



# Prolidase Enzyme Activity as a Potential Biomarker for Blast-Induced Traumatic Brain Injury: A Study in a Rat Model

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## Abstract

Blast-induced traumatic brain injury (TBI) poses a significant threat to individuals exposed to explosive events. We hypothesized that blast-induced neurotrauma is an oxidative stress to brain and hence prolidase (PD) enzyme, which is an antioxidant is recommended; its serum levels are better markers of degree of injury in the acute phase for TBI in a rat blast injury model. Results revealed that PD enzyme activity in the hippocampus showed a slight increase across high, medium, and low blast pressures, but remained lower than the sham group. However, serum PD enzyme activity levels were significantly higher in the blast-exposed groups compared to the sham group. Tau protein levels were significantly elevated in the blast-exposed groups. Longitudinal analysis demonstrated a decline in hippocampal PD activity over time, while tau protein levels progressively increased, suggesting a shift from initial oxidative stress to neurodegeneration. These findings suggest that blast injury triggers oxidative stress and subsequent neurodegenerative processes. The correlation with tau protein levels further supports the involvement of oxidative stress in neurodegeneration. In conclusion, this study provides insights into the underlying pathophysiological mechanisms of blast-induced TBI and highlights the potential utility of PD enzyme activity as a diagnostic marker.

## Keywords

- ▶ animal model
- ▶ blast-induced-traumatic brain injury
- ▶ neurodegeneration
- ▶ oxidative stress
- ▶ prolidase

## Introduction

Blast-induced neurotrauma (BINT) results from direct or indirect exposure to an explosive event.<sup>1</sup> Explosion leads to sudden release of energy that creates pressure above

atmospheric pressure, hence the term overpressure which consecutively compresses the surrounding medium (air or water), resulting in the propagation of a blast wave in a radial manner.

Prolidase (PD) enzyme has been proven to have antioxidant properties<sup>2</sup> and has been studied in several systemic pathologies. An increase in activity of PD enzyme activity is shown to be correlated with increased rates of

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collagen turnover.<sup>2</sup> To the best of our knowledge, it has not been studied with respect to traumatic brain injury (TBI).

In the current research, we hypothesized that, if oxidative stress is a key factor in pathophysiology of blast-induced TBI, can there be a role for PD in the process. Hence, we investigated the usefulness of PD enzyme as a marker for TBI in a rat blast injury model. Rats were subjected to different blast pressures, and their hippocampus samples and serum were analyzed.

## Methods and Materials

### Animals

Blast injury animal model used for the experiment was made using male Sprague Dawley rats aged 2 months and weighing 200–250 g. They were procured from Central Animal Research Facility (CARF) of our institute after ethical clearance from the Institutional Animal Ethics Committee and CARF. Approval number- AEC/65/386/NS-A

The rats were housed in 12-hour light and 12-hour dark cycle with access to standard rat chow and water ad libitum.

### Blast Exposure

Rats were exposed to overpressure via modified Reddy's gas-driven shockwave tube<sup>3</sup> (► Fig. 1).

Three groups of rats were exposed to different blast pressures of 81, 160, and 210 kPa as noted at 1 cm from opening of the driven section, ► Fig. 1, measured using a Piezoelectric sensor. One more group underwent sham blast.

### Sacrifice

The rats were sacrificed by manual cervical dislocation following which the skull caps were dissected out and the

brain was removed. The cerebral hemispheres were divided in the mid sagittal plane using a scalpel blade while on an ice-cold surface. One half of the brain was preserved in 10% neutral buffered formalin. From the other hemisphere, the hippocampus was dissected out and placed in an Eppendorf tube and frozen at  $-20^{\circ}\text{C}$ .

The hippocampi were homogenized in 50 mmol tris-buffered saline, pH 7.4 containing 0.5% TritonX-100 with pestle and sonicated with 3 pulses of 10 seconds each and centrifuged at 10,000 rpm for 15 minutes at  $4^{\circ}\text{C}$ , and the supernatants were collected and were then subject to tau enzyme-linked immunosorbent assay and PD assay using Chinnard's method<sup>4</sup> and via Cary 60 ultraviolet visible-vis spectrophotometer.

