Chapter

Edema Induced by sPLA2 from *Crotalus durissus terrificus* Involves PLC and PKC Signaling, Activation of cPLA2, and Oxidative Stress

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Abstract

sPLA2 from *Crotalus durissus terrificus* venom, free of crotapotin (Cdt sPLA2), purified and isolated sPLA2, was able to significantly increase lipid peroxidation, which occurred simultaneously with increased arachidonic acid (AA) metabolism. In addition, MDA and AA levels were elevated at 15 min after Cdt sPLA2 injection and after peak edema (negative control). Thus, oxidative stress and ROS play important roles in the inflammation induced by Cdt sPLA2. On the other hand, edema induced by sPLA2 involves the direct and indirect mobilization of arachidonic acid by the involvement of phosphokinase C (PKC) and phospholipase C (PLC), which indirectly stimulates cytosolic PLA2 (cPLA2). We also observed that the specific antivenin against Cdt venom had no significant effect on the neutralization of induced edema compared to the natural products 5-caffeine-linoleic acid (5CQA) and dexamethasone (AACOCF3). Our results also indicate that there was improvement in the inhibition of edema of natural polyphenolic compounds compared to antivenin or inhibition of the enzymatic activity of sPLA2 due to the fact that 5CQA is a potent antioxidant compound. Thus, our results show a clear correlation between increased arachidonic acid metabolism and oxidative stress.

Keywords: *Crotalus durissus terrificus* (Cdt), secretory snake venom phospholipase A2, edema, PKC, PLC, inflammation, oxidative stress

1. Arachidonic acid "dogma"

Arachidonic acid (ARA) is a 20-carbon chain fatty acid with four methylene-interrupted *cis* double bonds; the first, with respect to the methyl end (omega, ω or n), is located between carbons 6 and 7. Arachidonic acid (AA) has three possible

destinations: participating in the remodeling process of the cell membrane, release into the extracellular medium by diffusion, or its intracellular metabolism [1, 2]. In addition to AA, lysophosphatidic acid (lyso-platelet aggregation factor (PAF)) is another product of the enzymatic hydrolysis of membrane phospholipids, which, in the presence of lyso-PAF acyl transferase, is converted in PAF [3]. PAF is an extracellular lipid signaling molecule involved in a range of cellular activities, including survival, differentiation, cellular proliferation, morphological changes, and migration, among others [4]. Besides, its biological action is mediated by the presence of a cellular receptor (PAF-receptor (PAF-R)) (**Figure 1**). These physiological and pharmacological activities of PAF depend on the presence of its receptors, designated as PAF-R1 to PAF-R6. These receptors are G protein-coupled transmembrane receptors, and recent studies revealed that the PAF-R signaling pathway clearly affects different aspects of tumor progression [5, 6]. In the literature, it is well established that phospholipases A2 (PLA2s) are key enzymes involved in AA generation by hydrolytic digestion of membrane phospholipids. PLA2 is a superfamily

