Functions and mechanisms of cytosolic phospholipase A\textsubscript{2} in central nervous system trauma

Hao-Jie Zhang\textsuperscript{1,2,3,4}, Yi-Tuo Chen\textsuperscript{1,2,3,4}, Xin-Li Hu\textsuperscript{1,2,3}, Wan-Ta Cai\textsuperscript{1,2,3}, Xiang-Yang Wang\textsuperscript{1,2,3}, Wen-Fei Ni\textsuperscript{1,2,3,4}, Kai-Liang Zhou\textsuperscript{1,2,3,4}

https://doi.org/10.4103/1673-5374.346460

Date of submission: November 28, 2021
Date of decision: January 8, 2022
Date of acceptance: March 16, 2022
Date of web publication: June 2, 2022

From the Contents

Introduction ........................................................................................................................................ 258
Overview of Cytosolic Phospholipase A\textsubscript{2} .............................................................................. 259
Upstream of Cytosolic Phospholipase A\textsubscript{2}, Activation .............................................................. 259
Involvement of Cytosolic Phospholipase A\textsubscript{2} in Traumatic Brain Injury and Spinal Cord Injury 260
Potential Mechanisms of Cytosolic Phospholipase A\textsubscript{2} in Traumatic Brain Injury and Spinal Cord Injury ...................................................................................................................... 261
Anti-Cytosolic Phospholipase A\textsubscript{2} Therapies in Central Nervous System Trauma ......................... 262
Lisbon Declaration .......................................................................................................................... 263
Conclusion and Perspectives ........................................................................................................... 264

Introduction

Central nervous system (CNS) trauma, including traumatic brain injury (TBI) and spinal cord injury (SCI), is a leading cause of long-term disability and death worldwide (No authors listed, 2019a). The global burden of disease collaborator group study reported 0.93 million SCI cases and 27.08 million new TBI cases worldwide, while their global prevalence reached 27.04 million and 55.50 million, respectively (No authors listed, 2019b). To date, despite obvious clinical needs, no therapy has significantly improved the long-term rehabilitation outcomes after CNS injury. This may partially reflect an incomplete understanding of the complicated pathobiological mechanisms.

At present, we have knowledge of two events that occur during damage from CNS trauma: the primary mechanical injury involving direct mechanical tissue damage and secondary damage mediated by diverse pathogenic processes such as neuroinflammation, free radicals, calcium overload, and glutamate excitotoxicity (Hall, 1989; Saghazadeh and Rezaei, 2017; Gong et al., 2020). Overlapping boundaries exist between the effects mediated by these factors and other causal links driving the secondary injury (David and López-Vales, 2021). Among these, neural inflammation is an obvious characteristic of the reaction to central nervous system trauma (López-Vales and David, 2019). In addition, it is a vital marker of a variety of neurodegeneration illnesses where inflammatory events facilitate pathological development (Stephenson et al., 2018). CNS trauma-induced inflammatory reactions are intricate and revealing the causal links regulating such inflammatory events is pivotal for the purpose of developing valid therapies (David and López-Vales, 2021).

Cytosolic phospholipase A\textsubscript{2} (cPLA\textsubscript{2}), a key target of inflammatory response, is involved in neuroinflammation in SCI and TBI. Early studies have proven that it exists in both spinal cord and brain neurons (Bonventre, 1996; Ong et al., 1999a). Besides, collective evidence from many recent studies suggests that not only increased cPLA\textsubscript{2} activity but also cPLA\textsubscript{2}-generated mediators play an important role in acute inflammatory responses in the CNS (Farooqui et al., 2006). Biologically active lipids are a large family of multifunctional mediating factors for inflammatory events deserving extreme attention, and they involve prostaglandins and associated eicosanoids known to be predominant inflammatory modulators. Certain enzymes are required to transform aliphatic acids related to the cellular membrane into eicosanoids and the rest of the biologically active lipidic mediating factors (David and López-Vales, 2021). As the first enzyme in such processes, cPLA\textsubscript{2} can, in a preferential way, hydrolyze membranous phosphatides at the sn-2 position to produce lysophospholipids and arachidonic acid (AA) that contribute to various aspects of neuroinflammation through cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) pathways after CNS trauma (Li et al., 2019; Sarkar et al., 2020). Under physiological conditions, cPLA\textsubscript{2} participates in a variety of significant cellular responses, including phospholipid metabolism, signal transduction, and membrane remodeling (Kita et al., 2019; Wang et al., 2021; Hayashi et al., 2022). Nevertheless, under pathological conditions, elevated cPLA\textsubscript{2} activity and excessive free aliphatic acids including AA, proinflammatory mediators, and platelet-activating factor (PAF) might damage lysosome membranes and cause neuroinflammation and oxidative stress (Chuang et al., 2015).

