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

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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.

Key words: Blast exposure, head impact/acceleration, tauopathy, chronic traumatic encephalopathy, traumatic brain injury, tissue non-specific alkaline phosphatase

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 [19]. Although several biochemical and histopathological changes have been preclinically documented in the central nervous system after blast exposure

[6, 7, 15, 18, 25, 28, 32], 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 [9]. In particular, phosphorylated *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 hyperacceleration of the head may play an important role in the development of CTE-like neuropathology [9]. 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) [11, 14, 30]. 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 [11, 14, 30]. 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 [11, 14, 30]. Alkaline phosphatase showed significantly higher activity in dephosphorylating *pTau* compared to the other protein phosphatases studied [30].

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 [4, 10, 17, 26, 27, 29, 33]. Activation of mitogen-activated protein kinase by accumulated APP has been described as a mechanism yielding the phosphorylation of *Tau* protein [10]. 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 [17], 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 [5]. Briefly, after 4% isoflurane gas anesthesia in an induction box for 8 min (O₂ flow rate 1.5 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 (131kPa) with a 9 msec positive phase duration). Blast overpressure flow conditions were recorded using piezoresistive pressure transducers (Meggitt 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.* [20], the injury device consisted of a vertically secured 2.5 m long Plexiglas tube with a 19 mm inner diameter. A 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 plate 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*-nitrophenol 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 $\times g$ for 5 min at 4°C and the supernatants were used for the enzyme assay. Briefly, 5 μ l 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 (Molecular Devices, Sunnyvale, CA). The increase in optical density per minute was used for determination of enzyme activity.

2.6. *Western blot analysis of brain homogenates*

Brain homogenates (10% w/v) were prepared in T-PER tissue protein extraction buffer (Pierce Chemical Co, Rockford, IL) containing protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO) using an ultrasonic homogenizer. The homogenates were centrifuged at 5000 $\times g$ for 5 min at 4°C and 2 μ l each of supernatants were used for Western blotting. Rabbit polyclonal antibodies, which bind specifically to phosphorylated serine396 (S396) of *Tau* protein, were purchased from Abcam (Cambridge, MA) and used at dilution of 1 : 5,000. Mouse monoclonal antibodies against TNAP and β -actin were obtained from Sigma-Aldrich (St. Louis, MO) and used at a dilution of 1 : 2,000 and 1 : 40,000 respectively. Secondary antibodies

labeled with horse-radish peroxidase (HRP) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and used at a dilution of 1 : 2,500. SDS-polyacrylamide gel electrophoresis and Western blotting analysis were carried out as previously described by us earlier [0]. After Western blotting, the protein bands were detected using ECL-Plus Western blot detection reagent (GE Healthcare, Piscataway, NJ) and the chemiluminescence was imaged in an Alphamager reader (Cell Biosciences, Santa Clara, CA). The band intensity was measured by densitometry using AlphaView software (Cell Biosciences, Santa Clara, CA).

2.7. Statistical analysis

Statistical analysis was carried out by Repeated Measures Analysis of Variance (ANOVA) followed by Tukey's post-hoc test using HSD multiple comparisons (SPSS software, Version 20). Values were expressed as mean $\pm$ standard deviation (SD). A *p* value less than 0.05 was considered significant.

3. RESULTS

3.1. Expression of *pTau* in brain regions after injury

Western blot analyses of extracts from the different brain regions after either blast or weight drop showed significant increases in the expression of *pTau* (S396) at 6 and 24 hr post-insult (**Figure 1**), with increases ranging from 23 to 34 %. At 6 hr, the ratios (mean $\pm$ SD) of band intensities of *pTau*/actin for sham, blast and weight drop were 0.88 $\pm$ 0.06, 1.24 $\pm$ 0.17 and 1.34 $\pm$ 0.23 respectively in the brain stem, 0.74 $\pm$ 0.02, 0.97 $\pm$ 0.11 and 1.12 $\pm$ 0.21 respectively in the hippocampus, and 0.79 $\pm$ 0.05, 1.03 $\pm$ 0.15 and 1.15 $\pm$ 0.02 respectively in the cortex. At 24 hr, the ratios for sham, blast and weight drop were 0.84 $\pm$ 0.09, 1.09 $\pm$ 0.09 and 1.04 $\pm$ 0.06 respectively in the brain stem,

