Pathophysiology of traumatic brain injury

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ABSTRACT

تعد إصابة الدماغ الرضية وباء يقلل من صحة الإنسان. بالرغم من الجهود المبذولة المتزايدة لمكافحة هذه المشكلة الإكلينيكية فقد فشلت العلوم الطبية والتقنية في توفير علاج فعال لإصابة الدماغ الرضية مما يجعل عشرات الآلاف من مرضى إصابة الدماغ الرضية عرضة لعواقبه الضارة. تشير الدراسات إلى أن اصابات الدماغ الرضية تكون ثنائية الطور؛ حيث تقسم الإصابات إلى اصابات أولية وثانوية. تحدث الإصابة الأولية بشكل متزامن مع حدوث مسبب الإصابة مما يفسر عدم قابلية الإصابة للعلاج. بينما تتكون الإصابات الثانوية من ردة فعل مرضية تبدأ بعد ظهور الإصابة الأولية وتؤدي إلى عجز غير ميكانيكي لهيكل العصبون ووظائفه. في هذه المراجعة قمنا بتسليط الضوء على الآليات المرضية الأساسية التي تحدث في المراحل الأولية والثانوية لإصابات الدماغ الرضية.

Traumatic brain injury (TBI) is considered an epidemic that continues to compromise the welfare of humankind. Despite the extensive efforts invested in countering this clinical health problem, current clinical science and technology still fall short of providing a pharmacological cure for TBI rendering tens of thousands of TBI patients vulnerable to its detrimental sequelae. Over the past 30 years, the understanding of the pathophysiological mechanisms of TBI indicates that the pathology of TBI is biphasic. It comprises 2 injuries; the primary and the secondary injuries. The primary injury occurs simultaneously with the impact that caused the injury, which explains why this injury is not amenable to acute intervention. Whereas the secondary injury is a composite of interwoven pathophysiological responses that commence after the initial trauma leading to delayed, non-mechanical impairment of neuronal structure and function. In this review, we aim to highlight the main pathophysiological mechanisms that take place in the primary and secondary phases of traumatic brain injury.

Neurosciences 2013; Vol. 18 (3): 222-234

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Traumatic brain injury (TBI) strikes both in industrialized and developing countries at an alarming rate, posing itself as a global health problem that is the leading cause of mortality and morbidity among individuals under the age of 45 worldwide.[1,2] Advances in emergency and intensive care medicine have substantially ameliorated the mortality rate associated with TBI, but still every year tens of thousands of people die of TBI,[3] and those who survive the injury are left to deal with its catastrophic consequences including an array of neurological and neuropsychological problems.[4] Males between the age of 14 and 24 seem to be the group most commonly affected by TBI,[5] but females, children, and older age groups are still at risk. In general, gender differences regarding TBI seem to be in favor of females. Not only females less frequently sustain TBI, but also they seem to have better outcome compared to males. It is suggested that the better outcome in females may be due to lower levels of lipid peroxidation (LP) due to the antioxidant effects of estrogen and progesterone.[6,7] The TBI is precipitated by several etiological factors that have variable significance according to age. In the geriatric population, 51% of TBI cases are caused by falls, whereas in the younger population motor vehicle accidents and to a lesser extent assaults seem to be the dominant causes of TBI. Adolescents are particularly vulnerable to concussions precipitated by sports. On the other hand, pre-adolescent children are prone as pedestrians to TBI caused by motor vehicle accidents. Children below the age of 5 are at risk of TBI as a result of falls; while most of TBI cases in infants are inflicted by child abuse.[5] This review aims to highlight the main pathophysiological mechanisms that take place in the primary and secondary phases of traumatic brain injury.

The primary injury in traumatic brain injury. The primary injury represents the diffuse, and focal mechanical damage inflicted on the brain at time of the impact. The mechanical forces generating TBI occur in a short range of time estimated to be within 100 milliseconds, but they may destroy the cranial vault and sometimes cause further damage to the cerebrovascular structures by depressing fractured bone into the brain. This will contribute to shearing of axons and blood vessels that is initiated by the impact that caused the injury.[8] Intracerebral bleeding is the result of tearing of blood vessels and hemorrhage within brain parenchyma producing mass lesions. It is a common feature in moderate and severe TBI. It is generated whenever the impact produces sudden rotations of the head leading to a gliding motion of the brain within the skull in areas where the cranial vault topography is rough (opposing the frontal and the temporal lobes).[3] Such motion will contuse the brain parenchyma in the respected regions causing hemorrhage. Some clinical signs like deterioration of patient neurological status or uncontrolled intracranial hypertension might be suggestive of intracranial bleeding.[3] Most commonly, bleeding occurs within the subarachnoid space due to hemorrhagic contusions of the brain leading to the formation of a subarachnoid hemorrhage. It occurs frequently following TBI, and is related to poor neurological recovery.[1] Rupture of the cerebrovasculature and extravasation of blood following TBI may also be manifested by the formation of hematomas. The terminology of hematomas is based on location; epidural when the blood escapes between the cranial vault and the dura, or subdural when the blood is trapped between the dura and the subarachnoid membrane overlying the brain parenchyma. The 2 types of hematomas differ in incidence, etiology, and clinical presentation. Epidural hematomas are not common, occurring in less than 1% of all cases of head injury and seem to be more likely to occur in pediatric TBI. Most of the time they result from laceration of the middle meningeal artery and they are distinguished in CT scans by their biconvex or lenticular shape that is usually located in the temporal and parietotemporal areas of the brain.[3] Though epidural hematomas may predispose to neurological deterioration, their management by prompt evacuation has a favorable prognosis.[9] On the other hand, subdural hematomas are more common as they occur in one third of severe head injuries.[10] They often result from tears in bridging veins that cross the subdural space leading to extravasation of blood that will have a distinct crescent shape on CT scans. The subdural hematoma, through engorging the cranial vault, will often contuse or compress adjacent brain parenchyma, and in severe cases may cause midline shift; an index of poor outcome.[3]

