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Phospholipase A_2 and its Molecular Mechanism after Spinal Cord Injury

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Abstract

Phospholipases A_2 (PLA_2s) are a diverse family of lipolytic enzymes which hydrolyze the acyl bond at the sn-2 position of glycerophospholipids to produce free fatty acids and lysophospholipids. These products are precursors of bioactive eicosanoids and platelet-activating factor which have been implicated in pathological states of numerous acute and chronic neurological disorders. To date, more than 27 isoforms of PLA_2 have been found in the mammalian system which can be classified into four major categories: secretory PLA_2, cytosolic PLA_2, Ca^{2+}-independent PLA_2, and platelet-activating factor acetylhydrolases. Multiple isoforms of PLA_2 are found in the mammalian spinal cord. Under physiological conditions, PLA_2s are involved in diverse cellular responses, including phospholipid digestion and metabolism, host defense, and signal transduction. However, under pathological situations, increased PLA_2 activity, excessive production of free fatty acids and their metabolites may lead to the loss of membrane integrity, inflammation, oxidative stress, and subsequent neuronal injury. There is emerging evidence that PLA_2 plays a key role in the secondary injury process after traumatic spinal cord injury. This review outlines the current knowledge of the PLA_2 in the spinal cord with an emphasis being placed on the possible roles of PLA_2 in mediating the secondary SCI.

Keywords

Phospholipases A_2; Spinal cord injury; Inflammation; Oxidation; Apoptosis

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Introduction

Phospholipases A_2 (PLA_2s) are a diverse family of lipolytic enzymes which hydrolyze the acyl bond at the sn-2 position of glycerophospholipids to produce free fatty acids and lysophospholipids (Fig. 1) [1–3]. These products are precursors of bioactive eicosanoids and platelet-activating factor (PAF) which are well-known mediators of inflammation and tissue damage implicated in pathological states of numerous acute and chronic neurological disorders including spinal cord injury (SCI) [3–6]. The hydrolysis of membrane phospholipids by PLA_2 is a rate-limiting step for generation of eicosanoids and PAF [3, 7]. Stimulation of PLA_2 is thought to be an important event in production of lipid inflammatory mediators. Under physiological conditions, PLA_2s are involved in diverse cellular responses, including phospholipid digestion and metabolism, host defense, and signal transduction. However, in pathological situations, increased PLA_2 activity and excessive production of free fatty acids such as arachidonic acid (AA) and pro-inflammatory mediators such as eicosanoids and PAF, may lead to the loss of membrane integrity, inflammation, oxidative stress, and subsequent neuronal injury [2, 3, 5, 8, 9]. This review outlines the current knowledge of the PLA_2 in the spinal cord with an emphasis being placed on the possible role of PLA_2 in mediating the secondary injury after an initial trauma.

Classification, Structure, and Properties of PLA_2

To date, more than 27 isoforms of PLA_2s have been found in the mammalian system which can be classified into four major categories: secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), Ca^{2+}-independent PLA_2 ($iPLA_2$), and platelet-activating factor acetylhydrolases (PAF-AH; Table 1) [1, 2, 10, 11]. $sPLA_2$s, in which ten isozymes have been identified, have a low molecular mass of about 14–18 kD and require the presence of submillimolar to millimolar concentrations of Ca^{2+} for effective hydrolysis of a substrate phospholipid without any fatty acid selectivity [11–14]. They are synthesized intracellularly and then secreted into the extracellular space and can act extracellularly [15, 16]. $sPLA_2$ binds to two types of cell surface receptors, namely the N type, identified in neurons, and the M type, identified in skeletal muscles, of $sPLA_2$ receptors although this nomenclature is merely academic since neither receptor is limited to these tissues and the expression has been shown widely for both types [17]. Members of the $cPLA_2$ have a higher molecular mass (85–110 kD), selectively hydrolyze phospholipids containing AA, and require a submicromolar concentration of Ca^{2+} for optimal activity [2, 13, 18]. $cPLA_2$s consist of 6 isoforms, among which $cPLA_2\alpha$ plays an essential role in the initiation of AA metabolism. Intracellular activation of $cPLA_2\alpha$ is tightly regulated by Ca^{2+} and phosphorylation [11, 18]. $iPLA_2$s, containing seven enzymes, are intracellular enzymes with higher molecular mass ranging from 28 to 91 kD that shows no Ca^{2+} requiring for its activity. $iPLA_2$ is generally considered as a housekeeping enzyme for the maintenance of membrane phospholipids [2, 10, 11]. Recent evidence, however, suggests that $iPLA_2$ may also be involved in the pathogenesis of childhood neurological disorders [19]. PAF-AH family represents a unique group of PLA_2 that contains four enzymes exhibiting unusual substrate specificity toward PAF and/or oxidized phospholipids [2, 10, 11].