0.76±0.05, 0.86±0.02 and 0.87±0.03 respectively in the hippocampus, and 0.78±0.08, 0.96±0.01 and 0.97±0.01 respectively in the cortex. Although the increases resulting from the two different insults were not significantly different at these respective injury severities, the elevated levels of *pTau* in different brain regions of animals exposed to blast overpressure were generally less than those seen in rats subjected to weight drop injury. The levels of *pTau* in different brain regions were similar 24 hr after either insult and were significantly greater than measured in the sham controls. We have detected two adjacent bands in the Western blot at the expected position of *pTau*, which could be due to the difference in the number of amino acids phosphorylated or due to other post-translational modifications to *Tau* protein after injury. For densitometry analysis, the intensities of both bands were used.

3.2. Activity of TNAP in brain regions after injury

Figure 2 shows the activity of TNAP in different brain regions at 6 and 24 hr after blast or weight drop induced brain injury. At 6 hr, the TNAP activity (units/mg protein) for sham, blast and weight drop were 214.14±62.81, 119.20±16.66 and 106.86±17.29 respectively in the brain stem, 173.84±47.69, 118.31±19.24 and 107.21±22.23 respectively in the hippocampus, 209.07±116.54, 144.29±28.38 and 125.51±30.92 respectively in the cortex. At 24 hr, the TNAP activity for sham, blast and weight drop were 206.88±17.48, 166.02±23.17 and 159.58±18.25 respectively in the brain stem, 181.52±28.44, 140.93±11.56 and 133.80±22.52 respectively in the hippocampus, 203.18±20.59, 168.08±16.82 and 158.07±20.80 respectively in the cortex. Both insults significantly decreased the activity of TNAP at both 6 and 24 hr post-injury. At 6 hr post-injury, blast exposure decreased TNAP activity in brainstem, hippocampus, and cortex

by 45%, 32% and 31% respectively, whereas weight drop caused regional decreases of 51%, 39% and 40% respectively. At 24 hr, TNAP activity in brainstem, hippocampus and cortex remained decreased by 20%, 23% and 18%, respectively after blast whereas after weight drop, the decreases were 23%, 27% and 23% respectively. Although once again the changes resulting from the two insults were not significantly different, at these respective injury severities, weight drop consistently caused slightly greater decreases in TNAP activity than were seen after blast exposure, which were greater at 6 h than at 24 hr post-injury

Activity of alkaline phosphatase in the plasma

The baseline activities of alkaline phosphatase in the plasma for sham, blast and weight drop groups were 101.45 ± 15.27 , 97.61 ± 16.72 and 94.05 ± 10.97 respectively. At 6 hr, the activities of alkaline phosphatase in the plasma for sham, blast and weight drop group were 98.43 ± 19.78 , 88.56 ± 19.58 and 63.61 ± 3.85 respectively. At 24 hr, the activities of alkaline phosphatase in the plasma for sham, blast and weight drop group were 90.48 ± 4.07 , 83.08 ± 6.14 and 59.50 ± 5.30 respectively. Total alkaline phosphatase activity in the plasma decreased significantly at 6 and 24 hr after weight drop, in contrast to blast exposure, which did not significantly alter activity at either time post-injury (**Figure 3**). Weight drop caused 32% and 37% decreases in TNAP activity in the plasma at 6 and 24 hr respectively, yielding significantly different measurements than were seen in sham and blast-injured rats at these times.