### Statistical Analysis

The statistical analysis was performed using IBM SPSS Ver22. Analysis of variance was used to compare PD/tau levels in high-pressure, medium-pressure, and low-pressure groups.

Pearson correlation was performed to evaluate the linear relationship between PD activity and tau protein levels in hippocampus in various severity groups.

Dunnett t-test was conducted to identify the period of most significant change in enzyme activities.

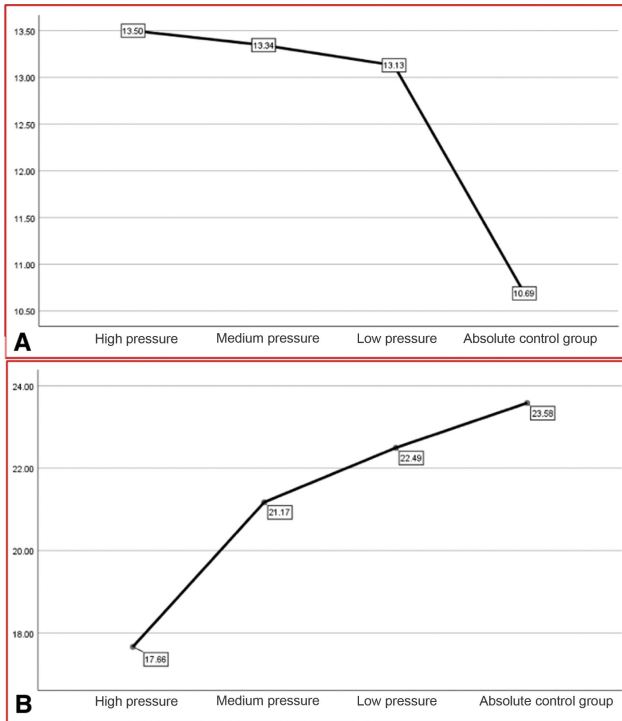
## Results

### PD Activity(U/mL)

The mean PD activity in hippocampus homogenates (HH) increases insignificantly between high, medium, and low pressures, but it remains less than the sham group ( $p=0.334$ ; ► Fig. 2A). Then, serum enzyme (PD) activity levels increase when compared with sham group significantly ( $p=0.002$ ; ► Fig. 2B).



**Fig. 1** (A) Modified Reddy's gas-driven shockwave tube. (B) Rat restrained in front of the open end of the modified Reddy's tube.



**Fig. 2** (A) Mean of prolidase (PD) enzyme activity in hippocampus homogenates (y-axis) and various pressure groups and controls (x-axis). (B) Mean of PD enzyme activity in serum (y-axis) and various pressure groups and controls (x-axis).

The correlation between enzyme (PD) activity in hippocampus and tau levels is significantly moderate (positive) in high ( $p=0.018$ ) and medium trauma ( $p=0.04$ ; ► **Fig. 3A** and **B**).

Fall of mean PD activity levels in HH is significantly different between 42 and 168 days ( $p<0.001$ ) and between 84 and 168 days ( $p=0.016$ ; ► **Fig. 4A**).

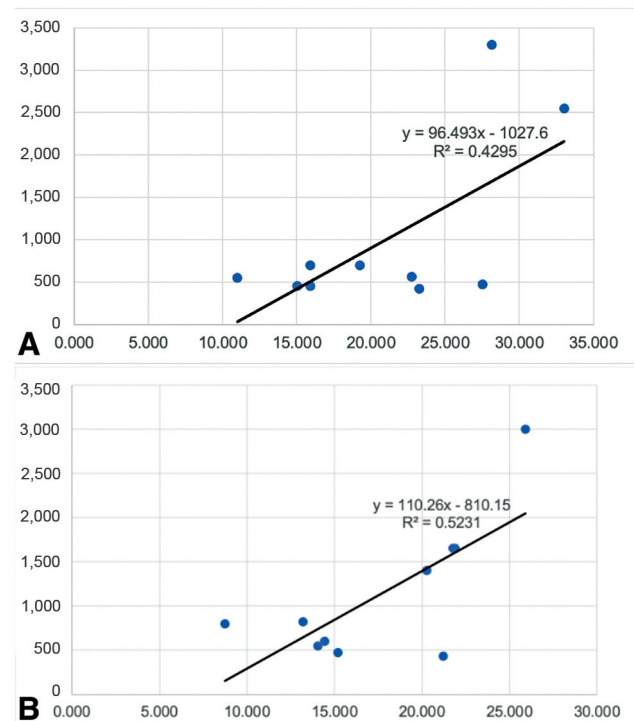
There is significant rise in mean tau activity between 42 and 84 days ( $p=0.001$ ) and between days 42 and 168 ( $p=0.001$ ; ► **Fig. 4B**).