In general, as a rate-limiting step of the inflammatory response, activated cPLA\textsubscript{2} will induce the production of more inflammatory factors, creating a cascade effect that promotes the progression of inflammation. Thus, as the critical component of signal transduction in the inflammatory response, cPLA\textsubscript{2} vitally influences TBI and SCI pathogenesis (Chao et al., 2018; Stewart et al., 2021). Although an increasing number of studies have confirmed the involvement of cPLA\textsubscript{2} in TBI and SCI pathogenesis, few have summarized the association between CNS trauma and cPLA\textsubscript{2}. In this paper, we have specifically highlighted the effects of cPLA\textsubscript{2} and its downstream products on inducing inflammatory events after SCI and TBI. We have also summarized the latest understanding of cPLA\textsubscript{2} in CNS trauma, with special focus on the potential effects of cPLA\textsubscript{2} in the mediation of secondary damage and the potential therapeutic prospects of cPLA\textsubscript{2}-related inhibitors.
Retrieval Strategy

Literature review was electronically performed using PubMed database. The following keywords were used to initially select studies that were located and evaluated: cPLA2; domain or structure; traumatic brain injury; spinal cord injury; mechanism or function; MAPK or calcium influx or upstream; brain or spinal. More than half of the selected studies were published from 2017 to 2022. We screened the abstract and full title and excluded studies that reported non-cPLA2-related experiments and reviews (Figure 1).

Overview of Cytosolic Phospholipase A₂

Phospholipase A₂ (PLA₂) is a family of enzymes with glycerophospholipid decomposition activity. By catalyzing the hydrolysis of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in the sn-2 position of the cell membrane, PLA₂ can produce free aliphatic acids and lysophospholipids, which act as lipidic secondary messengers (Peng et al., 2021). The physiological functions of PLA₂ include the transformation of the phospholipid structure, promotion of the autologous disappearance of necrotic tissues, and participation in the metabolism of alveolar surfactants (Kita et al., 2019). According to the classification of their biological activity, the PLA₂ family is subdivided into approximately four groups (Ong et al., 2010): (i) Secreted phospholipase A₂ (sPLA₂): sPLA₂ is a low molecular mass (14 kDa) enzyme with a rigid tertiary structure configured by disulfide bridges. It needs a millimolar concentration of calcium to exert its enzymatic action and has poor fatty acid selectivity when tested in vitro (Sun et al., 2021). Several studies have suggested that functionally active cPLA₂ is a prerequisite for sPLA₂-mediated AA release and prostanoid biosynthesis (Balsinde et al., 1998; Murakami et al., 1998; Shinohara et al., 1999) and that cPLA₂ regulates gene expression of sPLA₂ (Kuwata et al., 2000). (ii) PLA₂: mainly including cPLA₂α, cPLA₂β, and cPLA₂γ. cPLA₂α has a molecular weight of 85 kDa and is widely present in various bodily tissues. It normally requires the participation of a submicromolar Ca²⁺ concentration and the phosphorylation of upstream kinases during activation to selectively hydrolyze the phospholipid AA sn-2 position. Later, other downstream enzymes, including COX and leukotrienes among others, are responsible for the generation of endogenous eicosanoids and diacylglycerol (Farooqui et al., 2006). This gives PLCβ2 access to its enzyme action and has poor fatty acid selectivity when tested in vitro (Sun et al., 2021). Several studies have suggested that functionally active cPLA₂ is a prerequisite for sPLA₂-mediated AA release and prostanoid biosynthesis (Balsinde et al., 1998; Murakami et al., 1998; Shinohara et al., 1999) and that cPLA₂ regulates gene expression of sPLA₂ (Kuwata et al., 2000). (iii) PLA₂: mainly including cPLA₂α, cPLA₂β, and cPLA₂γ. cPLA₂α has a molecular weight of 85 kDa and is widely present in various bodily tissues. It normally requires the participation of a submicromolar Ca²⁺ concentration and the phosphorylation of upstream kinases during activation to selectively hydrolyze the phospholipid AA sn-2 position. Later, other downstream enzymes, including COX and leukotrienes among others, are responsible for the generation of endogenous eicosanoids and diacylglycerol (Farooqui et al., 2006). This gives PLCβ2 access to its enzyme action and has poor fatty acid selectivity when tested in vitro (Sun et al., 2021). Several studies have suggested that functionally active cPLA₂ is a prerequisite for sPLA₂-mediated AA release and prostanoid biosynthesis (Balsinde et al., 1998; Murakami et al., 1998; Shinohara et al., 1999) and that cPLA₂ regulates gene expression of sPLA₂ (Kuwata et al., 2000). (iv) Plasma-selective PLA₂: it has an apparent molecular mass of 39 kDa and mainly exists in the nucleus (Ong et al., 2010).