Another lesion characteristic of the primary injury of TBI is diffuse axonal injury. As the name 'diffuse' implies, this pathology exceeds the boundaries of the impact site and occurs throughout the brain. It is caused by inertial forces, generated by rotational acceleration/deceleration of the head at time of injury, which induces dynamic shear, tensile, and compressive strains that culminate in tissue deformation and damage in axonal networks deep in the brain. Such axonal damage is detected clinically by diffusion-weighted MRI. Diffuse axonal injury undermines the clinical outcome of TBI patients by escalating mortality and morbidity.[11,12] Although the trigger for this axonal damage is mechanical and considered part of the primary injury, much of the ensuing axonal damage occurs due to secondary injury mechanisms.[13]

The secondary injury in traumatic brain injury. The secondary injury is mediated by the pathophysiological responses that commence after the initial trauma leading to delayed, non-mechanical impairment of neuronal structure and function. It is believed that most of the brain dysfunction following TBI is not mainly related to the mechanical shearing of axons, but rather attributed to secondary injury mechanisms.[14] The delayed profile of secondary injury mechanisms allows potential pharmacological intervention. Secondary injury is a composite of interwoven mechanisms discussed below.

A. Role of hemodynamic and metabolic changes after traumatic brain injury. Pathological hemodynamic changes accentuate brain dysfunction mainly by inducing cerebral ischemia, which is a common complication in the majority of severe TBI cases.[15] Cerebral ischemia is related to poor outcome. and ischemic lesions are present at post-mortem in most patients who die from TBI.[16] The incidence of hypotension and hypoxia is critical for the evolution of cerebral ischemia after TBI, and is a major determinant of poor outcome.[15] Furthermore, cerebral blood flow (CBF) is reported to be approaching ischemic thresholds following TBI, and the collapse of CBF is even greater in the vicinity of contusions and epidural hematomas.[17-19] Accordingly, the hemodynamic status of TBI patients is commonly compounded by a lethal triad consisting of hypotension, deterioration of CBF, and the reported increased vulnerability of traumatized brain tissue to ischemia. Countering this triad by restoring normal blood pressure and normal cerebral perfusion pressure (CPP) to maintain CBF is crucial for TBI patient survival.[20] A closer study of the mechanics of

CBF reveals the factors responsible for its deterioration following brain injury. Under physiological conditions, CBF is proportional to CPP and inversely proportional to cerebral vascular resistance (CVR).[15] Since the latter is difficult to measure clinically, CPP is often used alone as an index of CBF. The CPP, a major determinant of CBF,[21] is defined as the pressure gradient created by the difference between the mean arterial blood pressure (MAP) and intracerebral pressure (ICP), which is the pressure within the rigid cranial vault relative to atmospheric pressure.[22] Physiological levels of ICP are <10 mm Hg. What often happens after severe TBIs is that CPP plummets due to a dual insult. First, the MAP is severely decreased as a manifestation of hypotension in most severe and sometimes moderate TBI patients. Second, ICP rises dramatically (intracranial hypertension) frequently after severe TBI. Intracranial hypertension following TBI is mediated by a plethora of causes including, extravasations of blood from cerebral blood vessels, vasogenic edema due to disruption of the blood-brain barrier (BBB) resulting in extravasation of fluids in brain extracellular space and cytotoxic edema due to accumulation of intracellular fluids.[23,24] Elements of the inflammatory response like microglial activation, leukocyte and lymphocyte migration, and production of vasodilatory mediators may make a prominent contribution to the formation of cerebral edema and hence contribute to intracranial hypertension following TBI.[25,26] The drop in MAP and the surge in ICP jointly causes a massive reduction in CPP. Under physiological circumstances, fluctuations of CPP within the range of 40 to 140 mm Hg are compensated by changes in vascular resistance to maintain a stable CBF sufficient to protect the brain against ischemic insults.[27] This physiological compensatory phenomenon is referred to as cerebrovascular 'autoregulation'. Basically, cerebrovascular autoregulatory mechanisms operate by inducing either vasoconstriction or vasodilatation in the cerebral microvessels to couple CBF to tissue metabolic demands and makes it insensitive to oscillations in CPP.[28,29] However, after severe or moderate TBI, cerebrovascular autoregulation is typically compromised and inadequate to maintain the coupling of CBF to brain metabolic demand.[21] The autoregulatory failure makes CBF exclusively sensitive to changes in CPP, which usually deteriorates after TBI. In summary, the drop in CPP and the lack of compensatory mechanisms can lead to deterioration of CBF precipitating ischemia in the traumatized brain tissue. These ischemic changes are aggravated in many severe or moderate cases of TBI by the incidence of constriction of the major cerebral vessels ("vasospasm"), which further compromises cerebral perfusion.[2] These ischemic changes are further complicated with cerebral edema. Cerebral edema arises as a result of the disruption of ionic gradients, which are accompanied by impairment of oxidative phosphorylation. This will increase the reliance on glycolysis to generate adenosine triphosphate (ATP) leading to accumulation of lactate.[5] High levels of lactate predispose for neuronal dysfunction by causing acidosis, membrane damage, disruption of the blood brain barrier, and cerebral edema. The compromise of energy production to fuel ATP- driven ionic pumps accentuates ionic imbalance and can worsen cerebral edema.[5] Edema contributes to very serious complications after TBI including distorting brain tissues, elevated intracranial pressure, vascular compression, and a likely fatal consequence, brain herniation.[15]

