Tau* (*pTau*) protein, which is essential for the preservation of neuronal microtubule assemblies and for protection against trauma-induced tauopathy and chronic traumatic encephalopathy (CTE), is primarily achieved in brain by tissue non-specific alkaline phosphatase (TNAP). Paired helical filaments (PHFs) and *Tau* isolated from Alzheimer's disease (AD) patients' brains have been shown to form microtubule assemblies with tubulin only after treatment with TNAP or protein phosphatase-2A, 2B and -1, suggesting that *Tau* protein in the PHFs of neurons in AD brain is hyperphosphorylated, which prevents microtubule assembly. Using blast or weight drop models of traumatic brain injury (TBI) in rats, we observed *pTau* accumulation in the brain as early as 6 h post-injury and further accumulation which varied regionally by 24 h post-injury. The *pTau* accumulation was accompanied by reduced TNAP expression and activity in these brain regions and a significantly decreased plasma total alkaline phosphatase activity after the weight drop. These results reveal that both blast- and impact acceleration-induced head injuries cause an acute decrease in the level/activity of TNAP in the brain, which potentially contributes to trauma-induced accumulation of *pTau* and the resultant tauopathy. The regional changes in the level/activity of TNAP or accumulation of *pTau* after these injuries did not correlate with the accumulation of amyloid precursor protein, suggesting that the basic mechanism underlying tauopathy in TBI might be distinct from that associated with AD.

1. Introduction

Advancements in far-forward medical care have greatly increased the survivability of traumatic brain injuries (TBIs), which in recent military conflicts have been primarily attributable to the widespread use of improvised explosive devices and other modern explosive weaponries. In particular, exposure to blast has been described as the major cause of TBI and associated disabilities in the recent wars in Iraq and Afghanistan [20]. Although several biochemical and histopathological changes have been preclinically documented in the central nervous system after blast exposure [7,8,16,19,26,29,33], the potentially complex pathophysiological mechanisms triggering long-term neurobehavioral abnormalities are not well understood, which has hampered the development of effective countermeasures and diagnostic approaches.

Recent studies indicate that chronic traumatic encephalopathy (CTE), a tau protein-linked neurodegenerative disorder which has been observed in several athletes with a history of multiple concussions, shares clinical symptoms and neuropathological features described in victims of blast exposure [10]. In particular, phos-

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phorylated *Tau* (*pTau*) protein neuropathology, with perivascular neurofibrillary degeneration, is recognized as a distinct feature of CTE and has been observed postmortem in the brains of blast victims and contact-sport athletes. Brains of mice exposed to blast overpressure in a shock tube also showed neuropathological features of CTE such as phosphorylated tauopathy, myelinated axonopathy, microvasculopathy, neuroinflammation and neurodegeneration, prompting postulation that blast-induced acceleration of the head may play an important role in the development of CTE-like neuropathology [10]. Phosphorylation of *Tau* protein disrupts microtubule assembly in neurons, which can result in tauopathy and the formation of neurofibrillary tangles seen in neurodegenerative disorders such as Alzheimer's disease (AD) [12,15,31]. Dephosphorylation of *pTau* is critical to prevent tauopathy and to restore microtubule assembly for neuroregeneration.

By dephosphorylating *pTau* in neurons, tissue non-specific alkaline phosphatase (TNAP) may play a major role in the etiology of brain disorders involving this neuropathological feature [12,15,31]. Paired helical filaments and *Tau* protein isolated from AD patients' brains were shown to form a microtubule assembly with tubulin *in vitro* only after treatment with alkaline phosphatase or protein phosphatase-2A, -2B and -1, suggesting that *Tau* protein in the paired helical filaments of neurons in AD brain is hyperphosphorylated, which prevents microtubule assembly [12,15,31]. Alkaline phosphatase showed significantly higher activity in dephosphorylating *pTau* compared to the other protein phosphatases studied [31].

A number of studies indicate that accumulation of amyloid precursor protein (APP) and β-amyloid peptides induces the phosphorylation of *Tau,* leading to microtubule disassembly, which is an accepted neuropathological feature of AD [5,11,18,27,28,30,34]. Activation of mitogen-activated protein kinase by accumulated APP has been described as a mechanism yielding the phosphorylation of *Tau* protein [11]. In a hybrid septal cell line, treatment with aggregated β-amyloid peptide resulted in accumulation of *pTau* and paired helical filaments and alkaline phosphatase treatment abolished the effect [18], emphasizing the potentially important role of β-amyloid peptide in triggering and TNAP in preventing *Tau* phosphorylation.