3.3. Expression of TNAP in the brain regions after blast and weight drop

Figure 4 shows the expression of TNAP in different brain regions at 6 and 24 hr post-injury. At 6 hr, the ratios (mean $\pm$ SD) of band intensities of *TNAP*/actin for sham,

blast and weight drop were 0.84 ± 0.06 , 0.70 ± 0.04 and 0.64 ± 0.07 respectively in the brain stem, 0.81 ± 0.04 , 0.69 ± 0.05 and 0.65 ± 0.06 respectively in the hippocampus, 0.76 ± 0.11 , 0.60 ± 0.01 and 0.59 ± 0.03 respectively in the cortex. At 24 hr, the ratios for sham, blast and weight drop were 0.80 ± 0.02 , 0.72 ± 0.03 and 0.71 ± 0.04 respectively in the brain stem, 0.77 ± 0.05 , 0.69 ± 0.02 and 0.69 ± 0.01 respectively in the hippocampus, 0.74 ± 0.01 , 0.68 ± 0.03 and 0.67 ± 0.02 respectively in the cortex. Compared to sham controls, both blast and weight drop significantly decreased the expression of TNAP at 6 and 24 hr post-injury. The magnitudes of the changes in different brain regions, ranged from 17 to 30 %, were quite comparable after either insult and were generally greater at 6 than at 24 hr post-injury.

3.4. Expression of APP in the brain regions after injury

The expression of APP in brain regions at 6 & 24 hr after blast or weight drop is shown in **Figure 5**. At 6 hr, the ratios (mean $\pm$ SD) of band intensities of *APP*/actin for sham, blast and weight drop were 0.99 ± 0.10 , 1.18 ± 0.27 and 1.09 ± 0.19 respectively in the brain stem, 0.94 ± 0.07 , 1.11 ± 0.16 and 1.05 ± 0.18 respectively in the hippocampus, 0.87 ± 0.07 , 1.01 ± 0.16 and 0.98 ± 0.16 respectively in the cortex. At 24 hr, the ratios for sham, blast and weight drop were 1.02 ± 0.06 , 1.51 ± 0.27 and 1.25 ± 0.12 respectively in the brain stem, 0.93 ± 0.02 , 1.16 ± 0.12 and 1.00 ± 0.02 respectively in the hippocampus, 0.86 ± 0.03 , 1.18 ± 0.02 and 1.00 ± 0.08 respectively in the cortex. The expression of APP in different brain regions was increased significantly after blast and weight drop compared to sham controls. Despite any statistical significance, the increased expression of APP in the brain regions was more after blast exposure compared to weight drop.

4. DISCUSSION

In this study, we report for the first time that brain injury causes an acute decrease in the protein level and activity of TNAP in the brain which is associated with an acute increase in the phosphorylation of *Tau* protein. In view of the previously reported role of this enzyme in the dephosphorylation of *pTau* protein, the acutely diminished level and /activity of TNAP after injury might promote the progressive accumulation of *pTau* as an early event leading to eventual tauopathy. Neither decreased TNAP activity nor increased accumulation of *pTau* in the brain after injury correlated closely with the deposition of APP, suggesting a distinct mechanism of tauopathy after brain injury compared to AD.

There are at least four distinct forms of alkaline phosphatase enzyme present in mammals - intestinal, placental, placental-like and TNAP (present in liver, bone, kidney, brain etc.). TNAP is sequenced in chromosome 1 and sequences for all other isozymes are located in chromosome 2. In the brain, several roles for TNAP have been reported, which include: (1) involvement in the development of the nervous system partly as an ectonucleotidase in neurogenic zones [16], (2) participation in neuronal migration by interaction with collagen [3, 21], (3) regulation of the synthesis of neurotransmitters such as γ -aminobutyric acid, dopamine and serotonin [22] and (4) dephosphorylation of *pTau* [11, 14, 30]. As described in the introduction, TNAP is the principal protein phosphatase enzyme present in the brain that is primarily involved in the dephosphorylation of *pTau*. Although other protein phosphatases such as PP-1, 2A, 2B and 2C can also dephosphorylate *pTau*, TNAP has been shown to have higher activity towards *pTau* than these other phosphatases [30]; hence, a decrease in TNAP level/activity alone can

result in significant accumulation of *pTau*. Non-phosphorylated *Tau* protein is essential for microtubule assembly with tubulin and accumulation of *pTau* disrupts this process, resulting in neuronal hypofunction and even neuronal death.