PLA_2 Isozymes in the Normal Spinal Cord

Multiple isoforms of PLA_2s have been found in the mammalian spinal cord. $sPLA_2$ activity was detected in the normal rat spinal cord homogenate [20]. Western blot analysis revealed the presence of $sPLA_2$ IIA and V in the normal rat spinal cord [20]. mRNAs of $sPLA_2$s IB, IIA, IIC, and V were also detected in the normal rat spinal cord [21]. We have recently shown that mRNAs for $sPLA_2$-IB, IIA, IIC, IIE, V, X, and XIIA were all expressed in the normal rat spinal cord [22]. Immunohistochemistry and Western blot analysis confirmed the expression of sPLA2-IB and IIA in the spinal cord at the protein level [22]. Immunofluorescence double staining revealed that $sPLA_2$-IB and IIA were localized to neurons, axons, astrocytes, and oligodendrocytes [22].

$cPLA_2$ activity was also detected in the cytosolic fraction of the rat spinal cord [23]. $cPLA_2$ immunoreactivity was found in the dorsal horn and motor neurons of the rat and monkey [24] and in the rat spinal cord shown by Western blot [25]. Lucas et al. [21] reported that $cPLA_2$ activity was present in the normal rat spinal cord homogenates and demonstrated that both the mRNA and protein of $cPLA_2$ are expressed in the normal rat spinal cord. Recently, we confirmed $cPLA_2$ expression in the normal adult rat spinal cord and defined its cellular localization in neurons and oligodendrocytes but not in astrocytes [26].

$iPLA_2$ mRNA is constitutively expressed in the human [27] and rat spinal cords [28]. Recently, Lucas et al. [21] demonstrated the activity as well as protein and mRNA expressions of $iPLA_2$ in the normal rat spinal cord using $iPLA_2$ activity assay, RT-PCR, and Western blot analyses.

PLA_2 Isozymes in the Injured Spinal Cord

Acute SCI triggers a secondary injury by multiple biological processes [29–32]. One such a critical process is the activation of PLA_2 which can result in the hydrolysis of membrane phospholipids, releasing free fatty acid, generation of oxygen free radicals, formation of eicosanoids, and ultimately leading to neuronal death [9, 33, 34].

SCI Induces Increases of PLA_2 Metabolites including Free Fatty Acids, Eicosanoids, and Lipid Peroxides

It has been demonstrated in several experimental SCI models that the degradation of membrane phospholipids, along with the generation of free fatty acids, eicosanoids, and lipid peroxides, increased following SCI [35–39], suggesting that PLA_2 activity increased following SCI. Phospholipids are main components of the cell membrane that play important roles in maintaining the structure and function of cell membranes. SCI resulted in an immediate decrease of total phospholipid content [37]. During the first minute of compression trauma to the spinal cord, 10% of the plasmenylethanolamine (PlsEtn) was lost with an overall loss of 18% found at 30 min after the injury [40].

One of the first pathophysiological events occurring following SCI is the release of free fatty acids due to the activation of PLA_2. Within the first few minutes after SCI, the level of free fatty acids has increased in the gray matter and later in the white matter to a lesser extent

[35, 41]. A time course study showed that there existed biphasic increases in the free fatty acid level in the spinal cord following injury [37]. The first increase occurred within 5 min after SCI which was declined at 30 min. The second increase occurred at 1 h after SCI, peaked at 24 h, and remained significantly high at 7 days after SCI [37].

Hydrolysis of membrane phospholipids by PLA_2 is a rate-limiting step for generation of pro-inflammatory eicosanoids and PAF [3, 7]. Following SCI, eicosanoids such as thromboxane A_2 and prostaglandin E_2 (PGE_2), metabolites of PLA_2, increased in the injured cord tissue within a few minutes after SCI, and persisted at significantly high levels for 72 h after injury [42, 43]. These eicosanoids and PAF are key mediators of tissue damage and cell death following SCI.

