Pre-clinical models for Traumatic Brain Injury: A Short Review

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Abstract

Traumatic brain injury (TBI) refers to a blunt, penetrating, or acceleration/deceleration force-derived craniocerebral injury. It may occur due to accidents, falls, injury or blow to head. It causes manifestations at cellular, molecular and organ level changing the lifestyle. It results in memory deficits, neurological or neuropsychological abnormalities and even death. It is considered as a major cause of deaths and disabilities. Due to its heterogeneous and complex nature, it is very challenging to explore pathogenesis of TBI and develop effective therapeutic measures for the management this neurotrauma. Various animal models for induction of TBI like fluid percussion model, weight drop method and blast induced injuries have been designed to study the diverse aspects of this neurotrauma. Most of these models have been quite successful in mimicking the conditions of human injury. However, keeping in view the ethical challenges, development of clinically relevant models needs to be encouraged.

Running title: Experimental models for TBI

Key words: Traumatic brain injury, neurotrauma, animal models, fluid percussion injury, controlled cortical impact.

Introduction

Traumatic brain injury (TBI) refers to a blunt, penetrating, or acceleration/deceleration force-derived craniocerebral injury. It is an insult to the brain generally caused by external mechanical forces e.g. blow or jolt to the head, head injury due to accidental mishaps, sports, blasts or penetration of objects etc. As per World Health Organization, the major cause of TBI is motor vehicle injuries and by 2030, TBI would be a leading cause of mortality as well as disability. TBI is a complex neurotrauma which may cause temporary or permanent damage to the brain thereby resulting in memory deficits, neurological or neuropsychological abnormalities and even death.[1] TBI is a leading cause of death among young adults and the number of TBI related deaths is increasing worldwide. Furthermore, it is a major cause of disabilities also as survivors of TBI often suffer from impairment of cognitive, physical and psycho-social functions which compromises their quality of life. These patients require long term care and incur economic cost to health systems. It is often regarded as a silent epidemic due to lack of awareness regarding its impact and magnitude. Due to a large number of deaths and longterm neuropsychological impairments, TBI is considered as a global public health concern which requires urgent attention. Currently surgery, neurocritical care, neurorehabilitation and various pharmacological interventions based on TBI associated impairments are the main treatment options.[2] Despite recent advancements, there is a lack of

pharmacological alternatives due to heterogeneous and complex nature of TBI. Therefore, there is a need to develop novel, effective preventive and therapeutic measures for the management of this critical health concern. For the development of novel and effective pharmacological agents, it is very crucial to explore the complex pathogenesis of TBI and to develop the clinically relevant animal models. So, the aim of this article is to review the models of TBI and explore the underlying pathogenesis.

Traumatic brain injury (TBI)

TBI is a brain injury, characterized by primary and secondary events. The primary event involves the physical damage to the brain due to forceful impact like road accidents, blow to head or fall from high buildings etc. This is followed by the secondary events which evolve over time and may be life threatening.[2] The secondary events include peri-lesional edema following TBI, increased levels of inflammatory mediators, increased oxidative stress, neuronal apoptosis, deficits in mitochondrial functioning, alteration in neurogenesis and cell signal induction pathways resulting in neuropsychological impairments.

TBI is classified on the basis of Glasgow Coma Scale (15point scale) into following three categories: mild (13-15), moderate (9-12) and severe (below 8). It can also be classified as focal injury or diffuse injury where the former one involves the localized injury like in case of skull fracture resulting in the tissue compression beneath the cranium at the site of injury. Diffuse injuries include axonal, microvascular and neural swellings.[3] Diffuse axonal injury (DAI) mostly affects the white matter in addition to axons and nerve fibers. Traumatic microvascular injury primarily damages the small blood vessels of brain. It may be a focal or diffusely generalized injury that causes structural damage to microvasculature. Microvascular injury alters the blood flow, disrupts the blood brain barrier, causes neuroinflammation, and hemorrhage leading to deficits in the functionality such as neurocognitive deficits and post traumatic seizures.[4]

Pathophysiology of TBI

TBI is a complex process which involves temporary or permanent neuronal deficits. Primary injury or impact causes damage to axons, vessels and brain cells. This is followed by secondary events like activation of inflammatory mediators, deposition of hyperphosphorylated tau proteins and oxidative stress that ultimately progresses into mental disability. The first stage is characterized by the cerebral damage followed by excessive cell membrane permeation and edema formation.[3] The second stage involves the over-expulsion of chemical messengers like glutamate and aspartate. In addition to this, there is an activation of voltage gated Ca^{+2} and Na^{+} channels as well as activation of enzymes like proteases, phospholipases or endonucleases which lead to increased levels of fatty acids and free radicals. This altogether damage the cells causing apoptosis and necrosis[5](Fig. 1).