The phosphorylation of *Tau* protein can occur on different amino acids at specific locations of the amino acid sequence. *Tau* protein, phosphorylated at serine396 (S396), is rich in the paired helical filaments which form neurofibrillary tangles observed in the brains of patients with AD [2, 12, 34]. Our results showed increased phosphorylation of S396 after blast and weight drop-induced brain injuries, pointing to the possibility of formation of neurofibrillary tangles after brain injury. Increased phosphorylation of *Tau* protein at S396 at 24 hr after blast exposure has been previously reported [13], supporting our current observation. In addition to S396, increased phosphorylation of several other amino acids in the *Tau* protein was observed in that study which could potentially also contribute to disrupted microtubule assembly after blast exposure. Using head impact-induced single and repetitive mild TBI in *hTau* mice, Ojo *et. al.* showed that repetitive, but not single, mild TBI also induced the phosphorylation of *Tau* at a number of amino acid sites [24]. The phosphorylation of *Tau* protein after single head impact in the current study could simply reflect a more severe insult in these subjects than was produced by a single impact in this earlier work.

In the present study, we have shown that the protein levels and activities of TNAP in the brain decreased significantly after blast exposure or impact-acceleration and were associated with a significant increase in the phosphorylation of *Tau* protein. At these respective injury severities, the decreases in TNAP activity were greater after weight drop than after blast and were concomitantly associated with comparably greater

increases in the levels of *pTau*, suggesting that decreased TNAP activity may in part be responsible for the accumulation of *pTau* after these brain insults. If persistent, decreased TNAP activity might promote progressive accumulation of *pTau*, culminating in tauopathy and conditions associated with chronic diseases such as CTE. Long-term studies evaluating the level/activity of TNAP and the accumulation of *pTau* in the brain are warranted to determine their roles in the development of chronic diseases. The decrease in the activity of the enzyme could be due to the decreased level of the protein or due to an inhibition of the enzyme activity. Western blot analyses of extracts from the three brain regions indicate that the TNAP protein level consistently decreased after the brain insults. The decreased protein level of TNAP in the brain regions could be due to decreased synthesis or increased degradation after injury, and further studies using messenger RNA levels are warranted to delineate the underlying mechanism.

The major alkaline phosphatase isozyme present in the blood is TNAP [23], and the decrease in the activity of TNAP in the brain was associated with a significant decrease in the activity of total alkaline phosphatase in the plasma after weight drop but not after blast exposure. Although it is not clear why plasma alkaline phosphatase activity declines after weight drop, this parallel with the brain points to the possibility that the two changes are related and that phosphatase activity in the circulation might be attributed to TNAP enzyme originating in the brain, which is diminished after injury. Unlike weight drop, where injury is localized to the brain, blast is a whole body insult affecting multiple organ systems; consequently, changes in total alkaline phosphatase activity in the circulation after blast may more generally reflect the net outcome of its composite effects on multiple organ systems and not just brain. In particular, since blast

exposure disrupts cell membranes in various organs resulting in the release of enzymes and other intracellular components [0, 1, 31], it is possible that unlike weight drop injury, blast exposure causes the release/leakage of different alkaline phosphatase isozymes into the plasma from peripheral organs such as liver, kidney, intestine, placenta, bone etc., all of which contribute to the alkaline phosphatase activity measured in plasma after blast exposure.

TNAP activity in the plasma has been reported to increase after 7 days post-injury in patients with different brain injury conditions such as post-resuscitation encephalopathy, ruptured cerebral aneurysms, subdural hematoma, cerebral contusion and non-traumatic intracerebral hemorrhage [35]. Although no change in TNAP activity was observed in those patients at early stages of injury, patients who died at early stages of injury had very low or undetectable TNAP activity in their plasma, which is in agreement with our observation of low TNAP activity at acute stages of injury.