B. Role of excitotoxicity after traumatic brain injury. The term "excitotoxicity" entails the release of excitatory amino acids in synaptic and extra synaptic regions (interstitial space) to the point they act as neurotoxins that can potentially kill nerve cells.[30,31] Excitotoxicity is a critically important neurodegenerative mechanism among most, if not all, acute and chronic neurodegenerative disorders including brain trauma,[30,31] and contributes to both necrotic and apoptotic neuronal cell death mechanisms.[32,33] Indeed, excitotoxic mechanisms are central to the secondary injuries that ensue following TBI.[34] These excitotoxic events are triggered by the release of excitatory amino acids, mainly glutamate, after TBI.[35] Excitotoxic mechanisms, initiated following TBI, feature persistent depolarization of neuronal cells accompanied by ionic influxes disrupting ionic balance across neuronal cell membranes.[35] Attempts of injured cells to restore normal ionic gradients increase brain metabolic demand for production of enough ATP to fuel the ATPases responsible for restoring ionic homeostasis. However, the ability to generate more ATP in the cell is incapacitated by the lack of adequate CBF, the reduction in tissue oxygenation and the progressive compromise of mitochondrial function following TBI.[14] Because of that, the excitotoxicity-induced ionic imbalance persists in the injured neural tissue and ultimately culminates in neuronal cell damage and death.[36]

Perhaps the most devastating element of excitotoxicity is massive calcium (Ca^{++}) influx. Loss of Ca^{++} homeostasis triggered by excitotoxic mechanisms will lead to axonal damage and culminate in neuronal cell death.[37] Disrupting Ca^{++} homeostasis in the cell is the central mechanism by which excitotoxicity contributes to downstream pathological events in the secondary injury of TBI like oxidative stress, mitochondrial

dysfunction, and cytoskeletal degradation.[15,38] In addition to that, influxes of sodium (Na+) seems to contribute to excitotoxic cell damage by mediating passive diffusion of water into neuronal cells causing cytotoxic edema.[39,40] Evidently, the initial insult after TBI is amplified in both expanse and severity by means of excitotoxic mechanisms.

Glutamate-mediated excitotoxicity is suggested as the main contributor to secondary injuries after TBI as glutamate is the most abundant and most rapidly released excitatory neurotransmitter in the brain following injury.[35,41] In addition to being released in synapses, glutamate has been also shown to accumulate in extrasynaptic extracellular spaces in both experimental and clinical TBI settings. The high levels of extracellular glutamate appear to potentiate excitotoxicity by depolarizing neighboring cells.[42] The potential sources of this increase in extracellular glutamate are: the immense exocytosis of glutamate into synaptic clefts, triggered by mechanical depolarization of glutamate-releasing neurons after TBI, may result in a flood of glutamate into brain synaptic and extra-synaptic spaces,[42] the disruption of the BBB after TBI is proposed to increase the entry of glutamate into the brain,[42] shearing of cerebral blood vessels leads to parenchyma hemorrhage around the impact site with concomitant extravasation of glutamate into the lesion site, and the possible formation of membrane micropores after injury is suggested to allow glutamate entry into the extracellular space.[42]

The levels of glutamate in synaptic and extrasynaptic spaces are mainly regulated by release and reuptake mechanisms because glutamate is not metabolized by extracellular enzymes.[43] Accordingly, disruption of glutamate transporters activity is expected to play a role in accumulation of glutamate in extracellular spaces leading to excitoxicity. There are 2 types of glutamate transporters; neuronal and astrocytic.[43-46] Compelling evidence suggests that most of the glutamate transport activity in the brain is carried out by astrocytic glutamate transporters; hence, their malfunction has a critical role in excitotoxicity.[47-49] Indeed, following experimental TBI, it has been documented that there is down-regulation of both astrocytic glutamate transporters levels in the injured cerebral cortex.[50] Down-regulation of astrocytic glutamate transporters is also evident after human TBI.[51] This decrease in astrocytic glutamate transporter activity has been shown to correlate with high levels of glutamate in the CSF after injury.[52] Moreover, hindering the expression of astrocytic glutamate transporters significantly aggravated neuronal loss after injury.[42] In conclusion, it is plausible to suggest that disruption of glutamate transport mechanisms contribute to build up of extracellular glutamate and subsequent glutamate-mediated neurotoxicity.