Following TBI, an inflammatory response starts with the activation of Adenosine Triphosphate, Heat Shock proteins and S100 proteins. They bind with the receptors by recognizing the specific patterns such as Toll-like Receptors on myeloid and dendritic cells. This leads to induction of ligands to trigger the pro-inflammatory factors like cytokines via signaling pathways or by forming or activating immune system sensors or receptors that activate pro inflammatory factors such as

IL-1β by inflammatory caspases. Activation of microglia aggravates neuronal inflammation. The reactive oxygen species (ROS) levels such as superoxide radical (O_2^-), Hydrogen Peroxide (H_2O_2), OH^- radicals are enhanced following the brain injury that causes the destruction of neurons by protein oxidation, peroxidation of lipids in membrane and DNA destruction. Brain is significantly prone to the damage by oxidation due to high lipid level, increased oxygen utilization, high metabolism and low levels of antioxidant enzymes[5]. Post traumatic injury, there is release of brain specific proteins due to injury to the neurons, axons, glial cells and blood vessels. The changes occur in the serum levels of proteins specific to brain such as S100B in astroglial cells and melanocytes, Glial fibrillary acidic protein (GFAP) in cytoskeleton of various cells in CNS astrocytes), tau proteins, Neurofilament Light (NF-L) protein and Ubiquitin C-terminal hydrolase L-1 enzyme (in repairing the neurons and axons). In the studies, it has been stated that S100B, GFAP levels are raised in serum in first 24 hours post injury tagged as acute biomarker while the levels of tau protein are found to be elevated from days to weeks tagged as sub acute biomarkers and the NF-L serum levels are raised from weeks to months post injury tagged as chronic biomarkers.[6] Thus, these biomarkers can be taken into considerations to determine the severity of the injury. Acute Amyloid β residues within the axons and diffused plaques extracellularly after TBI have been reported to cause neuronal damage and memory loss. The deposits of hyperphosphorylated Tau proteins and neurofibrillary tangles have been reported as markers of neuronal degeneration in TBI.[7]

Models for TBI

The models of TBI use the various species of rats or mice that ideally favor the similarities to humans in terms of behavioral activities, neurological functioning deficits, or underlying mechanisms. These models are discussed below (Fig. 2):

i) Fluid Percussion Injury (FPI) model

FPI uses a source of fluid to create hydromechanical pressure for injury. This fluid wave is used to induce the injury to the intact dura by performing the craniotomy, by striking the pendulum to the piston of fluid reservoir. The strength of the pressure can be directed by adjusting the height of the pendulum. A diffuse injury is induced in the midline location whereas focal injury at the lateral location. This method produces the TBI which can be marked by cerebral edema, intraparenchymal hemorrhage and injury to the neurons in cortex (Fig. 3). This has been remodeled to lateral FPI, that induces the injury into sub cortical regions for instance hippocampus and thalamus. This method is used to study stress response to TBI, focusing on Hypothalamus- pituitary- adrenal (HPA) axis.[8] This initiates the neuronal damage instantly after act and progression for 12 hours but does not expand to other regions by 7days following TBI. The alterations at the molecular level continue for longer period in the sub cortical region including septum pellucidum, thalamus, amygdala and striatum. This method is efficient as it induces the injury that mimics the condition of patients and induces the serious neurobehavioral and cognitive loss for 1year after TBI.[9] Other manifestations that have been recorded are bradycardia, hemorrhage, hypertension. FPI has high mortality rate as compared to other models due to brainstem compromised prolonged apnea. This model is used for the animals such as rabbit, cats, rats, mouse, and pig. The

mortality rate is more with the model and further increased according to the severity of the injury, with most magnitude of 3.6atm and 3.8atm levels of injury.[9]

ii) Controlled Cortical Impact (CCI) model

CCI induces traumatic brain injury by the pneumatic or electromagnetic impact device by performing craniotomy over parieto-temporal cortex. The method induces the injury with controlled velocity, depth and time. However, the impact velocity can be adjustment between the range 1.0 and 7.0 m/s.[10] The extent of injury and the speed of rod to make an impact may vary in different protocols of the experiments conducted (Fig. 4). Morganti et. al. proposed the CCI model with contusion upto 0.95 mm (from dura), speed 4.0 m/s, and the time period of 300 ms in the mice.[11] Izzy *et. al.* (2019) developed the model in mice with velocity of rod 6 m/s, depth of injury 0.6 mm, and duration of impact 100 ms.[12] It induces injury at several levels resembling the trauma of humans. Some studies have indicated that injury of less magnitude cause induce concussion like injury with acute neurological changes whereas high magnitude injury leads to chronic neurological alterations with brief structural damage.[10] The impact mimics the acute subdural hematoma, injury to axons, disruption of blood brain barrier, tissue loss in cortex region and even coma. The functional deficits that can be observed are cognitive impairments which last for 1 year and may be associated with the brain atrophy. In some reviews, it has been given the name of rigid indentation.[13] This method can be used to induce intracranial and extracranial injury in animals such as ferrets, rats, mice, swine, monkeys.