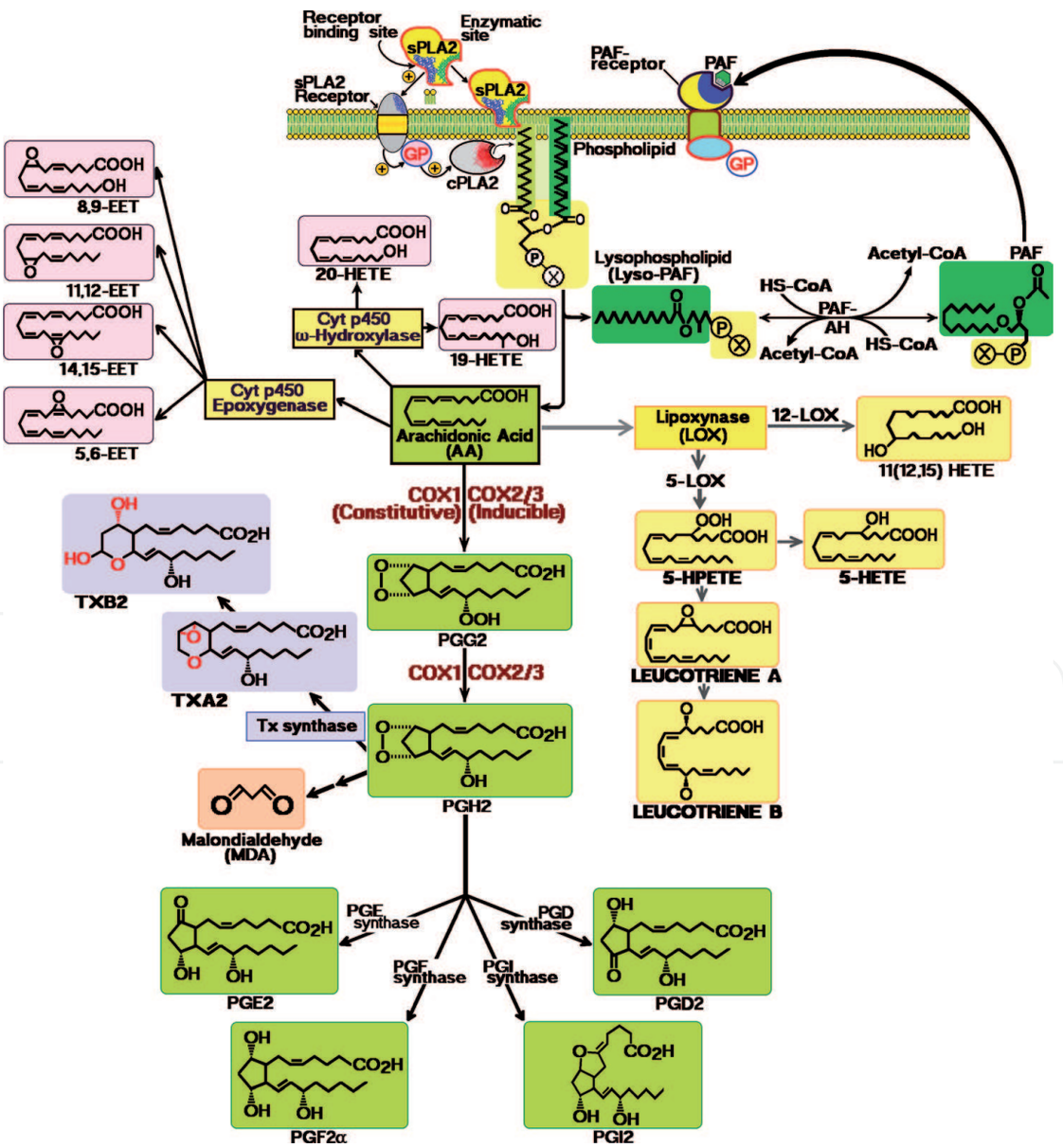

Figure 1.
Central dogma of arachidonic acid metabolism. AA cascade and its destination following three major oxidative pathways: (1) cyclooxygenase (COX), producing prostaglandins and related eicosanoids; (2) lipoxygenase (LOX), forming leukotrienes and related compounds; and (3) CYP450, forming arachidonic acid epoxides.

