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Original Article

Validation of a blast induced neurotrauma model using modified Reddy tube in rats: A pilot study

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ARTICLE INFO

Article history:
Received 23 July 2014
Accepted 20 November 2014
Available online 13 December 2014

Keywords:
Blast
Neuronal degeneration
Neurotrauma
Rat model
Reddy tube

ABSTRACT

Problems considered: Blast induced neurotrauma (BINT) is becoming increasingly prevalent. However the pathophysiology has not been elucidated. In animal models blast injury is mimicked by exposing the animals to shock waves produced by shock tubes or by exposing them to open field explosions. We have tried to validate a custom made handy, indigenous blast tube in producing blast injury in a rodent model.

Methods: Fifteen rats were subjected to increasing peak pressure blast waves using a modified Reddy blast tube. The pressure recordings were in keeping with the classic Friedlander waveform and were highly reproducible. One rat died immediately following exposure to the blast. At the end of 2 weeks all the live rats were sacrificed and subjected to histopathological examination.

Results: The pathology revealed neuronal degeneration and axonopathy in the cortex, cingulum, hippocampus, thalamus, brain stem and cerebellum with the severity of injury increasing with increasing blast pressure. These findings were consistent with that reported in the literature.

Conclusions: We have thus successfully validated the blast tube for production of BINT. The tube is handy, easy to use and can be made widely available for furthering the research in the field of BINT.

1. Introduction

Blast induced neurotrauma (BINT) is becoming a major focus of study following increasing military and civilian injury after exposure to blasts from improvised explosive devices. People who suffer from this injury have long term significant cognitive, memory and behavioral disturbance, the pathophysiology of which has not yet been understood completely.[1,2] Most of the studies in this regard are from developed countries, using sophisticated blast tubes which are extremely bulky and available only in major research facilities and in the

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http://dx.doi.org/10.1016/j.ijnt.2014.11.006

army research centers.[2] These tubes generate the shock waves which when exposed to experimental animals mimic a primary blast injury. Reddy Blast tube is an indigenous product from the Indian Institute of Science, Department of Aerospace Engineering, Bangalore, which is capable of providing the same type of blast mechanics as that of sophisticated equipments. In the present study we exposed experimental rats to the blast wave from modified Reddy tube and studied the neuropathological changes occurring in the rat brain when exposed to different blast peak pressures. The results were then compared to those reported in the literature.

2. Material and methods

2.1. *Experimental setup and procedure*

The experimental setup used in the present work consists of the modified "Reddy Tube" and a 2 L SS-304 cylinder. The details of the working of the Reddy tube has been described elsewhere.[3,4] However, a brief description of Reddy Tube is given below for making the paper self contained. It is a simple tube consisting of two sections (a high pressure-driver section, and a low pressure-driven section) separated by a diaphragm, generally made of a sheet of tracing paper of 90/95 grade. This is a recently invented system which produces moderate strength shock waves with associated blast overpressures manually. However since the overpressures generated in the original Reddy tube were not adequate to produce the BINT it was modified to operate using the compressed gas. When the Reddy tube was manually driven, the lengths of compression tube and driven sections were 0.49 m and 0.50 m, respectively.[3] The modified Reddy tube consisted of a 0.2 m long driver tube and 0.5 m long driven tube. Compressed nitrogen from the 2 L cylinder (filled to 80 bar) was used to rupture the paper diaphragm. The length of the driver section was shortened to obtain a blast profile at the end of the driven section of the shock tube. The modified Reddy tube was operated by admitting the driver gas from the high pressure cylinder in to the driver section using a safety valve. The high pressure gas in the driver section ruptures the paper diaphragm creating a planar shock wave traveling in to the driven section. The shock speed and the pressure jump across the primary shock wave traveling the driven section are measured using a couple of surface mounted pressure gauges on the driven section and the pressure behind the blast wave emerging from the open end of the shock tube was measured using pressure transducer mounted at a distance of 5 mm from the exit of the shock tube as shown in Fig. 1.