iii) Chimera- variant of CCI

Closed-head impact model of engineered rotational acceleration model, a pneumatically derived piston is used to induce flexion between cervical region of spine and angular acceleration of head. Trauma is induced under the anesthesia. This provides a compilation of cervical and accelerated head injury. This variant of CCI is preferred to induce the DAI with reactive microgliosis, release of inflammatory cytokines (TNF-α, 1-IL β) and hyperphosphorylated tau proteinopathy causing the neuromotor and behavioral deficits with possibility of cranial deformation and cervical spine injury.[4]

iv) Weight drop method

This model is concerned with the dropping of a known weight from a particular height on the exposed skull of the rat to precipitate the head injury. The method is quite useful in developing the open and closed type of head trauma, and in designing the repetitive TBI model (Fig. 5). R. Liu et. al. used the weight drop method on adult SD male rat (250-280g) by anaesthetizing maintaining the temperature using throughout the procedure. A right parietal craniotomy was performed by drilling under aseptic environment. A steel rod with flat end is allowed to fall freely from known height on the piston resting in the dura compressing the tissue of that region in brain to certain extent inducing the brain injury. The animals so far used are zebrafish, rats and mice. Marmarou et. al. developed this model also been called as impact acceleration model[8], in which he induced the injury in rats using the steel helmet on the rats head over which the plexiglass tube containing Brass weights in it to fall over the steel helmet from a predetermined height.[14] Sekar et. al.(2019) induced TBI in mice by weight drop method using about 60 g weight, 1 m vertical distance on the right side of

the head.[15] Shapira et. al. (1988) used the method in rats and dropped the rod on the head of the rats from the distance of 7 cm under the effect of the gravity.[16] The model is compatible in terms of expenditure, producibility however, is operator dependent and requires controlled forced and has chances of variations in the resulted injuries.

v) Blast Induced Traumatic Injury Model

The models that require the exposure of the animals to the blast wave mimicking the condition with the blasts occurred in the wars or in industries affecting the soldiers and people present at the places during blast. The problems encountered with the blast induced models are their cost and complications with the induction of only primary blast injury (Fig. 6). The various models to develop blast injury to cause primary blast induced injury include direct cranial blast injury,[17] or modified cranium only blast induced injury.[18]

a) Direct cranial blast injury (dCBI)

The method is based on exposing the rodents occipital region of skull to the wave created by blast with the help of COBIA (Cranium Only Blast Induced Apparatus).[17] The method is used to study abnormalities and deficits in neuromotor functioning, vestibulometer functioning, memory deficits prominently associated with microglial activation and neurodegenration in hippocampus.[17] However it is not painful procedure but still performed under anesthesia to eliminate pain after the injury.

b) Cranium only blast induced trauma in conscious rats

This is the modified design of dCBI that uses the Blast Dissipation Chamber Cranium Interface. Through this, the rats are trained to place their heads against the end of Chamber over the external occipital region for atleast 5 seconds, then the blast wave is applied that will not cause any damage to the skull, haemorrhage and skin lesions. The rats with injury in the posterior cranium are selected. The parameters such as number of seizures, forelimb and hind limb weakness by grip strength, anxiety using elevated plus maze model, spontaneous pain using rat grimace scale scoring for actions such orbital stress, folding of ears, flattening of nose and cheek, facial sensitivity to the heat.[18]

c) Primary blast injury model

This is used for inducing the diffuse injury to cause the damage via non-penetrating supersonic blast-wave impulse. This involves the high positive pressure created by the chemical reactions from the site followed by the negative pressure for longer period.[19] The complication encountered in determining the influence of blast trauma is the difficulty in creating the trauma to some organs like brain by low- level wave as compared to the air containing organs such as lungs.[20] As a consequence, the uncertain effects of primary blast makes difficult to identify the biological targets in the central nervous system. The blast injury model can be designed by production of blast waves using shock-tube in the controlled conditions with the help of compressed air or by explosion in the field[29] in which there is transmission of wave inside a cylindrical tube, in commonly used animal species include rodents, swine and primates. The released air from the compressor along cylindrical tube generates the pressure wave for precipitating the injury.[21] However, the former model