The trauma-induced disruption of glutamate release and glutamate transport mechanisms will lead to high levels of glutamate in extracellular spaces. This accumulated extracellular glutamate will propagate neurotoxicity by overstimulating glutamate receptors leading to downstream effects that put cell survival at risk. There are 2 main categories of glutamate receptors, ionotropic glutamate receptors, and metabotropic glutamate receptors. Ionotropic receptors are of 3 types: the NMDA receptor, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, and the kainate receptor.[53] The latter 2 receptors are not voltage-dependent and are mainly permeable to Na^+, potassium (K^+) and to a much lesser extent to Ca^{++}.[54,55] In contrast, the NMDA receptors are of particular interest because they are voltage-dependent ion channels that are preferentially permeable to Ca^{++},[56] and to a lesser extent Na^+. Such inherent qualities of the NMDA receptor suggest a significant involvement in the evolution of excitotoxicity following TBI. This stems from the knowledge that dramatic surges in intracellular Ca^{++} mediate much of the pathology ascribed to excitotoxicity in the CNS.[57] Many reports have confirmed that Ca^{++} dysregulation occurs following NMDA receptor activation of cultured neurons.[58-60] The important role of extracellular Ca^{++} overload in excitotoxicity was first validated by Choi and colleagues[39,40] who showed that glutamate-mediated neurotoxicity and death of cultured neurons were contingent on the presence of extracellular Ca^{++}. The role of NMDA receptors in excitotoxicity was further validated when it was shown by Tymianski[58] that Ca^{++} influx via NMDA receptors was neurotoxic to cultured neurons whereas influxes of Ca^{++} through other voltage-sensitive Ca^{++} channels were not equivalently catastrophic. It was hypothesized that the route of Ca^{++} influx will determine the destined subcellular localization of Ca^{++} and the signaling mechanisms triggered by its entry to the cell. Accordingly, the role of NMDA receptors in glutamate-mediated excitotoxicity is not only allowing influxes of Ca^{++} upon activation, but also linking Ca^{++} influxes to signaling cascades essential for downstream neurotoxicity. Some of these downstream neurotoxic effects are: mitochondrial dysfunction, igniting cellular enzymes like protein kinases, nitric oxide synthase, calpains and other proteases, calcineurins and endonucleases.[61] The activity of these enzymes will in turn boost the generation of free radicals, risk the integrity of the cytoskeleton, damage the DNA and activate cell death pathways.[61]

Metabotropic glutamate receptors (mGluRs) are also involved in mediating excitotoxicity. They are G-protein coupled receptors[62] and are classified into 3 groups; Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3), and Group III (mGluR4, mGluR6 mGluR7, and mGluR8).[63] Only Group I mGluR agonists were shown to contribute to excitotoxicity by disrupting Ca^{++} homeostasis[64,65] and boosting necrotic cell death. Interestingly, Group I mGluR agonists were also shown to mediate their excitotoxic detrimental affects by causing down-regulation of astrocytic glutamate transporters.[66]

Role of oxygen free radicals after traumatic brain injury. Generation of oxygen free radicals following TBI is one of the most confirmed aspects of secondary injury to brain tissues. By definition, a free radical is any chemical species capable of independent existence and containing one or more unpaired electrons.[67] Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which each includes both free radicals and compounds that can generate free radicals are produced by a plethora of pathways triggered after TBI, **Figure 1**. These reactive species are involved in the pathogenesis of post-TBI by inflicting damage to cerebrovascular tissues mainly by inducing membrane LP.

Superoxide radical is one of the primary radicals generated almost immediately following TBI.[68,69] A variety of sources contribute to superoxide radical ($O_2^{\bullet-}$) generation after TBI, each of which involves the one electron reduction of molecular oxygen (O_2 + $e^- \rightarrow O^{2\bullet-}$). For example, $O_2^{\bullet-}$ can be generated by the Ca^{++} induced activation of phospholipases and the downstream arachidonic acid cascade, conversion of xanthine dehydrogenase to xanthine oxidase, and by mitochondrial leak. In addition, enzymatic and/or autoxidation of biogenic amine neurotransmitters and the oxidation of extravasated hemoglobin may also be sources of $O_2^{\bullet-}$ after TBI. Infiltrating inflammatory cells including activated microglia, neutrophils, and macrophages are also additional sources of $O_2^{\bullet-}$.[70] The potential toxicity of $O_2^{\bullet-}$ can be halted by dismutation, which is an innate antioxidant mechanism that entails the conversion of $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2) in a reaction that is catalyzed by the enzyme superoxide dismutase.[71] However, $O_2^{\bullet-}$ can contribute to generation of more reactive free radicals like the hydro peroxyl radical (HO_2.) which is more lipid soluble and a more powerful oxidizing agent.[72] In aqueous environments, $O_2^{\bullet-}$ actually exists in equilibrium with $HO_2^{\cdot}$. However, under the acidic conditions that prevail in the injured neural tissue, the equilibrium between $O_2^{\bullet-}$ and HO_2. shifts in favor of $HO_2^{\bullet}$, which is much more reactive than $O_2^{\bullet-}$, particularly toward lipids.