of enzymes distributed throughout six major classes: secretory PLA2 (sPLA2), calcium-dependent cytosolic PLA2 (cPLA2), calcium-independent cytosolic PLA2 (iPLA2), lysosomal PLA2 (lPLA2), mitochondrial PLA2 (mPLA2), and, more recently, PAF-acetyl hydrolases (PAF-AHs). PAF-AHs are a small family of phospholipases A2 with a high specificity for the hydrolysis of the unsaturated fatty acid residue located at the sn-2 position [7, 8]. sPLA2 is considered a simple and primitive enzyme, acting as an inducer of the inflammatory process, besides being able to act as a pseudohormone. In addition to generating AA directly, this enzyme can also increase the activity of cPLA2 [9]. Furthermore, the produced AA usually follows one of three distinct enzymatic pathways involving cyclooxygenase, lipoxygenase, and cytochrome P450. Several products of these routes can modulate the functions of ion channels, protein kinases, and ion pumps. In addition, newly formed eicosanoids are excreted and mediate various physiological functions, including insulin secretion and muscle contraction, and most of these actions involve protein G. Ultimately, the products of AA metabolism are rapidly degraded [1, 10]. Briefly, AA, as well as other polyunsaturated fatty acids (PUFAs) generated at the cellular level, can be mobilized through the hydrolytic activity of various enzymes. It is possible to highlight the action of PLA2 through a single reaction pathway that produces AA and lysophospholipid (LysP), which is considered the classical pathway of AA generation—it is the most widely known and studied. In addition, AA is metabolized by cyclooxygenase (COX) and 5-lipoxygenase, resulting in the synthesis of prostaglandins and leukotrienes, respectively. These intracellular messengers play an important role in the regulation of signal transduction, leading to pain and inflammatory responses. Recently, the literature has shown that AA can follow a third pathway, resulting in its metabolism by cytochrome P450 enzymes—Cyt450 epoxygenase and Cyt450 omega hydroxylase. P450s are typical monooxygenases, which enzymatically cleave molecular oxygen, followed by the insertion of a single atom of oxygen into the substrate, while the remainder is released as water [11–14]. Cytochrome P450s metabolize AA to produce the collectively designated hydroxyeicosatetraenoic acids and epoxyeicosatrienoic acids; these bioactive compounds are generated in a tissue- and cell-specific manner, and numerous biological functions have been revealed (**Figure 1**).

2. Secretory phospholipase A2

Phospholipase A2 (EC 3.1.1.4, PLA2) belongs to the group of enzymes, which catalyze the hydrolysis of the ester bond at the sn-2 position of glycerophospholipids and, consequently, are capable of generating free fatty acids, including arachidonic acid (AA). Under physiological conditions, PLA2s are crucial for membrane phospholipid homeostasis, ensuring membrane stability, fluidity, and permeability, and they are involved in the regulation of transport processes through the cell membrane. Phospholipases A2 are enzymes widely diffused in bacteria, plants, venom (of various animals), and mammal cells. Several studies suggest that these enzymes can be classified into 19 groups, which have been identified in mammalian tissues. Besides, many of these groups exhibit significant A2 phospholipase enzymatic activity. At a high level, PLA2s can be classified into two groups: cytosolic PLA2 (cPLA2), and a large and diverse group of secretory PLA2s (sPLA2). Cytosolic PLA2 comprises calcium-dependent cPLA2 (cPLA2), calcium-independent cytosolic PLA2 (iPLA2), lysosomal PLA2 (lPLA2), mitochondrial PLA2 (mPLA2), and, more recently, PAF-acetyl hydrolases (PAF-AHs), which display a small family of phospholipases A2 with high specificity for hydrolysis of the unsaturated fatty acid residue located at the sn-2 position [7, 8, 10]. Several

studies suggest that the proinflammatory action induced by mammalian sPLA2 and even snake venom sPLA2 involve a significant increase of both oxidative activity and reactive oxygen species (ROS) in the cell. ROS are involved in processes such as lipid peroxidation and protein carbonylation, which, at certain levels, can lead to pathological events [15]. Studies conducted by Chiricozzi et al. (2010) [16] reveal that there is a relationship between the increased enzymatic activity of sPLA2, which belongs to the IIA family, and a significant cellular production of free radicals, which contribute strongly to the development of neurodegenerative diseases. Snake venom sPLA2 shares similar mechanisms of action and the same pathways of action with mammalian sPLA2. Experimental evidence in the literature demonstrates that both sPLA2 isoforms are able to induce inflammation and other similar biological activities [10, 17–19]. It is noteworthy that literature data demonstrate sPLA2 can activate signaling events that cannot be explained simply by its catalytic activity, and this fact emphasizes that sPLA2 could act essentially as a ligand of a receptor, rather than as an enzyme [20]. In contrast, studies suggest that products generated by sPLA2 may act as second intracellular messengers, and its enzymatic activity provides a crucial point in the biosynthesis pathways of several classes of inflammatory mediators [21]. In addition, studies performed with other sPLA2s suggest that, during the inflammatory process, leukocytes are recruited to the damaged site (via chemotaxis), where there are conditions necessary to produce a "respiratory explosion." This condition is characterized by high oxygen consumption and the production of reactive oxygen species (ROS), such as the superoxide anion radical ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2), which can generate the hydroxyl radical (•OH) directly or indirectly through chemical reactions, such as Fenton and Harber Weiss [22].