A recently published study has shown that PLA₂, cPLA₂, and sPLA₂ have a unique preference among the specific omega-3 fatty acids—eicosapentaenoic acid and docosahexaenoic acid—or the omega-6 AA, which are the precursors of most pro- and anti-inflammatory factors (Hayashi et al., 2021). Meanwhile, the study discovered that cPLA₂ selectively prefer AA, whereas iPLA₂ prefer prefer eicosapentaenoic acid, and sPLA₂ prefer docosahexaenoic acid as the substrate. Compared to the other three forms, cPLA₂ is the best characterized in terms of its enzyme properties and protein structure, while iPLA₂, sPLA₂, and plasma-selective PLA₂ need to be further clarified (Kita et al., 2019). cPLA₂ has two independent units that include an N-terminal C2 domain and a catalytic domain, and it is translocated to phosphatide membranes through the C2 domain at the nanomolar level of Ca²⁺, where it acts to catalyze the remaining phospholipase (Brown et al., 2021). Additionally, increasing evidence has shown that cPLA₂ is crucial for the release of AA and lysophospholipids induced by agonists such as cytokines and endotoxins and a series of downstream inflammatory mediators (Sun et al., 2021). Experiments performed using mice with cPLA₂ knockout (KO) have shown that cPLA₂ is the indispensable PLA₂ enzyme required for eicosanoid production in different inflammation illness models (Nagase et al., 2000b). Thus, most researchers now believe that cPLA₂ is the “valve” that regulates the inflammatory process (Chatterjee et al., 2021; Duro et al., 2022).

Upstream of Cytosolic Phospholipase A₂ Activation

It is generally accepted that the activation of cPLA₂ occurs only through the mechanism of postreceptor signal transduction and it depends on the concentration of many factors (Isenovic et al., 2011). It is evident that there are many phosphorylation sites on cPLA₂ (S431, S454, S505, and S727) that showed that cPLA₂ was a matrix for the rest of the kinases (de Carvalho et al., 1996). Thus, as a molecule in the complex intracellular regulating network, cPLA₂ can give “instructions” by sensing external signals, triggering a series of downstream effects, and playing an important role in connecting these signals with responses (Figure 2).

The extracellular signal molecule binds to its corresponding G-protein-coupled receptor to activate PLC. PLC cleaves the PI(4,5)P₂ in the cell membrane into DAG and IP3 and then activates PKC to cause a cascade reaction, which opens the calcium channel of the cell membrane and increases the intracellular calcium ion concentration. In addition, a second injury promotes cPLA₂ phosphorylation through the ras-MAPK signaling pathway. The increased intracellular calcium promotes the binding of the C2 domain of cPLA₂ to membrane phospholipids. Phosphorylated cPLA₂ hydrolyzes membrane phospholipids, producing downstream products (AA, Ly-so, and their subsequent metabolites) that trigger neuroinflammation. Phosphorylated cPLA₂ simultaneously binds to the lysosomal membrane, resulting in increased permeability of the lysosomal membrane. Lysosomal enzymes (CTSB, CTSD) can degrade TFAM and impair mitochondrial function. Exosomotic lysosomal enzymes break down digestive cells and cause neuronal cell death. Damaged lysosomal enzymes prevent the binding of lysosomal enzymes to autophagosomes and thus hinder the progress of autophagy (Ca²⁺). Intracellular calcium concentration; AA, arachidonic acid; CTSB: cathepsin B; CTSD: cathepsin D; DAG: diacylglycerol; IP3: inositol triphosphate; LPA: lysophosphatidic acid; LTB4: leukotriene B4; LXA4: lipoxin A4; Ly-so: lysophospholipids; MAPK: mitogen-activated protein kinase; PAF: platelet-activating factor; PG2: prostaglandin E2; PI2: phosphatidylinositol 4,5-bisphosphate; PKC: protein kinase C; PLC: phospholipase C; S-1-P: sphingosine 1 phosphate; TFAM: transcription factor A; TXA2: thromboxane 2.
Functions of cPLA2 in TBI

Two more than two decades ago, it was reported that cPLA2 activity was a vital contributing factor to deleterious cellular processes in the CNS (Mori et al., 1995). However, in an in vitro study, cPLA2 activity was more reduced in the P2* ischemia cortex than in the cPLA2 experiment (Naik et al., 2017). P38 MAPK and cPLA2 are considered initiating events (in 2 min) needed for radiation-triggered stimulation of ERK1/2 in vascular endothelial cells (Yazlovitskaya et al., 2008). In a study by Shletat et al. (2008) with primary neurons in cultivation, activation of the ionotrope glutamate receptor agonist caused reactive oxygen species (ROS) generation via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme and fast stimulation of ERK1/2 and cPLA2. Further research by Chuang et al. (2015) showed that in both primary and BV-2 microglia stimulated by lipopolysaccharide, the timepoint of increasing p-ERK1/2 was prior to that of p-cPLA2. The associations between p-ERK1/2 and p-cPLA2 were further verified by U0126, the MEK1/ERK1/2 inhibitor, which blocked cPLA2 activation. As a component of the MAPK family and mediates various types of cellular signaling processes such as cell proliferation, migration, and apoptosis (Li et al., 2004), increased cPLA2 activity has contributed to microglial activation in inflammation and the nervous system (Yang et al., 2009; Nasraadian and Van Den Berg, 2011). Hernández et al. (1999) found that TNF-α causes early stimulation of p38-MAP kinase and JNK in astrocytic glioma cells, which is before p-cPLA2 phosphorylation and the release of AA, suggesting that p-ERK1 and JNK are both upstream signaling pathways of cPLA2. In addition, an experiment in a liver injury model proved that gigantol ameliorated CCl4 triggered hepatic damage by avoiding stimulation of the JNK/cPLA2/12 LOX inflammation pathway (Xue et al., 2020).