In the present study, we utilized rat models of blast-induced TBI using a shock tube and impact acceleration-induced TBI using weight drop and explored the acute changes in the expression of *pTau* and level/activity of TNAP in different brain regions after these insults. We have also examined whether the alterations in the expression of *pTau* and TNAP are associated with a corresponding acute change in the expression of APP to examine a possible role of accumulated APP in the initiation and development of tauopathy after TBI.

2. Experimental procedures

2.1. Animals and blast injury

All animal experiments were conducted in accordance 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 (NRC Publication 2011 edition) using an Institutional Animal Care and Use Committee approved protocol. Male Sprague Dawley rats, 9–10 weeks old that weighed 300–350 g (Charles River Laboratories, Wilmington, MA) were housed at 20–22 °C (12 h light/dark cycle) with free access to food and water *ad libitum*. A total of 4 animals/group was used for each time point.

2.2. Brain injury by blast exposure

Blast exposure was carried out using a compressed air-driven shock tube as described earlier [6]. Briefly, after 4% isoflurane gas anesthesia in an induction box for 6 min (O_2 flow rate 2 L/min), rats were immediately tautly secured in a transverse prone position in coarse mesh netting 2.5 ft within the mouth of the 15 ft long and 1 ft internal diameter expansion chamber with the right side of the head/body facing the pressure chamber. Rats were exposed to single shockwave (peak static pressure of 19.0 psi (131 kPa) with a 9 ms positive phase duration). Blast overpressure flow conditions were recorded using piezoresistive pressure transducers (Meggit Inc., San Juan Capistrano, CA) mounted in the rat holder which provided measurements of total and side-on pressure waveforms. Sham control animals were included in all individual experiments and were treated in the same fashion without exposure to blast.

2.3. Brain injury by weight drop

As originally described by Marmarou et al. [21], the injury device consisted of a vertically secured 2.5 m long Plexiglas tube with a 19 mm inner diameter. A 0.014 inch Mylar helmet was mounted on the heads of the isoflurane-anesthetized rats to prevent any skull fracture during weight drop. The rats were placed in a prone position on a 12 × 12 × 43 cm foam bed (Type E manufactured by Foam to Size, Inc., Ashland, VA). After placing the rat on the foam bed, the bed was positioned directly under the tube. The rat's head and the cap were adjusted so that the striking plane was horizontal and parallel to the impacting face of the falling weight. The animal's body was held lightly on the form during impact. Brain injury was produced by dropping the cylindrical weight (500 g) from a predetermined height (150 cm). Rebound impact by the weight was prevented by sliding the foam bed and rat away from the tube immediately after impact/acceleration. As noted above, sham controls were anesthetized and handled without being subjected to brain injury.

2.4. Sample collection for analyses

Rats were euthanized 6 and 24 h post-injury and blood plasma and brain samples were collected. Cortex, hippocampus and brainstem were dissected and pooled across hemispheres over ice, immediately frozen and stored at −80 °C along with blood plasma until analyses.

2.5. Measurement of enzyme activity

The activity of TNAP in the brain and activity of total alkaline phosphatase in the blood plasma were determined using a diagnostic kit (Randox Laboratories, Kearneysville, WV) according to the manufacturer's instructions. In this assay, the colorless *p*-nitrophenyl phosphate is hydrolyzed to yellow colored *p*-nitophenol by alkaline phosphatase at alkaline pH in the presence of Magnesium ions. The absorbance of the yellow colored *p*-nitrophenol formed, which will be directly proportional to the activity of alkaline phosphatase, was measured at 400 nm. In the case of brain tissue, 10% homogenates were made in T-PER tissue protein extraction reagent (Pierce Chemical Co., Rockford, IL) containing protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO) using an ultrasonic homogenizer. The homogenates were centrifuged at 5000 × *g* for 5 min at 4 °C and the supernatants were used for the enzyme assay. Briefly, 5 μl of plasma or brain extract were added to the reaction mixture in the wells of a 96 well assay plate and the increase in optical density at 400 nm was measured every minute up to 5 min using SpectraMax M5 spectrophotometer