SCI Induces Increased PLA_2 Activity and Expression

Following a contusive SCI in adult rats, PLA_2 activity increased significantly which was peaked at 4 h post-injury and remained at a significant high level at 7 days [26]. The expression of $cPLA_2$, an important PLA_2 isotype, was also increased and peaked at 3 and 7 days post-injury [26]. Immunohistochemical studies revealed that $cPLA_2$ immunoreactivity was markedly increased in both the injured gray and white matter at 7 days after injury [26]. Immunofluorescence double labeling demonstrated that increased level of $cPLA_2$ was localized in neurons, swollen axons, oligodendrocytes, and a subpopulation of microglia [26].

In addition to $cPLA_2$, increased expression of a subset of $sPLA_2$ was found following SCI [22]. In this study, expression of ten $sPLA_2$ mRNAs including sPLA2-IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XIIA at 4 h after SCI was studied. Among them, seven were detected in naïve and spinal cord contused animals ($sPLA_2$-IB, IIA, IIC, IIE, V, X, and XIIA). Among these expressed $sPLA_2$ isoforms, $sPLA_2$-IIA showed the most dramatic increase after SCI. Increased expression of $sPLA_2$-IIE mRNA was also found in the injured cord in a similar pattern. In contrast, $sPLA_2$-X showed a decrease in signal intensity. No significant difference was found in expression patterns of $sPLA_2$-IB, IIC, V, and XIIA mRNAs between the sham and SCI groups. Real-time qRT-PCR revealed that $sPLA_2$-IIA mRNA expression had a significant fourfold increase at 1 h following contusion and remained highly elevated at 4 h after SCI [22]. Weston blot confirmed an increase in the expression of $sPLA_2$-IIA and IIE after SCI [22]. Immunohistochemistry showed that significantly more immunoreactivity of IIA and IIE was found in the injured cord at 4 h after SCI as compared to the sham control [22]. Finally, immunofluorescence double labeling revealed that $sPLA_2$-IIA was localized in neurons, axons, oligodendrocytes, astrocytes, and some myelin rings [22]. Thus, $sPLA_2$ isoforms are differentially expressed following SCI and are localized in both neurons and glial cells. Localization of specific $sPLA_2$ isoforms such as $sPLA_2$IIA in neurons and oligodendrocytes indicate that these molecules may play important roles in mediating neuronal and oligodendrocyte cell death following spinal cord injury.

PLA_2 Activation is Induced by Several Toxic Factors including Inflammatory Cytokines, Free Radicals, and Excitatory Amino Acids Generated in the Injured Spinal Cord

Although increased activity and expression of PLA_2s after SCI was observed, the mechanism(s) by which they increase remains unclear. Recent studies showed that PLA_2 activity and/or expression could be induced by several toxic factors such as inflammatory cytokines [2, 44], free radicals [45, 46], and excitatory amino acids [47–49]. All of these injury mediators were demonstrated to increase in the spinal cord following injury. It is therefore possible that PLA_2 may serve as a converging molecule that mediates the pathogenesis of these different injury mechanisms associated with spinal cord secondary injury [29–31].

Increased PLA_2 Results in Spinal Cord Tissue Damage and Behavioral Impairment

Increasing evidence suggests that PLA_2 and their metabolites may mediate inflammation, oxidation, and neurotoxicity following SCI. In vitro experiments showed that both PLA_2 and melittin, an activator of endogenous PLA_2, induced spinal neuronal death in a dose-dependent manner, an effect that could be substantially reversed by mepacrine, a PLA_2 inhibitor [26]. When PLA_2 was directly microinjected into the normal rat spinal cord, it induced tissue damage, demyelination, and sustained impairment in motor function. Such PLA_2-induced demyelination, however, could be effectively attenuated with mepacrine, a PLA_2 inhibitor [26]. Injections of PLA_2 also induced the expression of inflammatory cytokines TNF-α and IL-1β, as well as 4-HNE, a product of lipid peroxidation and a marker for oxygen free radical-mediated membrane injury [26]. PLA_2 has also been reported to mediate myelin breakdown and axonal degeneration [50].

In vitro experiments also showed that $sPLA_2$ induced spinal oligodendrocyte death in a dose-dependent manner [22]. Low levels of exogenously added $sPLA_2$-IIA (0.01 and 0.1 μM) result in a loss of processes extending from the soma and at higher dose (2 μM) triggers a complete loss of process and cell death. In contrast, 2 μM of $sPLA_2$-IIA had no effect on cultured Schwann cells and astrocytes, suggesting a specific sensitivity of oligodendrocytes to $sPLA_2$-IIA. In addition, $sPLA_2$-IIA mediates H_2O_2, IL-1β, and TNFα-induced oligodendrocyte cell death [22].