The over pressures generated at the shock tube exit was varied by increasing the number of layers of tracing paper diaphragm or by using an aluminum foil of 250 μm thickness.

Fifteen adult male Sprague Dawley rats (250–300 g) were used in this experiment. The experiments were conducted after obtaining clearance from our institution's animal ethics committee. All rats were housed in the Central Animal Research Facility, NIMHANS, in plastic cages and were provided with food and water *ad libitum*.

The animals were randomly divided into 3 groups depending on the peak over pressure they were exposed. The peak pressures used were 89 kPa, 160 kPa and 210 kPa and the number of animals exposed to each were 4, 4 and 7 respectively (Groups a, b and c respectively). During experiments the animals were restrained in a plastic triangular casing and secured on a board with rubber tourniquets. No anesthetic agent was used as it was thought that these agents may have a neuroprotective effect which could confound the results.[5] The snout was placed at the opening of the nozzle as shown in Fig. 1. After exposure to the blast the animals were replaced back in their cages and provided normal food and water. At the end of 2 weeks the animals were sacrificed with an overdose of anesthesia. A transcardiac perfusion with phosphate buffer saline and 4% paraformaldehyde pH 7.4 was done followed by brain fixation with 4% paraformaldehyde.

The brain was then sliced in axial plane for studying various neuroanatomical areas. The brain stem and cerebellum were also sliced serially in coronal plane. Slices were processed for paraffin embedding. Sections were stained with Hematoxylin–Eosin stains for delineating neuronal, glial and vascular pathology, if any. Serial sections at 4 micron thickness were

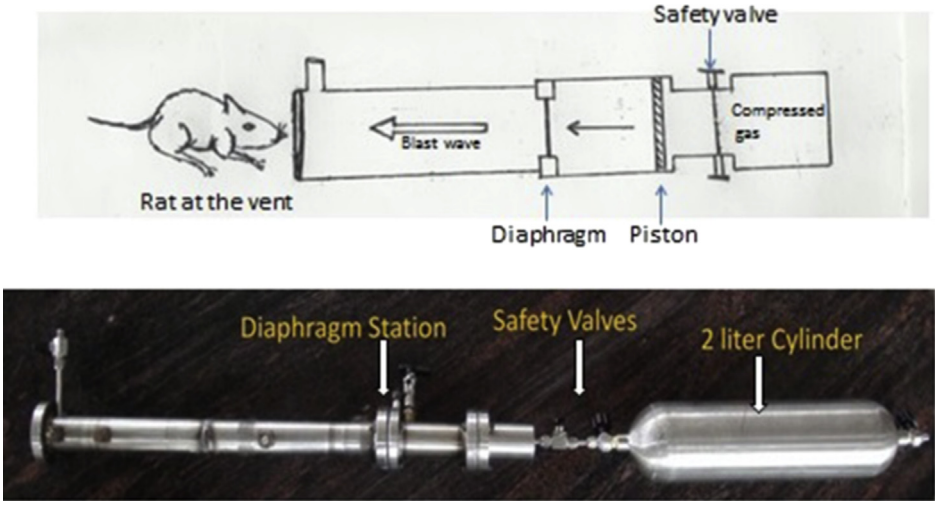

Fig. 1 – Modified Reddy tube assembled with the rat at the exit of the shock tube.