Involvement of Cytosolic Phospholipase A2 in Traumatic Brain Injury and Spinal Cord Injury

In a previous study, controlled BKs are reported to be important for the regulation of vasopermeability and inflammation processes after traumatic brain and spinal cord injury. In a pathways study, cPLA2 and LPO were identified via special suppressors and cPLA2 KO mice, whereas sPLA2 GIIA and iPLA2 were identified via special suppressors and cPLA2 KO mice, which suggested that cPLA2 may take part in the early stage of brain injury.

Functions of cPLA2 in SCI

In 1999, high levels of cPLA2 activity were noted to exist in the rat spinal cord (Ong et al., 1999b). Later, in an in vitro experiment, cPLA2 immune reactivity was found to be remarkably upregulated in nerve cells undergoing apoptosis (Hornfelt et al., 1999). This finding suggested that cPLA2 was pivotal for certain causal links participating in or tightly associated with neuron death. Later, Liu et al. (2007) found that the usage of annexin A1 (a nonselective PLA2 suppressor) can inhibit SCI-triggered inflammatory events and decrease tissue damage. Another study showed that both cPLA2 and mellitin (an activation agent of endogenetic cPLA2) caused spinal neuronal death in vitro, which was remarkably treated by melipaque, a cPLA2 suppressor (Liu et al., 2006). When cPLA2 or mellitin was delivered into the lumbar spinal cord via microinjection, the former triggered confirmed myelinolysis and the latter caused diffuse tissue necrosis. These two microinjections caused oxidative stress, inflammatory, membrane integrity, apoptosis, autophagy, and functional outcomes following SCI.

It was then revealed that suppressing cPLA2 (AACOCF3) or gene knockout (cPLA2 KO C57/6 mice) can improve motor deficiency and cause less tissue injury after SCI (Liu et al., 2014). In Malada’s study, an in vitro experiment in microglia showed that cPLA2 can upregulate CD40 protein expression by activating the NOX2-NADPH oxidative enzyme and NF-κB. The outcomes indicated that cPLA2 might be an essential magnifier of the inflammatory reaction after CNS trauma (Malada-Edelstein et al., 2017). Recently, a study confirmed that upregulation of cPLA2 can also damage the lysosomal membrane and thus impair autophagic flux (Li et al., 2019), suggesting that activation of cPLA2 by SCI can destabilize the lysosomal membrane. Overall, these data reveal a crucial effect of cPLA2 on regulating oxidation, inflammation, membrane integrity, apoptosis, autophagy, and functional outcomes following SCI.

However, an interesting study discovered there were complicated effects of diverse PLA2 enzymes (cPLA2, sPLA2, and iPLA2) for alleviating or worsening the injury after SCI (López-Vales et al., 2011). Although all PLA2s were upregulated after SCI, contrary to Liu et al. (2006), cPLA2 was found to benefit SCI, which was identified via special suppressors and cPLA2 KO mice, whereas sPLA2 GIIA and iPLA2 were deleterious for SCI. López-Vales et al. (2011) postulated that the strain of mice and the genetic background of different strains of mice were the main reasons for the discrepancy. A study focused on lumbar spinal stenosis showed that inhibition of cPLA2 can reduce proinflammatory lipid mediator generation and tissue compression (López-Vales et al., 2015). This suggests that cPLA2 is adequately expressed in cells in a wide range of nervous systems, such as the CNS and peripheral nervous system, and it is regulated by cPLA2-related inhibitors.
Effect of cPLA2 on neuroinflammation in CNS trauma

cPLA2 is vital for the production of diverse inflammatory mediators: a variety of inflammatory mediators are directly or indirectly involved in the pathogenesis of CNS trauma, some of which induce each other and some are antagonistic to each other, thereby jointly building a complex network. A previous study showed that CNS trauma increased cPLA2 metabolites such as free aliphatic acids, eicosanoids, and lipid peroxides (Abu Hamdeh et al., 2018). First, oxidized free aliphatic acids are a primary product of lipid peroxidation. In a traumatic brain injury (TBI) rat model, oxidative peak was delayed at 60 minutes after CCI and gradually weakened at the 4- and 24-hour time points (Anthony-muthu et al., 2017). Sixty minutes after TBI, enzyme lipid peroxidation was the primary causative link with 15-LOX, leading to almost total complete oxidation of aliphatic acids. Proinflammatory lipid mediators were elevated at 1 and 4 hours after TBI and were restored to basic levels after 24 hours, which coincides with the time of early cPLA2-mediated macrophage/microglia activation, thus reducing neuroinflammation. The metabolism of free aliphatic acids and lysophosphatides leads to the loss of essential phospholipids. Thus, it may also function with a detergent-like effect on the membranes of the neurons and influence nerve conduction (Faure et al., 2014). Moreover, free aliphatic acids could deplete oxidative phosphorylation (OXPHOS), leading to mitochondrial function disorder (Papa et al., 2012). Arachidonic acid is the predominant product of phospholipids degraded by cPLA2, and subsequently bio-transforms through different pathways to diverse inflammatory mediators such as prostaglandins, leukotrienes, and eicosanoids (Wang et al., 2021). cPLA2 can be stimulated by some pivotal pathological conditions, such as inflammatory cell factors, free radicals, excitatory amino acids, which are increased after CNS trauma (Vichai et al., 2005; Lin et al., 2015; Wang et al., 2021). Increased cPLA2 activity in CNS trauma not only modulates the neuronal membrane, further increasing the release of inflammatory, oxidative, and excitatory amino acids. This suggests that metabolites of cPLA2 might be pivotal in creating the positive feedback loop induced by trauma injury. The effector system that controls cPLA2 and other relevant mobilization of AA ensures that the pathological conditions closely related to arachidonic acid cascade activation.