Annexin A1 (ANXA1) is an endogenous nonselective inhibitor of PLA_2. Our experiment showed that administration of ANXA1 inhibited SCI-induced increases in PLA_2 and myeloperoxidase activities [51]. In addition, ANXA1 administration reduced the expression of interleukin-1β and activated caspase-3 at 24 h post-injury and glial fibrillary acidic protein at 4 weeks post-injury [51]. Furthermore, ANXA1 administration significantly reversed PLA_2-induced spinal cord neuronal death in vitro and reduced tissue damage and increased white matter sparing in vivo, compared to the vehicle-treated controls [51]. Fluoro-Gold retrograde tracing showed that ANXA1 administration protected axons of long descending pathways at 6 weeks post-SCI [51]. ANXA1 administration also significantly increased the number of animals that responded to transcranial magnetic motor-evoked potentials [51].

A recent study showed that intravenous administration of arachidonyl trifluoromethyl ketone ($AACOCF_3$; 7.13 mg/kg), a cPLA2 inhibitor, at 30 min after SCI significantly increased the number of surviving neurons and oligodendrocytes at 7 days post-SCI as well as improved behavior recovery [52]. This finding suggests that PLA_2 activation plays a critical role in mediating secondary SCI.

Increased Metabolites of PLA_2 Result in Neurotoxicity and Demyelination

Metabolism of free fatty acid represents a source of reactive oxygen species (ROS). Generation of free fatty acids in SCI is closely associated with increases in free radical formation observed in the lesioned region of injured spinal cord [53, 54]. Several studies showed that free fatty acids activated NADPH oxidase, a key enzyme mediating ROS production [46, 55–57]. Application of pathophysiological concentrations of free fatty acids has been demonstrated in vitro to induce oxidative injury to spinal cord cell cultures [58]. Low concentrations of free fatty acid, AA, support cultured neurons to survive whereas higher concentrations are neurotoxic [59]. Neurotoxic effects of AA have also been observed in hippocampal neurons and cortical neurons [59, 60] as well as oligodendrocytes [61].

The bioactive eicosanoids such as thromboxanes, prostaglandins, and leukotrienes from AA, induced by PLA_2, have been implicated as mediators of secondary injury via a host of mechanisms [62, 63]. For example, thromboxane A_2 stimulates platelet aggregation and vasoconstriction. PGE_2 and leukotrienes B_4 (LTB_4) increases vascular permeability. LTB_4 also is a potent stimulator of polymorphonuclear leukocyte chemokinesis and chemotaxis.

In contrast to the above-mentioned eicosanoids, another AA-derived lipid mediator, lipoxin, has been shown to have an anti-inflammatory effect through modulating key steps in leukocyte trafficking [64]. However, changes of lipoxins and its possible role following SCI remain to be determined.

Lysophosphatidylcholine (Lyso-PC) and PAF are also metabolic products mediated by PLA_2. Injection of Lyso-PC into the spinal cord causes demyelination as well as expression of a number of chemokines and cytokines [65, 66], which occurred in the injured cord after SCI. PAF levels have been shown to increase 20-fold after spinal cord injury induced by stroke [67]. Intrathecal administration of PAF leads to reduced spinal cord blood flow and motor deficits, an effect which can be blocked by the PAF receptor antagonist, WEB 2170 [68]. Treatment with WEB 2170 after acute spinal cord contusion resulted in significant increases in white matter sparing as well as decreases in pro-inflammatory cytokine mRNA levels within the lesion epicenter [69, 70]. Treatment of a PAF receptor antagonist BN52021 also improves behavioral recovery after SCI [71]. In vitro experiments showed that low concentrations of PAF resulted in neuronal differentiation and sprouting, while higher concentrations were neurotoxic [72]. PAF not only induced neuronal death in a dose-dependent manner in vitro [73, 74] but also death of oligodendrocytes and astrocytes [69].