In AD, the accumulation of amyloid plaques have been reported to precede and promote tauopathy [10, 17, 26, 27, 29, 33]. In particular, formation of amyloid β ($A\beta$) protein has been shown to enhance the phosphorylation of *Tau* protein, resulting in neurofibrillary tangle formation in an APP and *Tau* double transgenic mouse model [27]. The phosphorylation of *Tau* protein has been further proposed to involve the activation of mitogen-activated protein kinase by accumulated APP or $A\beta$ protein [10]. In contrast, previous shock tube studies with rats revealed a divergence in the changes of these proteins in brain after blast exposure. Whereas the expression of APP increased in the brain at 24 hr after blast and increased further after 1 week, the levels of $A\beta$ peptides progressively decreased during this time [8], indicating that increased accumulation of

A β peptides may not trigger the phosphorylation of *Tau* protein after blast exposure. In the present study, comparison of the accumulation of *pTau* (S396) in brain after weight drop and blast injury (**Fig.1**) with the expression of APP after these insults (**Fig.5**), reveals a similar divergence in these measurements, reinforcing interpretation that accumulated APP may not play a substantial role promoting the phosphorylation of *Tau* protein after blast exposure. Blast or weight drop didn't increase the expression of APP in the brain at 6 hr post-injury. At 24 hr, both insults significantly increased expression of APP, but the greatest increase in APP was observed after blast exposure (**Fig.5**) unlike *pTau*, where the greatest increase was evoked by weight drop (**Fig.1**). Although these disparities do not rule out APP promoting phosphorylation of *Tau*, they nevertheless do raise the possibility that the neurobiological mechanisms yielding tauopathy after TBI might be distinct from those of AD.

CONCLUSION

Brain injury after blast exposure or weight drop results in a significant decrease in the expression and activity of TNAP which is associated with a significant increase in the accumulation of *pTau* in different brain regions. In view of the known function of TNAP in dephosphorylating *pTau*, the accumulation of *pTau* after brain injury could be attributed to the decreased TNAP activity/levels in the brain after the injury. The decreased activity of TNAP in the brain after injury was associated with a significantly decreased total alkaline phosphatase activity in the plasma. The accumulation of *pTau* after brain injury did not show a positive correlation with the expression of APP in different brain regions, indicating that the mechanism of tauopathy after brain injury could be distinct from that of AD.

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Figure Legends

Figure 1. (A) Western blotting showing the expression of *pTau*, with phosphorylation at serine396, in different brain regions at 6hr and 24hr after blast or weight drop. The antibody detects *Tau* protein only when the serine396 is phosphorylated.

Representative figures from two rats out of four in each group are shown. (B)

Densitometry analysis showing the ratio of band intensities of *pTau* and β -actin. Values are expressed as mean $\pm$ SD. * $p < 0.05$.

Figure 2. Activity of TNAP in different brain regions at 6hr and 24hr after blast or weight drop. One unit of enzyme activity is the μ moles of *p*-nitrophenol liberated per minute.

Values are expressed as mean $\pm$ SD. $n = 4$, * $p < 0.05$, ** $p < 0.01$.

Figure 3. Activity of alkaline phosphatase in the plasma at different intervals after blast or weight drop. One unit of enzyme activity is the μ moles of *p*-nitrophenol liberated per

minute. Baseline – plasma collected before injury. Values are expressed as mean $\pm$ SD.

$n = 4$, * $p < 0.05$, ** $p < 0.01$.

Figure 4. (A) Western blotting showing the expression of TNAP in different brain regions at 6hr and 24hr after blast or weight drop. Representative figures from two rats out of four in each group are shown. (B) Densitometry analysis showing the ratio of band intensities of TNAP and β -actin. Values are expressed as mean $\pm$ SD. * $p < 0.05$.

Figure 5. (A) Western blotting showing the expression of APP in different brain regions at 6 and 24hr after blast or weight drop. Representative figures from two rats out of four in each group are shown. (B) Densitometry analysis showing the ratio of band intensities of APP and β -actin. Values are expressed as mean $\pm$ SD. * $p < 0.05$.

Figure 1

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