Nucleic acids, proteins, and lipids are important targets of ROS, and their attack may lead to an increased risk of mutagenesis due to the modification of these molecules. Moreover, during the inflammatory process, they synthesize soluble mediators, such as arachidonic acid metabolites, cytokines, and chemokines, which lead to the recruitment of more cells that are involved in the inflammatory process to the injured site, thus increasing ROS production. These key mediators may activate signal transduction cascades and induce changes in transcription factors, such as nuclear transcription factor κ-β (NFκ-β) and signal transducer/transcriptional activator 3 (STAT 3), which mediate the response to cellular stress. In addition, induction of cyclooxygenase-2 (COX2) was reported to contribute to nitric oxide synthesis by the enzyme inducible nitric oxide synthetase (iNOS), besides the increased expression of tumor necrosis factor (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and alterations in the expression of specific microRNAs [23, 24]. It should be noted that nitric oxide can form reactive nitrogen species (RNS) that are highly damaging to cells [25, 26]. Signaling of inflammation is recognized globally by IL-1, IL-6, and TNF-α through Toll-like receptors (TLRs), which belong to the IL-1R family. IL-1 and TNF-α represent the proinflammatory cytokine archetypes that are readily released in response to tissue injury or infection, and they represent a programmed recognition system to trigger inflammation [27–29]. It is important to note that although nitric oxide ($NO^{-\bullet}$), generated by iNOS, has been revealed to have an essential role as a cellular marker, in an environment with oxidative stress, it can react with $O_2^{-\bullet}$ to generate peroxynitrite (NOO^-) and other harmful RNS species [26, 30]. Some authors suggest that preventing the formation of NOO- or inducing its efficient decomposition in inflammatory processes may result in a new therapeutic strategy for the treatment of inflammatory processes [30]. In this context, enzymes such as glutathione peroxidase (Gpx) and peroxiredoxin (Prx) appear to have great importance, since they respond to NOO^- decomposition with high efficiency [30–33].

3. Edema induced by sPLA2 from *Crotalus durissus terrificus* involves oxidative stress signaling

There is no significant evidence that enzymatic toxins from snake venom are able to increase cellular oxidative stress during inflammation [34]; there has been neither a molecular nor a physiological connection shown between edema and other pharmacological activities induced by secretory phospholipase A2 from *Crotalus*