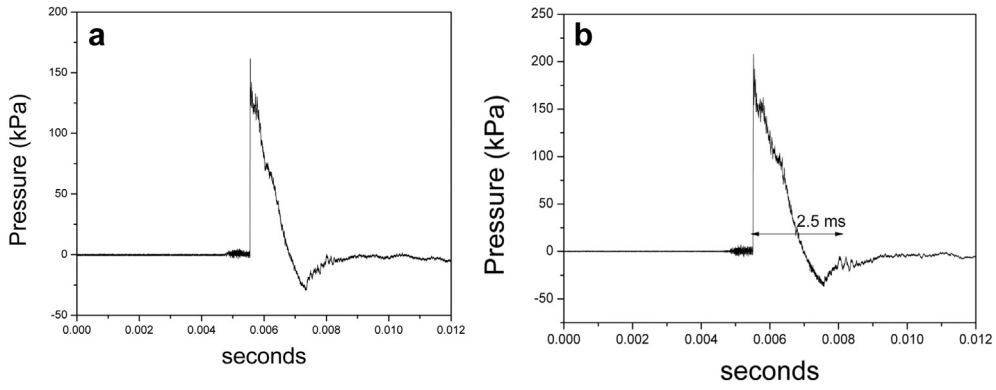

Fig. 2 – a,b: Pressure waves and signals obtained at the exit of the shock tube (160 and 210 kPa). Duration of blast wave ≈2.5 ms.

stained with Nissl stains for neuronal alterations and Luxol fast blue stains for determining myelin alterations.

3. Results

The output of the shock tube was similar to the Friedlander wave which is produced by standard shock tubes. The pressure profiles obtained using the pressure transducer mounted on the sidewall of the shock tube at a distance of 5 mm from the exit of the driven section is given in Fig. 2. The waveform and over pressures obtained were found to be highly reproducible.

All animals except one survived the blast. One rat exposed to a 210 kPa pressure had seizures and died immediately after the blast. Six of the rats exposed to 160 and 210 kPa peak pressures had eye, ear and/or nasal bleed immediately after exposure to blast. The injury was from soft tissue and there was no globe rupture. Six rats were "stunned" for a couple of seconds with immediate recovery.

All the live rats recovered well and had no gross behavioral changes and had normal food intake. At the end of 2 weeks they were all sacrificed.

3.1. *Histopathological examination*

Gross examination of the brains was done. The surface was normal in all the brains with no evidence of subdural, subarachnoid or parenchymal haemorrhages. There was no evidence of macroscopic intraventricular or cisternal bleed either.

The brains were systematically examined for laterations in neurons, axons and myelin and blood vessel alterations, if any. The alterations in neuronal, axonal and myelin changes progressively increased with increase in pressure.

The rat that died immediately following blast injury revealed hemorrhage in the basal cisterns and lateral ventricles. There was also evidence of acute ischemic changes in neurons and perineuronal vacuolation reflecting seizure induced phenomena along with tissue tears indicating severe injury.

In all the other rats the striking features noted on histopathology were neuronal degeneration with axonopathy. The severity of the damage correlated well with increasing blast overpressures.

Group a: The group of rats exposed to 89 kPa peak pressure revealed mild alterations. There was eosinophillic change with shrinkage of neurons seen focally in the cingulate and upper layers of frontal cortex (Fig. 3), sparing the parietal and temporal cortex, hippocampus as well as thalamic nuclei. There was mild vacuolation of the dendritic processes. Myelin stains revealed preserved myelinated fiber tracts with no axonal//myelin alteration seen on routine and Luxol Fast blue stains. No inflammation or microglial response was noted. Vessels appeared normal. Sections through brain stem and

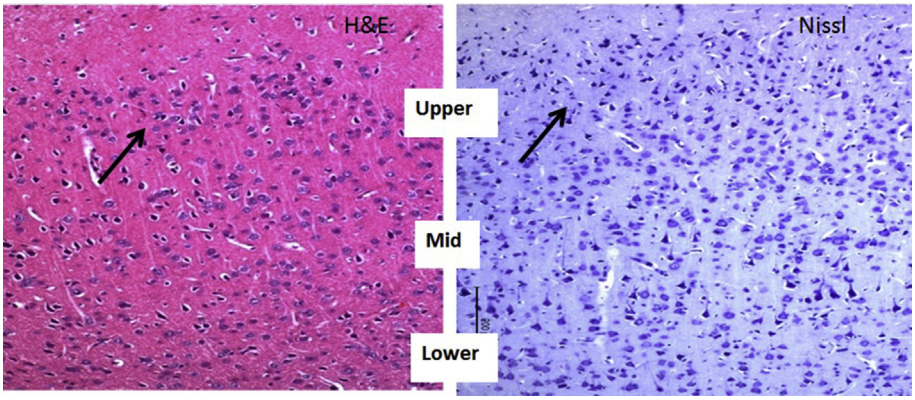

Fig. 3 – Group a: Frontal cortex showing neuronal degeneration in upper layers (arrow), H&E and Nissl stain.