Effect of cPLA2 on the hemato-endothelial barrier in CNS trauma

Phosphatidylserine is a key constituent of the cell membrane of brain cells, and the glia cells affected the neuron status. In cultured astrocytes, it was found that BK-induced inflammation after TBI primarily occurred with the motion of microglia (Zahiri et al., 2021), suggesting that S-1-P can be involved in the inhibition of cPLA2 targets based on a negative feedback mechanism.

Apart from AA, another major product mediated by cPLA2 at sn-2 of gycerocephospholipids is lysosphospholipids, the downstream metabolites of which can also lead to secondary CNS trauma. Lysosphospholipids can activate cPLA2 may be different in various cells after CNS trauma. In astrocytes, cPLA2 interacts with mitochondrial antiviral-signaling protein to boost nuclear factor kappa B (NF-κB) and induce proinflammatory responses (Chao et al., 2019). In microglia, cPLA2 activity is linked to ROS and nitric oxide production during cell activation mainly through the MAPK pathway (Chuang et al., 2015). As mentioned above, although cPLA2 is widely expressed after CNS trauma, the specific role of cPLA2 on different cell types in CNS trauma are limited because of the complex cellular composition of the nervous tissue. However, with recent advances in techniques for separation and isolation of specific cell types in the brain and spinal cord tissue, understanding of the microglia function of cPLA2 has greatly increased. The specific underlying mechanism requires further investigations (Sun et al., 2021).

Potential Mechanisms of Cytosolic Phospholipase A2 in CNS Trauma

Specific effects of cPLA2 in cell types after CNS trauma

Once CNS trauma occurs, a variety of numerous cells such as neurons, astrocytes, and microglia will participate in the inflammatory response (Li et al., 2019). The BK receptor is a significant factor within secondary injuries in TBI. A previous study showed that reactive astrocytes (the major component of the glial scar) played a more important role than neurons in BK-cPLA2-AA inflammation (Chao et al., 2018). These authors thought that injury-induced inflammation after TBI primarily occurred with the glia cells, and the glia cells affected the neuron status. In cultured astrocytes and microglia, TNF-α and IL-1β can induce the activation of cPLA2, leading to PGE2 production and AA release (Hernández et al., 1999). AA triggers Ca2+-dependent cell death through mitochondrial permeability transition in both cells (Penzo et al., 2004). PGE2 exerts neurotoxicity via astrocyte glutamate release (Stachowicz, 2021) and microglia cytokine release (Bhatta et al., 2017). Those showed that cPLA2 and its metabolites played different toxicological roles in specific cell types. In addition to inducing cPLA2 to activate PLA2 may be different in various cells after CNS trauma. In astrocytes, cPLA2 interacts with mitochondrial antiviral-signaling protein to boost nuclear factor kappa B (NF-κB) and induce proinflammatory responses (Chao et al., 2019). In microglia, cPLA2 activity is linked to ROS and nitric oxide production during cell activation mainly through the MAPK pathway (Chuang et al., 2015). As mentioned above, although cPLA2 is widely expressed after CNS trauma, the specific role of cPLA2 on different cell types in CNS trauma are limited because of the complex cellular composition of the nervous tissue. However, with recent advances in techniques for separation and isolation of specific cell types in the brain and spinal cord tissue, understanding of the microglia function of cPLA2 has greatly increased. The specific underlying mechanism requires further investigations (Sun et al., 2021).