PLA_2 Mediates Excytotoxic Neuronal Death and Tissue Damage

It has been shown that the release of high levels of excitatory amino acids (EAA) such as glutamate and aspartate in experimental SCI is an important mechanism of secondary

injury [30]. Growing evidence suggests that PLA_2 mediates EAA-induced neuronal death and tissue damage. Marked increases in PLA_2 activity and AA release have been reported after treatments of neuronal cultures with glutamate, *N*-methyl-D-aspartate (NMDA), and kainic acid (KA) [47, 75, 76]. This increased PLA_2 activity can be inhibited by a PLA_2 inhibitor, mepacrine as well as a KA/AMPA receptor antagonist, CNQX [76]. Recently, phosphorylated $cPLA_2$ expression and AA release have been reported to increase in cultured primary neurons after NMDA stimulation [77]. The NMDA-induced AA release was inhibited by a $cPLA_2$ inhibitor methyl arachidonyl fluorophosphonate and a NADPH oxidase-ROS pathway has been demonstrated to mediate NMDA-induced $cPLA_2$ phosphorylation [77]. It has been hypothesized that the glutamate release triggers a sequence of events including NMDA receptor activation, increases of intracellular Ca^{2+}, activation of PLA_2, and eventually neuronal death [49]. It has been shown that glutamate release in the spinal cord can be suppressed by PLA_2 inhibitors such as indomethacin by 40%, $AACOCF_3$ by 45%, and 4-bromophenacyl bromide by 36%, suggesting that PLA_2-induced EAA release mediates the pathogenesis of secondary injury in a positive feedback manner [78]. Thus, excessive stimulation of NMDA receptors, as occurs in the spinal cord trauma, may result in stimulation of PLA_2 activity leading to alterations in membrane composition, permeability, and fluidity which could cause neuronal and glial death. Indeed, in vivo and in vitro experiments showed that exogenous administration of PLA_2 induced neuronal death and tissue damage [79–81].

Possible Mechanisms of PLA_2 Action after Spinal Cord Injury

To date, mechanisms underlying PLA_2-mediated SCI remain unclear. Several hypotheses, however, have been proposed to interpret PLA_2-mediated injury. These possible mechanisms include the effect of PLA_2 on membrane damage, release of pro-inflammatory mediators, generation of free radicals, release of excitotoxic neurotransmitters, and enhancement of apoptosis (Fig. 2) [3, 49, 63, 82, 83].

Injury to the neural membrane can be a result of PLA_2's direct action. Phospholipids are the main components of a neural cell bi-layer membrane. They not only constitute the backbone of neural membrane, but also provide the membrane with suitable environment, fluidity, and ion permeability, which are required for the proper function of integral membrane proteins, receptors, and ion channels. PLA_2 activation induces phospholipid degradation and membrane breakdown directly through hydrolysis of neural membrane phospholipids, resulting in alteration of membrane function such as fluidity and permeability, behavior of transporters and receptors, ion homeostasis, and eventually leading to functional failure of excitable membranes [3, 49, 84]. Once the neural membranes are destroyed, the functional loss may become irreversible.

Induction of abnormally high levels of PLA_2 metabolites could be another indirect mechanism of PLA_2-mediated injury. PLA_2 metabolites mediate inflammation, oxidation, and neurotoxicity following SCI. For example, metabolism of free fatty acids represents a source of ROS. A number of studies showed that free fatty acids activated NADPH oxidase, a key enzyme of ROS production [46, 55–57]. Application of pathophysiological concentrations of free fatty acids has been demonstrated to induce oxidative injury to spinal

cord cell cultures [58]. In addition, several well-known bioactive mediators of inflammation such as eicosanoids (prostaglandins, thromboxanes, leukotrienes, and lipoxins) and PAF induced by PLA_2 have been implicated as mediators of secondary injury [62, 63, 68–71].

PLA_2 could also mediate damage induced by EAA. Application of PLA_2 to the rat ischemic cerebral cortex resulted in a significant increase in EAA levels and a PLA_2 inhibitor mepacrine significantly decreased the ischemia-evoked efflux of EAA into cortical superfusates, suggesting the involvement of PLA_2 in EAA release [82]. The release of high levels of EAAs is an important mechanism of secondary injury after acute SCI [30].