is more reproducible and more precisely controlled as compared to latter for producing the determined level of blast wave. This type of injury is also induced by the use of advanced blast simulator by generating the single pulse shock waves.[20] To eliminate the stress to the whole animal from primary blast, a new method was approached to consider the biological aspects due to primary blast culturing the cell or tissue samples isolated from the animals under controlled environment. Different types of cultures can be derived from different organs of different species to determine the impacts of blast. Though, limitations are there considering the boundary opposition due to culture vessel surfaces or walls. However, the direct influence of shock wave on the various parts of brain and its cells can be studied.[20]

vi) Repetitive TBI

This method uses the pneumatic piston cylinder as mechanical impactor to hit for defined number of times in rats, this is mostly achieved by weight drop method hence the mortality rates are more. However, it does not require any invasive procedure.[22] This includes the reduction in body weight and body growth as compared to single impact. This method will mimic chronic traumatic encephalopathy caused due to head injury several times in sports, the injury occurs at mild level with minimal or no deaths.[22] This model helps in inducing the injury to mimic the injury due to child or woman physically abused with primary hit to the head. This is responsible for the abnormalities in the neurocognitive functioning, abnormalities at the cellular level in cortex and hippocampus. These types of models provide more stress to the animals due to repetitive induction.

vii) Intracranial hypertension model

This involves the filling of autologous blood in the epidural space of animal, resembling the epidural hematoma. This results in the increased cellular apoptosis and cleaved caspases-levels in hippocampus, hypothalamus and pituitary. However, this model is not recommended much and is quite uncommon to develop.

viii) Dynamic cortical deformation

The method has its utilization in inducing the lesions by the application of vacuum pulse to the cortex region in rats approximately for 25 milliseconds performed under anesthesia mimicking the rapid tissue deformation. The device for DCD consists of a solenoid valve connected to a vacuum source, designed aluminium coupled via Leur-Lok fitting (Fig. 7). The magnitude, duration and shape of vacuum pulse is independently controlled providing the moderate injury with 4psi magnitude while severe injury with 8 psi magnitude.[23] This method produces both focal and diffused injury reported by Mathew et.al.(1996).[24] It is responsible for producing the hemorrhagic lesions, extensive gliosis. Moreover, it produces the controlled sized lesions, hence, is helpful in examining the range of lesions in the cortex with different sizes. The method has also been used by Shreiber et al.(1996) to estimate the mechanical damage that occurs to the brain regions and disruption of blood brain barrier which is primarily dependent on the magnitude of impact that has been applied and deformation of the tissue.[23]

ix) Drosophila model using high impact trauma (HIT) device

HIT uses the device to cause injury which consists of a wooden board whose one end is attached to a metal spring and another end placed over a polyurethane or Styrofoam pad. Flies anatomy is quite similar to humans such as heart, adipose tissue, a respiratory network, an excretory system, a brain with barrier having complex junctions and CNS with glial cells. So, Drosophila can be used as model for TBI for its similarities to humans and less maintenance cost.[25] The unanaesthetized dosophila are put into a standard vial of plastic nature connected to a spring and flies are forced to be at the vial's bottom inserting the cotton plug from top (Fig. 8). Deflections and movement of the spring causes the vial to come in contact with the pad, the alterations in the motions cause the head as well as full body trauma. The force to every fly is varied due to difference in the exposure to force. The immediate outcome is ataxia resembling the condition of humans suffered from traumatic injury in vehicle crash or sports.[25]

x) Sensorimotor cortex ablation model

In this model, a tissue from sensorimotor cortical region is isolated by sucking through a propylene tip causing TBI with less mortality rate and leading to cortical tissue loss and inducing various deficits in the neurocognitive functioning. P. Brkic et. al. (2012) performed the ablation by determining the atlas as 2 mm anterior, 4mm posterior to the bregma and 4mm lateral from the mid-line in rats under anesthesia. The suction was carried out to the depth of white matter. This method is quite advantageous in producing highly reproducible lesions, with limited inflammatory responses and gliosis, maximal behavioral deficits.[26] The incision is made from caudal to the eyes to caudal to the ears. By deflecting periostium, craniotomy is performed. Then the cortex is removed by sucking with a gentle negative pressure through a fine glass pipette until white matter is visible.[27]

Conclusion

The developed models provide path to test the novel ideas for treatment of any disease and it is very crucial to develop clinically relevant models. But due to ethical issues developing such models that are quite similar to humans sufferings in terms of injury, morphological, intracranial pressure or functional changes, is very challenging. Moreover, before performance of induction during experiments, many attempts have been conducted to know the defined force that could actually cause TBI with least mortality rate, due to which many animals had lost their lives for the perfection to come. So, models with no or least involvement of the application of force or pressure should be developed to avoid any unethical treatment to the animals. In case of blast injury models, the blast wave inducing procedure is quite costly, so some economical alternative methods need to be explored.

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