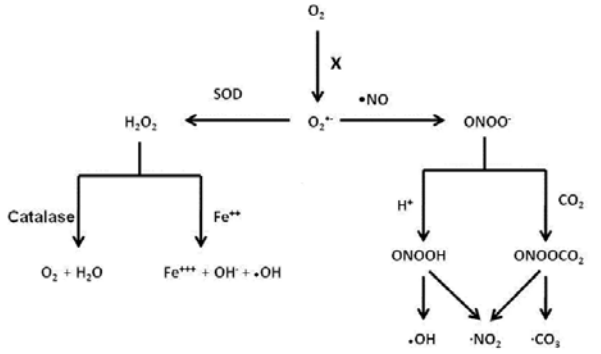

Figure 1 - Formation of reactive oxygen species and reactive nitrogen species in the injured brain. O_2 - molecular oxygen, $O_2^{\bullet-}$ - superoxide anion, $ONOO^-$ - peroxynitrite, •NO - nitric oxide, SOD - superoxide dismutase, H_2O_2 - hydrogen peroxide, Fe^{++} - ferrous iron, Fe^{+++} - ferric iron, ONOOH - peroxynitrous acid, $ONOOCO_2^-$ - nitrosoperoxocarbonate, •OH - hydroxyl radical, •CO3, carbonate radical. X stands for: xanthin oxidase activity, hemoglobin, mitochondrial leak, microglial activation, and arachidonic acid activation.

Iron is abundant in the CNS, and is in part responsible for the increased susceptibility of the brain to oxidative damage, particularly LP.[73] Because it is a strong inducer of LP, iron is tightly regulated in the neural tissue under physiological conditions. Nonetheless, following TBI iron homeostasis is disrupted by acidosis and hemorrhage. Acidosis increases iron solubility and mediates its delocalization from an inactive to an active redox state.[74,75] Moreover, extravasated hemoglobin as a result of rupture of cerebral blood vessels and hemorrhage is another source of iron. Hemoglobin itself is capable of inducing oxidative damage by stimulating $O_2^{\bullet-}$ production, but more importantly hemoglobin-mediated oxidative damage is inflicted by iron within the released heme complex.[76,77] Ultimately, the released iron following TBI contributes to the generation of $O_2^{\bullet}$ when ferrous iron undergoes autoxidation. Released iron also contributes to the production of hydroxyl radical (•OH) by means of the Fenton reaction in which ferrous iron is oxidized by its reaction with H_2O_2. The hydroxyl radical is an extremely aggressive oxidant that can attack most biological molecules. The relevance of •OH in TBI pathogenesis was revealed using the salicylate trapping method, which captured an early surge of •OH levels in the brain following both focal and diffuse TBI.[78,79] The increase in •OH levels immediately preceded an increase in LP and disruption of the BBB following TBI.[80] The cerebral microvasculature seems to be the initial source of •OH production.[68,80]

Peroxynitrite (PN; $ONOO^-$) is a free radical donor that has been suggested to be the principal reactive species involved in producing tissue injury in a variety of neurological disorders.[81] It is the result of $O_2^{\bullet-}$ reaction with nitric oxide (•NO), the latter produced by nitric oxide synthase (NOS).[82] Both $O_2^{\bullet-}$ and •NO are not very reactive radicals and their interaction with biological molecules is limited in aqueous environments, but their chemical union leads to the formation of unstable intermediates that decay to yield yet more reactive free radicals. Specifically, the reaction between $O_2^{\bullet-}$ and •NO forms the peroxynitrous anion ($ONOO^-$), which is largely protonated at physiological pH levels to form peroxynitrous acid (ONOOH), a rather unstable compound that decomposes to give •OH and nitrogen dioxide ($\bullet NO_2$) radicals. Peroxynitrite anion can also react with carbon dioxide (CO_2) leading to the formation of nitrosoperoxocarbonate ($ONOOCO_2$), which readily decomposes into $\bullet NO_2$ and another highly reactive radical, the carbonate radical ($\bullet CO_3$). Collectively, the decomposition of peroxynitrite yields •OH, $\bullet NO_2$ and $\bullet CO_3$ radicals. The relatively long half-life of peroxynitrite and its ability to diffuse across cellular membranes is thought to explain the incidence of oxidative damage in areas distant to peroxynitrite synthesis sites. Each of the PN-derived radicals (•OH, $\bullet NO_2$, and $\bullet CO_3$) is capable of abducting an electron from a hydrogen atom bound to an allylic carbon in polyunsaturated fatty acid (LH) leading to LP cellular damage,[83] or reacting with susceptible amino acids (for example, lysine, cysteine, arginine) causing protein carbonylation.[84] Moreover, $\bullet NO_2$ can nitrate the 3 position of tyrosine residues in proteins, which leads to the formation of 3-nitrotyrosine (3-NT); a specific biological marker of PN-induced oxidative damage. Peroxynitrite-mediated protein nitration can be augmented by lipid peroxyl or alkoxyl radicals, which are capable of mediating the initial oxidation of a tyrosine moiety,[85] a rather critical event that sets the stage for nitration by $\bullet NO_2$.