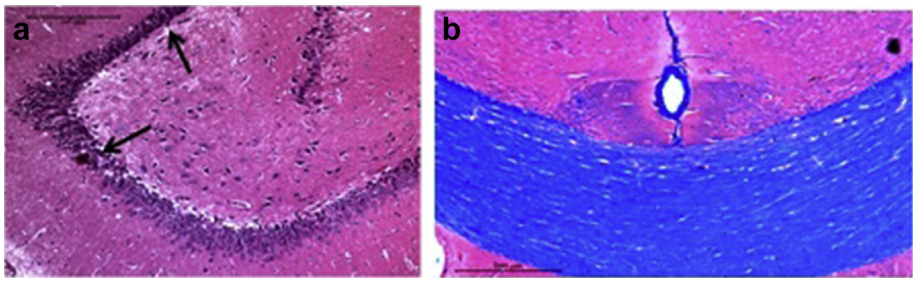

Fig. 4 – Group b: a) focal neuronal degeneration in hippocampus (H&E stain), b) Corpus callosum: mild demyelination and vacuolation (Luxol-Fast Blue).

cerebellum were also relatively normal except for mild pallor of molecular layers. Purkinje neurons and granular layer were well preserved as also the white matter of cerebellar cortex.

Group b: With increase in pressures to 160 kPa, the neuronal eosinophilia and pyknosis was more extensive with involvement of bilateral cingulate, frontoparietal cortex and extending to involve the hippocampus, though in segmental fashion with mostly the dentate gyrus and CA3 neurons showing shrinkage of neurons and vacuolation of the dendritic processes suggesting mitochondrial damage. The thalamic neurons also revealed shrinkage and eosinophilia of neurons. The brain stem and corpus callosum revealed focal vacuolation of white matter tracts with vacuolation of myelin sheaths and focal axonal dilatations suggesting deafferentation (Fig. 4). The molecular layer of cerebellum appeared pale with vacuolation of apical dendritic processes coursing through the molecular layer while there was focal demyelination of cerebellar deep white matter. No inflammation or microglial or astrocytic proliferation was noted. One of the rats showed focal hemorrhage into the third ventricle.

Group c: Neuronal alterations with eosinophillic shrinkage and pyknosis evident on cresyl violet stains was maximal with exposures to high pressures of 210 kPa involving full thickness of cingulate cortices and extending to involve lower layers in frontoparietal and temporal cortices. Hippocampus also revealed more extensive changes with longer lengths of dentate and CA1–CA3 pyramidal neurons showing shrinkage while others revealed pale cytoplasm reflecting fibrillar protein accumulation. Similarly, thalamus also showed more extensive neuronal pyknosis. The brain stem white matter and cerebellar white matter showed foci of demyelination and axoplasmic distensions (Fig. 5). The molecular layer of cerebellum also revealed pallor and vacuolation of apical dendrites. No inflammation, reactive astrocytosis or microgliosis was detectable in any of the cases.