Increasing evidence suggests the involvement of multiple isoforms of PLA_2 in apoptosis, which has been identified as an important mechanism of cell death in many neurological disorders including SCI [85–87]

In summary, PLA_2 can be activated by several key injury mediators such as inflammatory cytokines, free radicals, and excitatory amino acids that have been shown to increase following traumatic SCI. Increased PLA_2 activity can in turn hydrolyze neural membrane and further increase inflammation, oxidation, and excitatory amino acid release. This indicates that PLA_2 activation may play a central role in this positive feedback loop triggered by traumatic SCI. Such activation may eventually lead to neuronal and glial cell death, tissue damage, and electrophysiological and behavioral impairments. Thus, PLA_2 may act as a convergence molecule that mediates multiple injury mechanisms after SCI and blocking PLA_2 action may represent a novel and efficient strategy to block multiple injury pathways that occur following SCI.

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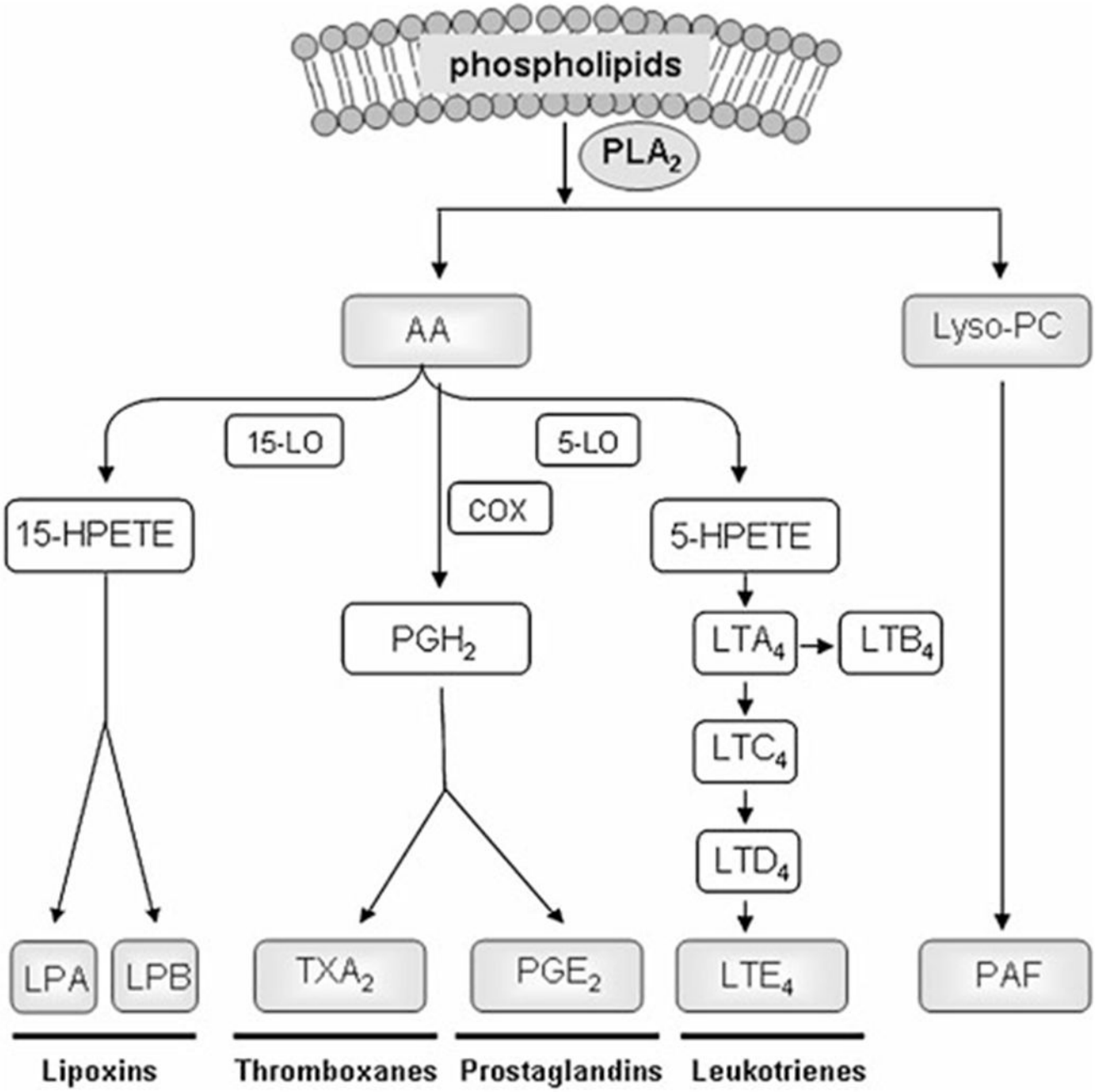

Fig. 1.