Currently, several lines of evidence indicate that PN has a critical role in the pathogenesis of secondary injury of TBI. The increased activity of all 3 NOS isoforms following TBI shed light into the possible formation and involvement of PN in the secondary injury of TBI.[86-88] That notion was supported by the emergence of biochemical footprints of PN-mediated damage in rodent TBI paradigms including an increase in 3-NT levels,[89,90] and adenosine diphosphate ribosylation. Moreover, the isolation of mitochondrial NOS suggested that PN is also the main ROS generated in mitochondria.[91] The involvement of PN in TBI pathogenesis was further validated in recent work by Deng-Bryant et al[92] who showed that scavenging peroxynitrite derived free radicals, by the nitroxide drug tempol, ameliorated the accumulation of 3-NT in injured brains and concomitantly improved neurological recovery.

Lipid peroxidation is one of the most frequently studied mechanisms of oxidative damage in the neural tissues following TBI. The abundance of polyunsaturated fatty acids[79] and high iron contents in the brain[73] put neuronal cell membranes in the brain at increased risk of lipid peroxidative damage. High levels of LP are a consistent finding following TBI in humans and seem to be proportionate to injury severity.[93] The process of LP is triggered in neuronal cell membranes by a plethora of ROS including PN-generated free radicals,[83] **Figure 2**. Initiation of LP occurs when a reactive radical species such as ·OH reacts with an allylic carbon (carbon surrounded by adjacent double bonds) and extracts a hydrogen (and its single electron) from a LH. In the process, the initiating radical is quenched by receipt of the hydrogen electron from the LH. This, however, converts the LH into a lipid or "alkyl" radical (L•). This "initiation" phase sets the stage for a series of "propagation" reactions, which begin when the alkyl radical reacts with molecular oxygen creating a lipid peroxyl radical (LOO•). The LOO• then reacts with a neighboring LH within the cell membrane and steals its electron forming a lipid hydroperoxide (LOOH) and a second alkyl radical. The formation of lipid

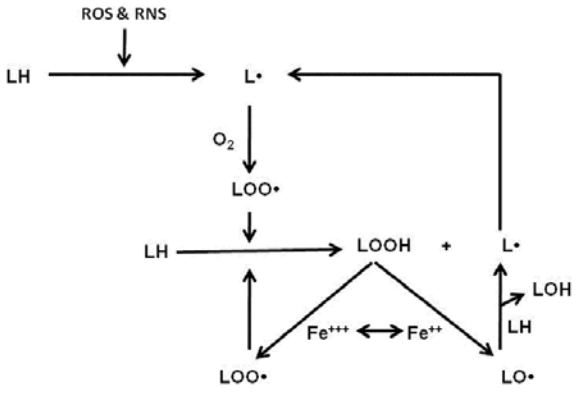

Figure 2 - A flow chart of free radical induced iron catalyzed lipid peroxidation process in polyunsaturated fatty acids (LH). ROS - reactive oxygen species, RNS - reactive nitrogen species, O_2 - molecular oxygen, L• - lipid alkyl radical, LO• - lipid alkoxyl radical, LOH - lipid alcohol, LOO• - lipid peroxyl radicals, LOOH - lipid hydroperoxide, Fe^{++} - ferrous iron, Fe^{+++} - ferric iron.

hydroperoxides is critical for propagating LP since the propagation phase of LP involves decomposition of LOOH through reactions with either ferrous iron (Fe^{++}) or ferric iron (Fe^{+++}). In the case of Fe^{++}, the reaction results in the formation of a lipid alkoxyl radical (LO•). If, however, the reaction involves Fe^{+++}, the LOOH is converted back into a lipid peroxyl radical (LOO•). Both reactions of LOOH with iron have acidic pH optima causing them to be augmented by tissue acidosis. Either alkoxyl (LO•) or peroxyl (LOO•) radicals arising from LOOH decomposition by iron can initiate so called lipid hydroperoxide-dependent LP resulting in chain branching reactions;

$$LOO\bullet + LH \rightarrow LOOH + L\bullet$$
$$LO\bullet + LH \rightarrow LOH + L\bullet$$

Ultimately, the LP process is terminated by fragmentation or scission reactions that liberate neurotoxic aldehydes like 4-hydroxynonenal (4-HNE) from cellular membranes. The LP-derived 4-HNE produces neurotoxicity by binding to basic amino acids such as lysine or histidine as well as sulfhydryl-containing cysteine residues in cellular proteins. The resulting chemical modifications, which have been shown to inhibit the function of a variety of structural and enzymatic cellular proteins, are implicated in neuronal degeneration.[94] Moreover, 4-HNE has been shown to interact with nucleic acids forming DNA adducts that increase the risk of mutations.[95]

Compelling evidence suggests that lipid peroxidative damage contributes to cytosolic Ca^{++} toxicity following TBI by several mechanisms, **Figure 3**. First, it has been documented that accumulation of 4-HNE inhibits astrocytic glutamate transporters,[96,97] an effect that can potentially extend glutamate-mediated neurotoxicity hence prolonging NMDA receptor-mediated Ca^{++} influxes. Secondly, lipid peroxidative damage has been also shown to contribute to Ca^{++} dysregulation by damaging the Ca^{++} ATPase in the cell membrane rendering the cell unable to restore ionic homeostasis.[98] Thirdly, LP has been also reported to mobilize Ca^{++} from intracellular stores like the endoplasmic reticulum.[99] Fourthly, the incidence of LP in mitochondrial membranes contributes to mitochondrial dysfunction,[100,101] which compromises mitochondrial capacity to sequester Ca^{++}.[100,102] Accordingly, lipid peroxidative damage aggravates loss of Ca^{++} homeostasis, which is a central mechanism of neuronal cell injury following TBI.