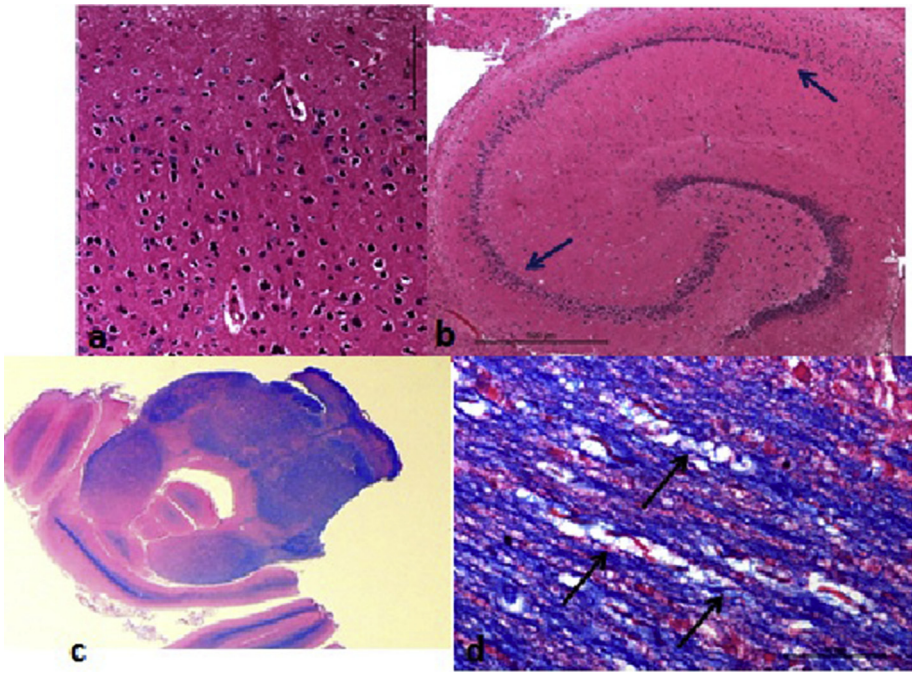

Fig. 5 – Group c: Shows neuronal degeneration in a) all the layers of the frontal cortex and b) in the hippocampus (arrows). Myelin stain (Luxol-Fast Blue) shows axonal fragmentation and vacuolation of surrounding myelin sheaths in brain stem tracts (arrows).

4. Discussion

4.1. *Blast mechanics*

With increasing terrorism worldwide and with use of sophisticated explosive devices the number of military personnel and civilians being subjected to blast induced neurotrauma is increasing by the day. With the use of body armor, primary injury to the lungs and the hollow viscera has reduced but this has led to an increase in the incidence and awareness of primary blast injury to the brain. Hence there has been an impetus in the research in the field of pathophysiology and outcome following bTBI. Secondary and tertiary mechanisms are somewhat clear hence studies are now ongoing into the pathophysiology of primary blast injury.[1,6–8]

Primary blast injury is caused by the fast moving transient pressure wave following explosion. Blast induced neurotrauma occurs following transmission of this pressure to the brain through the skull. Prior to the use of body armors it was thought that brain injury occurred via transmission of the wave from the thoracic cavity upwards through vessels. However the exact pathophysiology has not been understood.[1,8,9] A blast wave is characterized by a rapidly rising overpressure with an exponential decay and then a brief negative phase called the Friedlander wave. Shock tubes producing this wave have been extensively used to study blast induced neurotrauma in animals.[1,9] In experimental conditions small animals (rats and mice) are exposed to peak over pressures produced by air-driven shock tubes which are designed to provide the Friedlander curve. The shock tubes are bulky, long and measure between 15 feet and 6 m and have a cross sectional area of $9 \times 9''$ in which the animal is kept. These tubes are available only in sophisticated laboratories and cannot be transported easily to other facilities. The animal has to be anaesthetized and then kept within it and if not protected the entire body is exposed to the blast thus confounding the primary brain injury.[1,2,10]

The Reddy tube is compact and easy to handle. It can be easily transported and manual compression or pressurized gas can be used to produce the blast. The blast waves and pressure recordings have been shown to be reproducible.[3,4] Due to the small opening of 29 mm diameter only the head of the rat is exposed to the blast beyond which the pressure falls rapidly therefore the body is not exposed to the blast. The blast pressure curve follows the Friedlander distribution. The duration of the blast is $\approx$ 2.5 ms.