Regardless of the specific metabolic pathways undertaken by AA and other metabolites, cPLA2 is vital in the first step of the synthesis of these eicosanoids (Wang et al., 2021). cPLA2 can be stimulated by some pivotal pathological conditions, such as inflammatory cell factors, free radicals, and excitatory amino acids, which are increased after CNS trauma (Vichai et al., 2005; Lin et al., 2015; Wang et al., 2021). Increased cPLA2 activity in CNS trauma not only modulates the neuronal membrane, further increasing the release of inflammatory, oxidative, and excitatory amino acids. This suggests that metabolites of cPLA2 might be pivotal in creating the positive feedback loop induced by trauma injury. The effector system that controls cPLA2 and other relevant mobilization of AA ensures that the pathological conditions closely related to arachidonic acid cascade activation.
survival of terminal differentiation cells such as nerve cells. Mice with nerve tissue-specific KO of the vital autophagic genes Atg5 (autophagy-associated 5) or Atg7 (autophagy-associated 7) exhibit serious neural degeneration, with impaired motor functions and reflexes (Hars et al., 2006; Koss et al., 2006). Autophagic damage is associated with neurodegenerative diseases such as Parkinson’s, Alzheimer’s, and Huntington’s and related to lysosomal storage diseases (LSDs) (Shintani and Klionsky, 2004; Klionsky, 2006). Previous studies confirmed that autophagy is impaired after SCI or TBI, thereby contributing to secondary neurological cell death (Klionsky and Emr, 2000; Shintani and Klionsky, 2004; Mizushima and Komatsu, 2011; Li et al., 2019). Autophagy consists of several steps, such as the formation of the phagophore, formation of autophagosomes, and fusion of autophagosomes with lysosomes to produce autophagic lysosomes followed by decomposition of the autolysosome (Zhang et al., 2021; Rickman et al., 2022). Autophagy flux is a kinetic process in which these steps occur persistently in cells (Kim et al., 2020). If any obstacle occurs during autophagic flux, the autophagic process cannot be completed. As cPLA2 can exert its lysosomal function to generate lysosomal dysfunction above, cPLA2-induced lysosomal dysfunction can also impair the autophagy process, thus contributing to cell death. Several recent studies have proven that elevated cPLA2 activities and its transfer to the lysosomal fraction are essential factors during lysosome impairment and the subsequent damage to autophagic flux after CNS trauma (Li et al., 2019; Sarkar et al., 2020). LC3 serves as an autophagic biomarker. In the course of autophagy, cytoplasmic LC3 (LC3-II) enzymatically hydrolyses a small portion of polyenepeptides and then transforms into the autophagosome membrane type (LC3-II) (Kocak et al., 2021). The LC3-II/I ratio could be used to predict autophagy levels. Meanwhile, P62 is a selective autophagy receptor, forming a bridge connecting LC3 with ubiquitinated substrates to be degraded (Li et al., 2021). P62 binds to ubiquitinated proteins and enters the autophagophore, where it eventually fuses with lysosomes to form autolysosomes and is then cleared. The P62 content increases when autophagic flux is inhibited but it decreases when autophagic flux is activated. After treatment with C-1-p (a cPLA2 activator), neuroglioma cells exhibited higher LC3II/LC3I and P62 levels. Additionally, upstream autophagy regulators remain unaltered (Sarkar et al., 2020). These results show that cPLA2-induced impairment of autophagy activity may be achieved by blocking autophagy flux. However, a recent study showed that cPLA2-activated lipid mediator pathways are involved in autophagy induction (Qi et al., 2021) and confirmed that cPLA2 appears to be able to trigger or amplify autophagy responses. The authors hypothesized that the initiation of cPLA2-activated autophagy may be ATG5-dependent and independent of autophagy flux suppression. It should be noted that this research only detected the autophagy marker LC3 but did not detect p62. LC3II/LC3I levels alone cannot fully reflect changes in autophagic flux. In fact, acute autophagic dysfunction can be compensated for by the different types of cells (one was investigated in neural cells and the other in macrophages). Based on the aforementioned research, the role of cPLA2 in autophagy needs to be explored further. As in the CNS, microglia are the resident immune cells of the brain, which function as macrophages and primarily take part in surveillance and phagocytosis (Stewart et al., 2021). Therefore, more research is required to investigate the effect of cPLA2 on the levels of autophagic flux in microglia after CNS trauma.

### Anti-Cytosolic Phospholipase A2 Therapies in Central Nervous System Trauma

CPLA2 has a great impact on the mediation of inflammation after CNS trauma. As discussed, researchers have developed many inhibitors targeting CPLA2. Meanwhile, suppression of the upstream activators and downstream target of CPLA2 has also been explored as a therapeutic possibility. Although there are currently no clinically approved drugs, it may imr re inflammation after CNS trauma (Kopper et al., 2021). Although PACOCF3 is a high-selective CPLA2 inhibitor, it has off-target effects, as it can bond with IPLA2 and sPLA2 (Kopper et al., 2021). Besides, Jan et al. (2000) raised an important issue: PACOCF3 may alter cellular functions by affecting Ca2+ signaling in a manner independent of cPLA2 inhibition. Thus, more potential mechanisms of PACOCF3 therapy need to be studied.