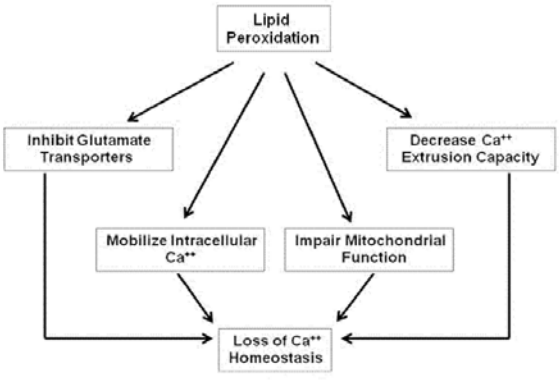

Figure 3 - Simplified representation of lipid peroxidation contribution to downstream neurotoxic mechanisms leading to dramatic increase in cytosolic Ca^{++}, a prominent pathology of the secondary injury following traumatic brain injury.

Role of mitochondrial dysfunction after traumatic brain injury. Mitochondria are membrane-bound intracellular organelles. They are bound by 2 membranes; the outer membrane is permeable for ions and small molecules whereas the inner membrane is almost impermeable. The inner membrane houses the electron transport chain (ETC) protein complexes and is also equipped by several ion channels and transporters including the Ca^{++} uniporter, K^{+} ATPase channels and Na^{+}/Ca^{++} exchanger. Since one of the most important tasks of mitochondria is the production of ATP, they are often referred to as the "powerhouse" of the cell. In a typical vertebrate cell, almost 95% of the ATP demand is generated by mitochondria.[103] Neurons, particularly, seem to be highly dependent on sound mitochondrial function to support the metabolic demands of membrane excitability, which is essential for impulse conduction, neurotransmission, and other processes like plasticity.[104,105] Maintaining ionic gradients and neurotransmission creates a huge demand on ATP by neuronal cells, which rely on oxidative phosphorylation carried out in mitochondria to meet ATP demand. Accordingly, mitochondrial function is crucial for neuronal survival,[106] and aberrant mitochondrial function are often involved in neurodegeneration.[107]

Mitochondrial damage is a prominent pathology after TBI. Experimental studies carried out with electron microscopy have revealed that cristae membranes are destroyed in mitochondria isolated from traumatized brains.[108] Moreover, traumatized mitochondria are swollen with signs of discontinuity in their membranes.[102,109] With the use of antibodies against specific markers of oxidative damage, it was proved that the structural dis-integrity in mitochondria was accompanied by high levels of LP. Experimental work on isolated mitochondria from rodents has

revealed that controlled cortical impact TBI induces high levels of 4-HNE and 3-nitrotyrosine (3-NT), markers of LP and protein nitration.[102] Proteomic analytical data have confirmed that most of the proteins within the ETC complexes, which are central for ATP synthesis, are targeted by post-traumatic oxidative damage.[110] This is coincident with the impairment of oxidative metabolism and depletion of ATP stores that take place following TBI.[111-114] Obviously these catastrophic changes in energy metabolism are ascribed to mitochondrial dysfunction, which ensues following TBI.[115-118] Mitochondrial dysfunction in neuronal cells seems to be precipitated by glutamate neurotoxicity.[36] Glutamate release following TBI induces an immense intracellular Ca^{++} accumulation as a result of NMDA receptor activation.[57] This, in turn, is accompanied by overloading mitochondria with Ca^{++},[119,120] which put mitochondria at risk of further damage. Such Ca^{++} perturbation aggravates mitochondrial dysfunction and energy failure following TBI.[121,122] Moreover, the resulting mitochondrial Ca^{++} load has been shown to induce mitochondrial generation of ROS, RNS, and their highly reactive free radicals.[123-125] Thus, Ca^{++}-loaded neural mitochondria become both the main source and target of these free radicals, which initiate mitochondrial membrane LP and protein modifications resulting in irreversible loss of mitochondrial functions such as mitochondrial respiration, oxidative phosphorylation, and ion transport,[126,127] and decreasing mitochondrial Ca^{++} buffering capacity.[102] However, the most devastating sequelae of Ca^{++} load on mitochondria is the induction of mitochondrial permeability transition pore (mPTP) in the inner mitochondrial membrane and dumping of the matrix Ca^{++} pool back into the cytoplasm.[106] The mPTP is a sudden increase in the inner mitochondrial membrane permeability allowing solutes of molecular mass less than 1500 Daltons to pass freely across the inner mitochondrial membrane.[128] This mitochondrial collapse amplifies the rise in cytosolic Ca^{++} and contributes to delayed Ca^{++} dysregulation.[120,129] The formation of the mPTP appears to be vital for Ca^{++}-induced generation of free radicals by mitochondria.[125] It is accompanied by a drastic collapse of the mitochondrial membrane potential, an event that will not only abolish ATP synthesis by mitochondria but may also lead to functional reversal of the ATP synthase.[120] This will exacerbate energy failure by depleting the cytoplasmic pool of ATP.