Balmaan et al. have generated blast waves in their experiments using an air-driven shock tube measuring 1.25 m long. Here the rat was placed inside the tube, body protected with only the head being exposed to the blast wave.[11] Similar smaller tubes which exposes only part of the body to the blast are also available.[12,13]

4.2. *Pathology of BINT*

Apnea for 20–37 s, bradycardia and hypotension has been described to occur following rat exposure to blast.[1] In our study on visual inspection the rats were stunned (lay listless) for 2–7 s after which they recovered rapidly. Primary BI can cause eye injury globe rupture, serous retinitis and hyphema.[7] Six rats had eye or ear bleed which was from soft tissue and there was no evidence of rupture of the globe. Possibility of injury from the fragments of the ruptured diaphragm could not be ruled out. A variety of pathological changes have been reported in animals exposed to bTBI depending on the intensity of the blast. On the surface subdural, subarachnoid haemorrhages and parenchymal injuries to the cortex, cerebellum and medulla have been noted.[14]

Microscopic evaluation findings are normal brain architecture, reactive gliosis, neuronal swelling, cytoplasmic vacuolation and degeneration, axonal damage either focal or diffuse. Presence of activation and proliferation of microglia, astrocytes and inflammation 1–14 days after injury has also been demonstrated. Loss of blood brain barrier, increased oxidative stress has been reported. All these findings contribute to the pathophysiology of BINT.[1,8–11,14–16]

Balman et al. exposed rats to low blast pressures (74 kPa). The rats had memory dysfunction in object recognition and pathology revealed no gross changes except for shortening of the axonal initial segment.[11]

Garman et al. demonstrated multifocal axonal degeneration with deep cerebellar and brainstem white matter tract lesions along with neuronal degeneration at 2 weeks post blast exposure suggestive of diffuse axonal injury.[8] Neuronal degeneration was seen in the cortex, hippocampus CA1 region and the cerebellar cortex. Long et al. demonstrated cortical cell loss, gliosis and infiltration, hemorrhage and extensive necrosis in the brains of rats exposed to 147 kPa overpressure at 2 weeks post exposure, while there was widespread fiber degeneration (especially in commissural fibers and other fiber tracts) best seen in silver stains at all cross sections with no cell loss at overpressures of 126 kPa. At 114 kPa overpressure there were no changes in the rat brain.[1]

Gama Sosa et al. (BOP) reported acute (<24 h) and long term (>4 months) changes in the brain. Acute findings included intraventricular and choroid plexus bleed suggesting the high vulnerability of the choroid plexus to blast overpressure. Long term changes included focal rips or tears in the brain tissue suggestive of shear stress induced changes. These lesions followed penetrating cortical vessels suggesting that the blood vessels may represent the fault lines along which blast pressure is transmitted. Like in other studies they also showed microglial activation and proliferation. The neurons and their nuclei were elongated trying to align along the direction of the blast pressure wave.[15]

In our study we were able to produce increasing severity of neurotrauma following exposure to increasing peak overpressure waves. The changes of neuronal degeneration, axonal damage both focal and diffuse, hippocampal involvement could be seen. Cerebellar and brainstem injury was also seen. As we have used only limited stains further characterization of neuronal and axonal alterations cannot be done. It requires application of immunohistochemistry to determine cytoskeletal damage and accumulation of dystrophic, cytoskeletal proteins in neurons and degenerating axons as well as glial and microglial alterations.

5. Conclusions

We have been successful in developing a model for blast induced neurotrauma in rats using an indigenous shock tube called modified Reddy tube. The histopathological findings included neuronal degeneration and focal/diffuse axonal injury which were consistent with that reported in literature. This opens new avenues for further research into the pathophysiology, prognosis and treatment of BINT. As the shock tube designed is handy and easily transportable and it has a potential of being made widely available and can be used in many not so high end research facilities.

Conflicts of interest

All authors have none to declare.

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