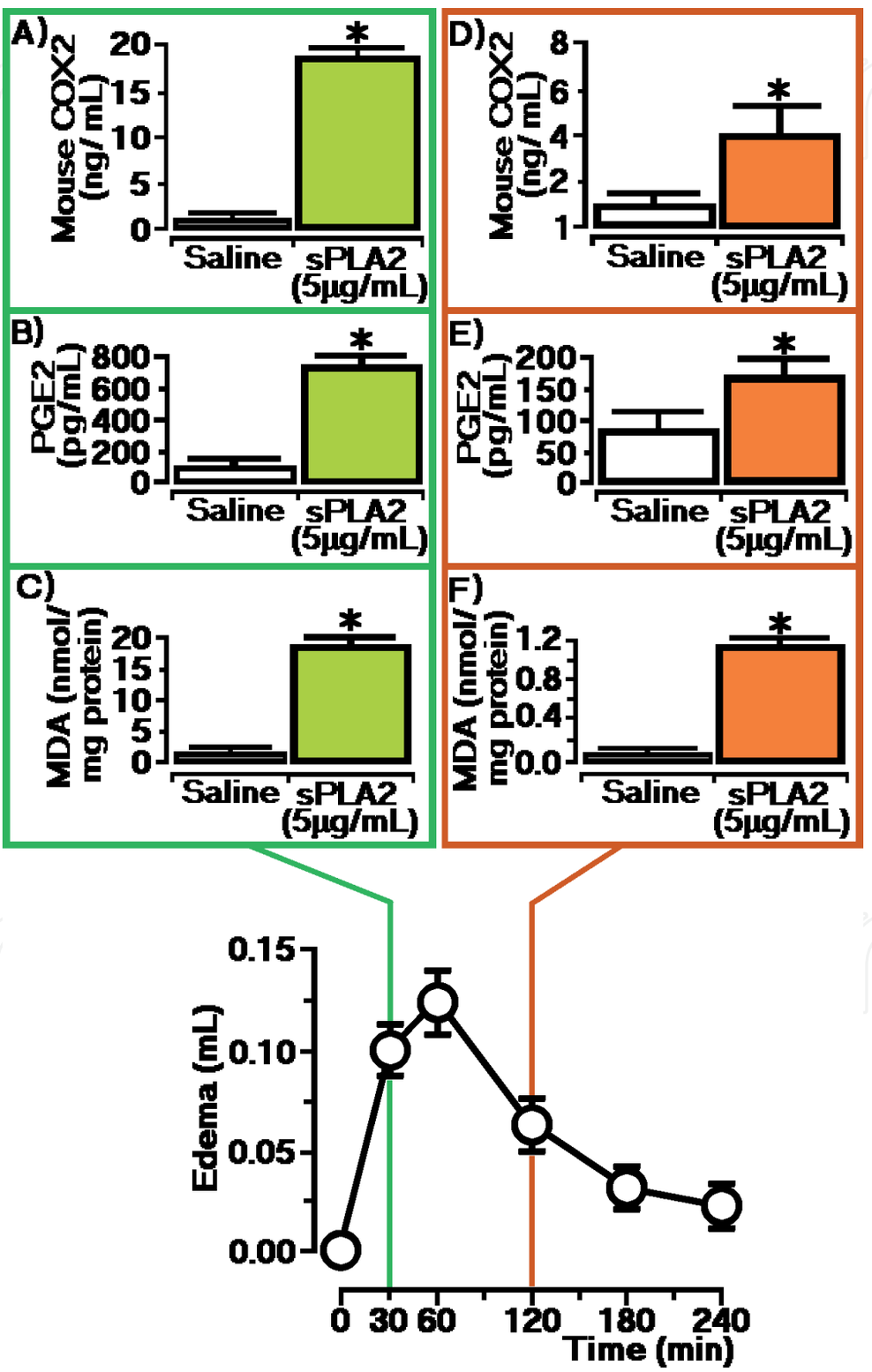

Figure 2.
Edema values induced by Cdt sPLA2 at the adjusted concentration of 10 µg/site (n = 5). Blood and tissue samples were collected from the animals in two phases: at 30 min (B–D) and 90 min (E–F). Measurements of COX2, PGE2, and MDA levels are representative of the analysis of five animals.

durissus terrificus (Cdt sPLA2). However, our results show there is a biochemical, physiological, and temporal connection between the AA metabolism induced by sPLA2, culminating in edema, and the increase of cellular oxidative stress, which was evaluated by measuring malondialdehyde (MDA) content. MDA is a highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and AA metabolism. This compound produced by oxidative stress can interact with several molecules, including proteins, lipoproteins, and DNA. The main source of MDA in biological samples is the peroxidation of polyunsaturated fatty acids with two or more methylene-interrupted double bonds [35, 36]. H_2O_2 represents a messenger capable of altering redox homeostasis, contributing, at various levels, to related inflammatory diseases. Although H_2O_2 is not an inherently reactive compound, it can be converted into highly reactive and deleterious products that kill cells. In this context, several studies have shown that plant phenolic compounds have great neutralization capacity toward hydrogen peroxide, because these compounds can donate electrons to hydrogen peroxide and neutralize it as water [37, 38].