Mitochondrial dysfunction in general is implicated in neuronal cell death following TBI.[118,130] It is suggested that the status of mitochondrial functions determines the mode of cell death, necrotic versus apoptotic following glutamate neurotoxicity.[36] Elements of oxidative stress that are induced by the formation of mPTP following injury seem to pave the way for apoptosis by enhancing cytochrome c release from mitochondria.[131] Mitochondrial dysfunction and the formation of mPTP contributes to post-TBI neurodegeneration as revealed by studies employing inhibitors of mPTP like cyclosporine A and it's non immunosuppressive analogue NIM811, which have been proved to preserve mitochondrial function,[109,118] and ameliorate neurodegeneration in controlled cortical impact-TBI models.[132-134]

C. Role of calpain-mediated proteolysis after traumatic brain injury. Calpains are Ca^{++} dependent cysteinyl/thiol intracellular proteases that function at neutral pH,[135] and are believed to be important mediators of cellular damage following TBI.[136] Even though the physiological significance of calpains has not been fully elucidated, some reports suggest they play a role in synaptic plasticity and cytoskeletal protein turnover.[137,138] The dependence of calpains' activity on increased cytosolic Ca^{++} suggests that conditions manifesting dramatic increase in intracellular Ca^{++} will feature calpain activity. Indeed, it is a strong consensus now that cytosolic Ca^{++} overload is a key pathology in experimental TBI and is considered a central destructive pathway leading to delayed neuronal cell damage and cell death after TBI.[139,140] Accordingly, igniting calpain activity could be one possible mechanism to explain the detrimental effects of intracellular Ca^{++} overload in experimental TBI. Calpains' activity was detected in TBI models by tracing their favorite substrates; the cytoskeletal proteins like spectrin, neurofilament, and MAP2 proteins.[141] Interestingly, it was shown by several studies that each of the aforementioned proteins was subjected to degradation in TBI models.[141] More robust evidence for calpains activity following TBI was obtained by using antibodies against cytoskeletal breakdown products unique to calpains since degradation of cytoskeletal proteins can be mediated by other cellular proteases. Spectrin breakdown products (SBDPs) have been extensively utilized for that purpose.

Spectrin is an integral component of the cytoskeleton, especially in axonal membranes and presynaptic terminals.[142] Calpain-mediated degradation of spectrin lead to the formation of breakdown products of 2 distinctive molecular weights; 150 kDa and 145 kDa,[143] which are considered footprints of calpain activation.[144-146] Both calpain activation (autolysis) and calpain-mediated proteolysis of spectrin occur in experimental TBI[147] earlier than significant cell loss.[148] Studies carried out with a rat TBI model[149] suggested

that calpain-mediated spectrin degradation ultimately culminates in overt damage to the cytoskeleton leading to irreversible axonal damage and probably axotomy. Several studies highlighted the value of SBDPs as a convenient marker of calpain activity[144-146] that can be easily detected by western blotting. Furthermore, the reliability of SBDPs as potential biomarkers of calpain activity has increased over the years as it was shown that the level of SBDPs can be a convenient predictor of outcome after trauma,[93,133,145] not only in experimental TBI but also in clinical TBI.[150] Recently, it has been shown by a clinical database study that calpain-mediated SBDPs in CSF correlated with longer elevations of intracranial pressure and poorer Glasgow coma scale sores,[151] suggesting their pathophysiological relevance.

The validation of both calpain's contribution to secondary injuries after TBI and the value of SBDPs as a reliable footprint of calpain activity were followed by several studies aiming at defining the role of calpains in the pathophysiology of TBI and elucidating the therapeutic promise of targeting calpains after injury. Perhaps the best strategy that was employed in this regard was to use calpain inhibitors and evaluate their ability to enhance the outcome in experimental TBI models. Many studies were conducted employing calpain inhibitors to elucidate their therapeutic potential. For example, work carried out with a rat mechanical compression TBI model showed that arterial infusion of calpain inhibitor II protected the cytoskeleton by impeding degradation of spectrin and neurofilament.[152] Moreover, the calpain inhibitor SNJ-1945 significantly reduced spectrin degradation in the mouse TBI model.[153] In addition to protecting the cytoskeleton, calpain inhibitors were also shown to enhance functional recovery after injury. Studies carried out in a rat TBI model by Saatman et al[154] revealed that the calpain inhibitor AK295 attenuated both motor and cognitive deficits after injury. Subsequently, it was shown in a mouse diffuse TBI model that the calpain inhibitor SJA6017 can improve motor functional recovery.[155] In conclusion, a huge body of literature supports the notion that calpains are key mediators of the pathophysiology of secondary injury after TBI. Furthermore, its activation by increased intracellular Ca^{++} is implicated in axonal damage and neuronal cell loss leading to functional impairment after TBI suggesting that a therapeutic strategy that halts calpain activation after injury may confer neuroprotection.

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