### Discussion

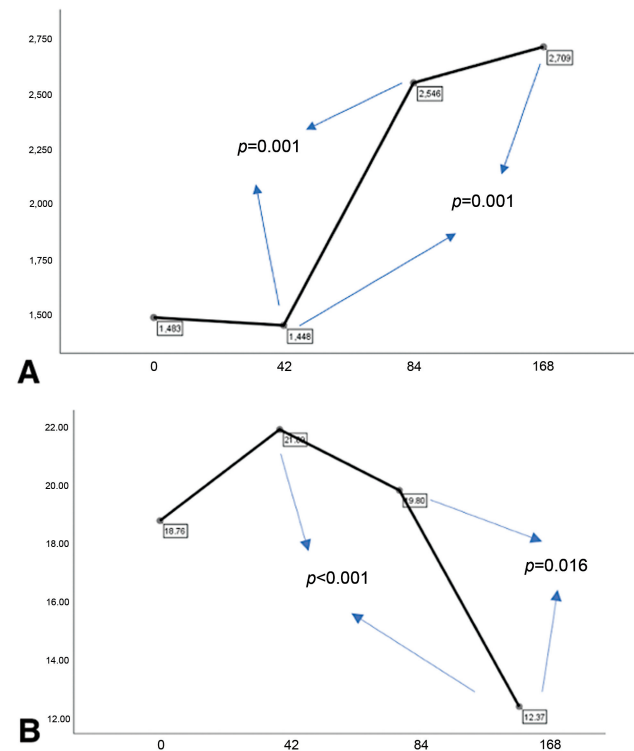
BINT is primarily seen in soldiers and civilians residing in war-inflicted regions. The main mechanism by which blast waves (overpressure and under pressure phase) interact with the body is by spallation, inertial effects, and implosion (creates cavitation).<sup>5</sup>

This study looked for biomarker for TBI. PD enzyme activity was studied in HH and serum samples from these subjects. PD enzyme activity levels were correlated with tau protein concentration.

Misiura and Miltyk<sup>6</sup> in their review on the role of PD in cellular mechanisms explained that PD has both enzymatic and nonenzymatic activity. PD regulates collagen synthesis by providing proline via enzymatic pathway and regulated the function of p53 intracellularly via its nonenzymatic activity while acting as a ligand. During cellular stress (e.g., blast injury and TBI) and after activation of reactive oxygen species, the PD-p53 complex dissociates and p53



**Fig. 3** (A) Correlation between prolidase (PD) activity (x-axis) in hippocampus homogenates and tau activity (y-axis)—high pressure. (B) Correlation between PD activity (x-axis) in hippocampus homogenates and tau activity (y-axis)—medium pressure.



**Fig. 4** (A) Correlation between prolidase activity in hippocampus homogenates (y-axis) and days since the blast (x-axis). (B) Correlation between tau activity in hippocampus homogenates (y-axis) and days since the blast (x-axis).

translocate to mitochondria and results in cell death. In this study, a significant rise in serum PD activity levels was observed when compared with the sham blast group.

Toklu et al<sup>7</sup> concluded that BINT increases inflammation and oxidative stress in neural tissues. PD activity in HH increases from 0 to day 42 and then starts to fall. It suggests initial possible oxidative stress decreases after day 42. However, it was seen that tau activity (marker of neurodegeneration) increases from day 42 onward. It is also having positive correlation with high- and medium-pressure blast injury groups. Effects of tau protein accumulation and its effects on memory function are already proven in Alzheimer's disease research. It signifies that neurodegeneration starts after initial period of oxidative stress.

## Conclusion

Blast injury results in oxidative stress and neurodegeneration. PD, being an antioxidant, increases significantly in serum and hippocampus in blast groups. It also correlates with tau levels that are a marker of neurodegeneration.

### Note

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None.

## Conflict of Interest

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