PLA_2 mediates the production of lipid mediators. *PLA_2* hydrolyzes the membrane phospholipids to produce a free fatty acid such as arachidonic acid (*AA*) and a lysophospholipid such as lysophosphatidylcholine (*Lyso-PC*). *AA* can give rise to eicosanoids via cyclooxygenases (*COX*), 5-lipoxygenase (*5-LO*), and 15-lipoxygenase (*15-LO*) enzymes. Eicosanoids such as thromboxanes (TX), prostaglandins (PG), and leukotrienes (LT) are potent mediators of inflammation which can increase vascular permeability and induce chemotaxis of immune cells. Lipoxin is also an AA-derived lipid

mediator. However, in contrast to above eicosanoids, it has an anti-inflammatory effect. In addition, platelet-activating factor (*PAF*) is biosynthesized from *Lyso-PC* and acetyl CoA by enzyme Lyso-PC acetyltransferase. *Lyso-PC* is a myelinolytic agent and can act as a chemoattractant for immune cells. *PAF* is a potent phospholipid activator and a mediator of many leukocyte functions, including platelet aggregation, inflammation, and anaphylaxis. *5-HPETE* 5-hydroperoxyeicosatetraenoic acid, *PGH_2* prostaglandin H_2

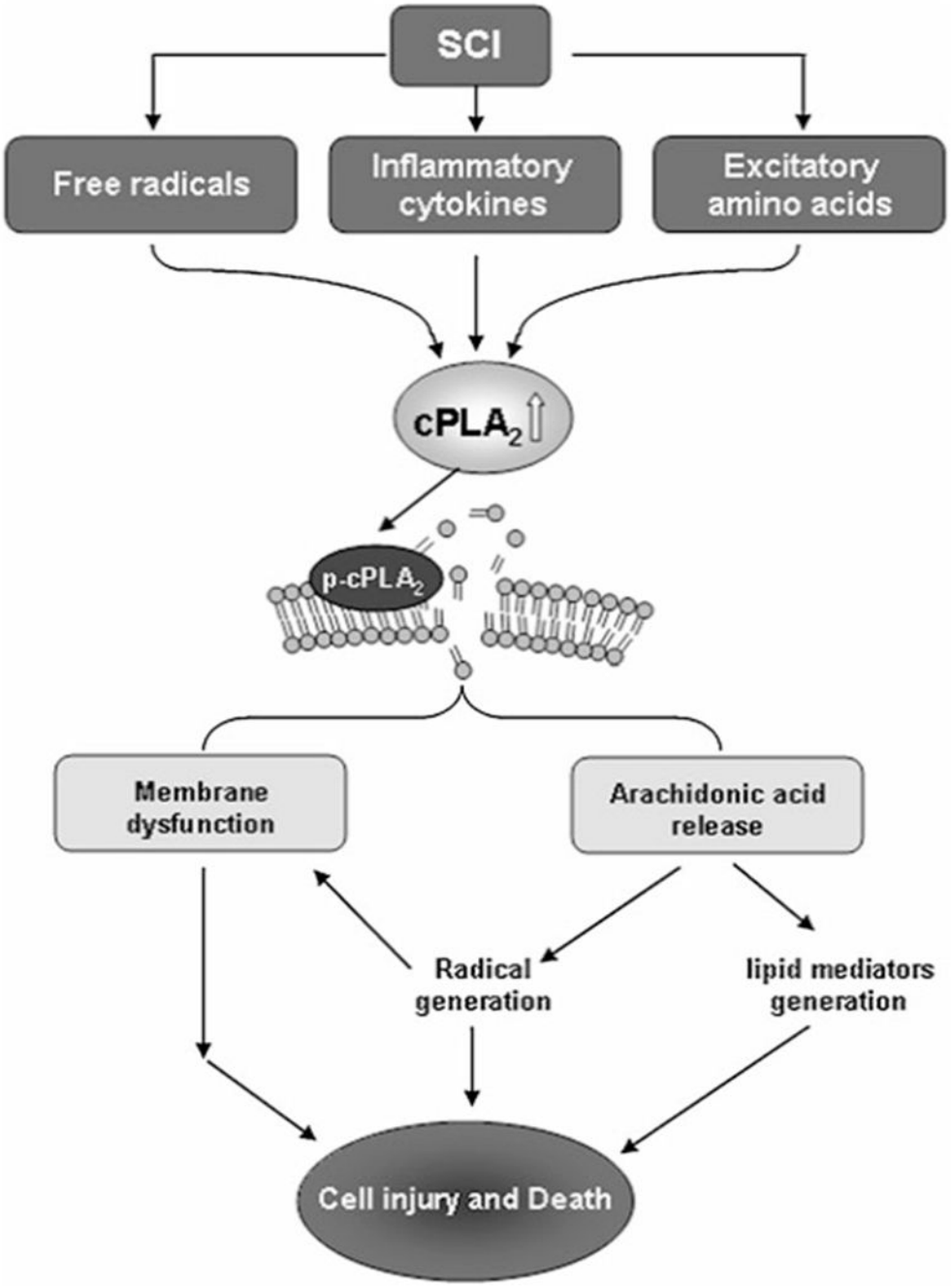

Fig. 2.

Possible mechanisms underlying PLA_2-mediated secondary spinal cord injury. Acute traumatic SCI triggers a secondary injury process mediated by multiple injury inducers including inflammatory cytokines, free radicals, and excitatory amino acids; all these inducers may activate PLA_2. Over-activation of PLA_2 may enhance membrane phospholipid hydrolysis, arachidonic acid release, oxygen free radical generation, eicosanoids production, and ultimately lead to neuronal death

Table 1

A summary of mammalian PLA_2 enzymes