### Pyrophosphonate

Pyrophosphonate is an inhibitor of cPLA2 derived from pyrophosphate. A previous study showed that pyrophosphonate suppressed sera-activated cPLA2 C2 domain-to-Golgi transfer by blocking calcium mobilization (Yun et al., 2016). It binds to the cPLA2 via a variety of hydrophobic pyrophosphonate residues located distally from the active parts (Burke et al., 2009). Treatment with pyrophosphonate can reduce cPLA2 expression levels and thus suppress lipopolysaccharide- and IFN-activated NO generation within BV-2 cells (Chung et al., 2020). However, due to the toxicity of some therapies that are under development and have shown great promise (Table 1).

### Table 1

| Drug         | Target          | Disease                  | Therapeutic effects                                      | References            |
|--------------|-----------------|--------------------------|---------------------------------------------------------|-----------------------|
| AACOCF3      | cPLA2           | Spinal cord injury       | Reducing membrane injury and inflammation, decreasing tissue injury, and improving behavioral recovery | Liu et al., 2014      |
| PACOCF3      | cPLA2 and Ca2+  | Spinal cord injury       | Restoring autophagic flux, weakening cortical cell deaths, and ameliorating movement and cognition functions | Kopper et al., 2021   |
| ANXA1        | Membrane phospholipids | Spinal cord injury         | Improving tissue repair, reconstruction, regeneration, decreasing white matter sparing, and protecting axons of long descending pathways | Li et al., 2007       |
| U0126        | ERK1/2          | DCS spinal injury        | Attenuating oxidative stress and inflammatory response, and improving motor function by upregulating heat shock proteins | Quan et al., 2021     |
| SB203580     | MAPK kinase-2&3 | Spinal cord injury       | Preventing the delayed progressive degeneration of oligodendrocytes and promoting recovery of motor function | Hikide et al., 2003   |
| Celecoxib    | LOX/COX-2       | Spinal cord injury       | Attenuating oxidative stress, apoptosis, and inflammation | 2020                  |
| Salalate     | LOX/COX-2       | CCI induced              | Blocking pro-inflammatory gene expression and nitrite secretion by microglia | Lagrou et al., 2017    |

CCl: Controlled cortical impact; C2: cycloxygenase-2; cPLA2: cytosolic phospholipase A2; DCS: deoxynribosylation injury; EAE: experimental autoimmune encephalomyelitis; IFN: interferon; IPLA2: intracellular phospholipid-associated lipase 2; LOX: lipoxygenase; TBI: traumatic brain injury.
and secondary injury by several bioprocesses. cPLA2, the heavyweight component of cPLA2 in CNS trauma, pharmacological inhibitors of cPLA2 were commonly used in previous studies. However, these inhibitors are not specific. Therefore, using cPLA2-KO mice is a better choice in future research studies.

Conclusion and Perspectives

This review has several limitations. First, the current review mainly focused on cPLA2α in CNS trauma, and further understanding of other cPLA2 subtypes, such as cPLA2β and cPLA2γ are also essential. Second, we discussed the effects of cPLA2 on lysosome membrane in nerve cells following TBI and SCI, but its potential roles on other membranous organelles such as the mitochondria and endoplasmic reticulum, should also be elaborated in further studies. Third, based on the limited literature, the mechanism of cPLA2 on autophagy in CNS trauma was discussed in this review. Many other types of programmed cell death such as ferroptosis, necroptosis, or pyroptosis may be also related to cPLA2. Therefore, an extensive investigation in this field is needed. Finally, to determine the function of cPLA2 in CNS trauma, pharmacological inhibitors of cPLA2 were commonly used in previous studies. However, these inhibitors are not specific. Therefore, using cPLA2-KO mice is a better choice in future research studies.

Conclusion and Perspectives

Accumulating evidence has proven that acute SCI and TBI can cause a decrease in iNOS, TNF-α, IL-1β, and COX-2 expression (Piao et al., 2003). In addition, the p38 MAPK suppressor SB203580 has been applied to CNS trauma models. Intrathecal SB203580 administration into the cerebral ventricle had a neuroprotective effect after tFCl and was correlated with a decrease in iNOS, TNF-α, IL-1β, and COX-2 expression (Piao et al., 2003). In addition, treatment with SB203580 ameliorated hindlimb functions in a rat model (Horiiuchi et al., 2003) and attenuated BBB extravasation and subsequent edema in a TCI model in rats (Nito et al., 2008). In contrast to the above consequences, a different study showed that intrathecal SB203580 administration did not ameliorate functional results after a middle conus traumatic SCI (Stirling et al., 2008). The discrepancy between the outcomes may be related to the different degrees of damage used in the different studies.

Limitations

This review has several limitations. First, the current review mainly focused on cPLA2α in CNS trauma, and further understanding of other cPLA2 subtypes, such as cPLA2β and cPLA2γ are also essential. Second, we discussed the effects of cPLA2 on lysosome membrane in nerve cells following TBI and SCI, but its potential roles on other membranous organelles such as the mitochondria and endoplasmic reticulum, should also be elaborated in further studies. Third, based on the limited literature, the mechanism of cPLA2 on autophagy in CNS trauma was discussed in this review. Many other types of programmed cell death such as ferroptosis, necroptosis, or pyroptosis may be also related to cPLA2. Therefore, an extensive investigation in this field is needed. Finally, to determine the function of cPLA2 in CNS trauma, pharmacological inhibitors of cPLA2 were commonly used in previous studies. However, these inhibitors are not specific. Therefore, using cPLA2-KO mice is a better choice in future research studies.