Family	Group	Other name	Size (kD)	Ca^{2+} requirement	Catalytic site	*sn*-2 FA Preference	Spinal cord localization	Human chromosome
$sPLA_2$	IB	Pancreatic PLA_2	14	mM	His/Asp dyad	No	+	12q23-24
	IIA	Synovial PLA_2	14	mM	His/Asp dyad	No	+	1p34-36
	IIC		15	mM	His/Asp dyad	No	+	1p34-36
	IID		14	mM	His/Asp dyad	No	NRA	1p34-36
	IIE		14	mM	His/Asp dyad	No	+	1p34-36
	IIF		16	mM	His/Asp dyad	No	NRA	1p34-36
	III		55	mM	His/Asp dyad	No	NRA	22q
	V		14	mM	His/Asp dyad	No	+	1p34-36
	X		14	mM	His/Asp dyad	No	+	16p12-13
	XII		19	mM	His/Asp dyad	No	+	4q25
$cPLA_2$	IVA	$cPLA_2\alpha$	85	μM	Ser/Asp dyad	AA	+	1q25
	IVB	$cPLA_2\beta$	110	μM	Ser/Asp dyad	To be confirmed	NRA	15
	IVC	$cPLA_2\gamma$	60	None	Ser/Asp dyad	To be confirmed	NRA	19
	IVD	$cPLA_2\delta$	92-93	μM	Ser/Asp dyad	To be confirmed	NRA	15
	IVE	$cPLA_2\varepsilon$	100	μM	Ser/Asp dyad	To be confirmed	NRA	15
	IVF	$cPLA_2\xi$	96	μM	Ser/Asp dyad	To be confirmed	NRA	15
$iPLA_2$	VIA1	$iPLA_2$	84-85	None	Ser/His/Asp triad	No	+	22q13.1
	VIA2	$iPLA_2\beta$	88-90	None	Ser/His/Asp triad	No	+	22q13.1
	VIB	$iPLA_2\gamma$	88-91	None	Ser/His/Asp triad	No	NRA	7q31
	VIC	$iPLA_2\delta$	146	None	Ser/His/Asp triad	No	NRA	NRA
	VID	$iPLA_2\varepsilon$	53	None	Ser/His/Asp triad	No	NRA	NRA
	VIE	$iPLA_2\xi$	57	None	Ser/His/Asp triad	No	NRA	NRA
	VIF	$iPLA_2\eta$	28	None	Ser/His/Asp triad	No	NRA	NRA
PAF-AH	VIIA	Plasma PAF-AH	45	None	Ser/His/Asp triad	Acetate	NRA	N.D.
	VIIB	PAF-AH II	40	None	Ser/His/Asp triad	Acetate	NRA	N.D.
	VIIIA	PAF-AH-Iα_1 (Ib)	26	None	Ser/His/Asp triad	Acetate	NRA	N.D.
	VIIIB	PAF-AH-Iα_2 (Ib)	26	None	Ser/His/Asp triad	No	NRA	11q23

ND not determined, *NRA* no report available, *SC* spinal cord. Adopted from Liu et al. [83] with modifications