Acknowledgments

We would like to thank the Medical Editor, Dr. Ai Chen, for her valuable comments and suggestions on the manuscript. This study was supported by the National Natural Science Foundation of China (81871817), the Ministry of Science and Technology of China (2018YFC1108700), the Shanghai Science and Technology Commission (17JC1403000), and the Shanghai Key Laboratory of Mental Health Research (19XJ1403000).
Review

Piao CS, Kim JB, Han PI, Lee JK (2003) Administration of the p38 MAPK inhibitor SB203580 affords brain protection with a wide therapeutic window against focal ischemic insult. J Neurosci Res 73:537-544.

Piwowarek K, Rzeszotarska A, Korsak J, Juszkwiec A, Chciałowski A, Kruszewski J (2021) CXCR4 regulates autophagy and enhances neuronal survival in the hippocampus. J Neuroinflammation 18:149.

Qi HY, Daniels MP, Liu Y, Chen XJ, Alsally S, Levine SJ, Sheltamer JH (2011) A cytosolic phospholipase A2 (cPLA2) initiated lipid mediator pathway induces autophagy in macrophages. J Immunol 187:5286-5292.

Resnick D, Graham SH, Dixon CE, Marion DW (1998) Role of cyclooxygenase 2 in acute spinal cord injury. J Neurotrauma 15:1005-1013.

Rickman AD, Hilyard A, Heckmann BL (2022) Dying by fire: noncanonical functions of cytosolic phospholipase A2 in cytoprotection. FEBS Lett 598:1147-1162.

Shelat PB, Chalimoniuk M, Wang JH, Strosznajder JB, Lee JC, Sun AY, Simonyi A, Sun GY (2018) Cyp2c12 regulates cerebral ischemia/reperfusion-induced mitochondrial damage and autophagy in hippocampal neurons. J Neurochem 144:1359-1373.

Shibata N, Kato Y, Inose Y, Hiroi A, Yamamoto T, Morikawa S, Sawada M, Kobayashi M (2011) Cyp2c9 protects against mitochondrial dysfunction in H9c2 cardiomyocytes. FEBS Open Bio 1:240-252.

Shimada A, Arai K, Hattori T, Tanaka H, Takezawa M, Toyama K (2021) Chronic cerebral hypoperfusion enhances cerebrovascular cytosolic phospholipase A2 activity in hypoxic preconditioning. J Neurotrauma 38:506-515.

Sissons PJ, Cote SM, Colvin MM, Yilmaz D, Rong Y, Wang M, Song L (2016) Changes in cerebrovascular cytosolic phospholipase A2 are associated with ischemic brain injury in middle cerebral artery occlusion. Stroke 47:2635-2643.

Tian Q, Zhang X, Wang Y, Yu X, Zhang M, Li F, Jiang R, Wang Y, Sun G, Dong Y, et al. (2021) Cyp2c12 deficiency aggravates cerebral ischemia/reperfusion injury in mice. J Neuroinflammation 18:385.

Wang L, Li Z, Li X, Wang H, Han X, Mi Q, Zhang H, Wang J, et al. (2019) Cyp2c12 deficiency increases cerebral ischemia/reperfusion injury in mice. J Neuroinflammation 16:282.

Yang D, Ji HF, Zhang XM, Yue H, Lin L, Ma YY, Huang XN, Fu J, Wang WZ (2014) PLA2G4A/cPLA2-mediated lysosomal membrane damage leads to inhibition of autophagy and neurodegeneration after trauma brain. Autophagy 10:2963.

Yin XJ, Chen ZY, Zhu XN, Hu JJ (2017) Loss of PAFR prevents neuroinflammation and brain edema after experimental traumatic brain injury. J Neuroinflammation 14:132.

Yu X, Sha L, Liu Q, Zhao Y, Fang H, Cao Y, Zhao J (2021) Recent advances in cell membrane biomarker of anaphylaxis: Cross-sectional study. PLoS One 16:e0256168.

Zeng G, Li X, Ren X, Feng X, Shi X, Zhao T, et al. (2021) Cyp2c12 deficiency increases cerebral ischemia/reperfusion injury in mice. J Neuroinflammation 18:385.

Zhu XN, Hu JJ, Yin XJ, Wang X, et al. (2017) Loss of PAFR prevents neuroinflammation and brain edema after experimental traumatic brain injury. J Neuroinflammation 14:132.

Zhu Q, Meng X, Huang G, Y H, Zheng J, Zhang K, Hu W (2021) MEK1 inhibition synergistically enhances the preventive effects of normobaric oxygen on spinal cord injury in decompression sickness rats. Front Physiol 12:674430.

C-Editor: Zhao M; S-Editor: Li CH; L-Editors: Li CH, Song LP; T-Editor: Jia Y