The edema values plotted in **Figure 2A** were obtained by subtracting the edema values induced by saline (negative control). In this work, we evaluated the activity of COX2 and quantified PEG2 and MDA in blood and tissue samples collected at two different time points—30 and 90 min after sPLA2 administration. **Figure 2A** shows that the amount of COX2 present in swollen tissue after a 5 μg/site Cdt sPLA2 injection was 18.7 ± 1.23 ng/mL (n = 5), compared to values resulting from saline injection that were close to zero. In **Figure 2B**, quantification of PGE2 in the blood of animals collected after Cdt sPLA2 injection (5 μg/site) reveals a concentration of 783 ± 32.4 pg./mL (n = 5), while the saline treatment resulted in 65 ± 18.6 pg./mL (n = 5). Thus, the amount of PGE2 was 12-fold higher than the control values. MDA, produced during lipid peroxidation, is widely used for determining oxidative stress, and the results (shown in **Figure 2C**) indicate that the amount of MDA in plasma was 17.82 ± 8.65 nmol, whereas the amount of MDA released after the saline injection was 0.58 ± 0.22 nmol (n = 5). The results presented in **Figure 2A–C** were obtained before the edema peak, and they show that COX2, PGE2, and MDA levels were extremely high in comparison with the control. However, the samples from the material collected at 90 min or after the peak of edema showed that the COX2, PGE2, and MDA levels did not significantly vary from the control (saline), as shown in **Figure 2D–F**.

4. Edema induced by sPLA2 from *Crotalus durissus terrificus* involves PLC and PKC signaling

The metabolism of AA is a crucial point in the course of proinflammatory secretory phospholipase A2 (sPLA2). These enzymes basically have two distinct molecular domains, one involved in catalysis and the other responsible for receptor interaction, which allows sPLA2 to mobilize other enzymes involved in the production of proinflammatory mediators. In addition, studies indicate that sPLA2 receptors can mediate their activity through G-protein, and therefore, they can trigger the activation of phospholipase C (PLC), activating the phosphokinase C (PKC) signaling pathway and leading to potentialization of cytoplasmic PLA2 (cPLA2) and COX2. In **Figure 2A**, we show the effect of the different treatments on edema induced by sPLA2 of *Crotalus durissus terrificus* (Cdt sPLA2). In **Figure 2A**, the results clearly show that the edema peak induced by sPLA2 produces an increase of 0.278 ± 0.016 mL (5 μg/site; n = 5). About 20 μL of PKC inhibitor (GF109203X; Tocris 30 mg/kg, dissolved in 0.5% DMSO) was injected by endovenous route 30 min (n = 5) before administering sPLA2. The PKC inhibitor was able to significantly reduce edema induced by sPLA2, which was 0.123 ± 0.018 mL (n = 5).

About 20 μL of PLC inhibitor (U73122; Tocris; 30 mg/kg, dissolved in 0.5% DMSO) was injected intravenously 30 min prior to application of sPLA2, revealing that the peak of edema was 0.167 ± 0.021 mL (n = 5), which was significantly lower than the edema peak induced by sPLA2. In **Figure 2B**, we show the effect of the specific inhibitor against cPLA2 and COX2. To assess the effect of arachidonyl trifluoromethyl ketone (AACOCF3) (Sigma-Aldrich, 30 mg/kg, dissolved in 0.5% DMSO), each animal received 20 μL of the compound by endovenous route 30 min (n = 5) before injecting sPLA2; there was a significant decrease in the edema, revealing a maximum edema of 0.218 ± 0.018 mL (n = 5). About 20 μL of *N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonamide (NS-398) (Cayman Chemical, 30 mg/kg, dissolved in 0.5% DMSO) was injected intravenously 30 min prior to application of sPLA2, and the peak of the resulting edema was 0.146 ± 0.021 mL (n = 5), which is also significantly lower than the edema peak induced by sPLA2.

The **Figures 1** and **2** show that sPLA2 triggers proinflammatory activity by a signaling pathway involving PKC and PLC. In the case of PLC, two products are generated, diacylglycerol (DAG) and inositol triphosphate (IP3), which can induce

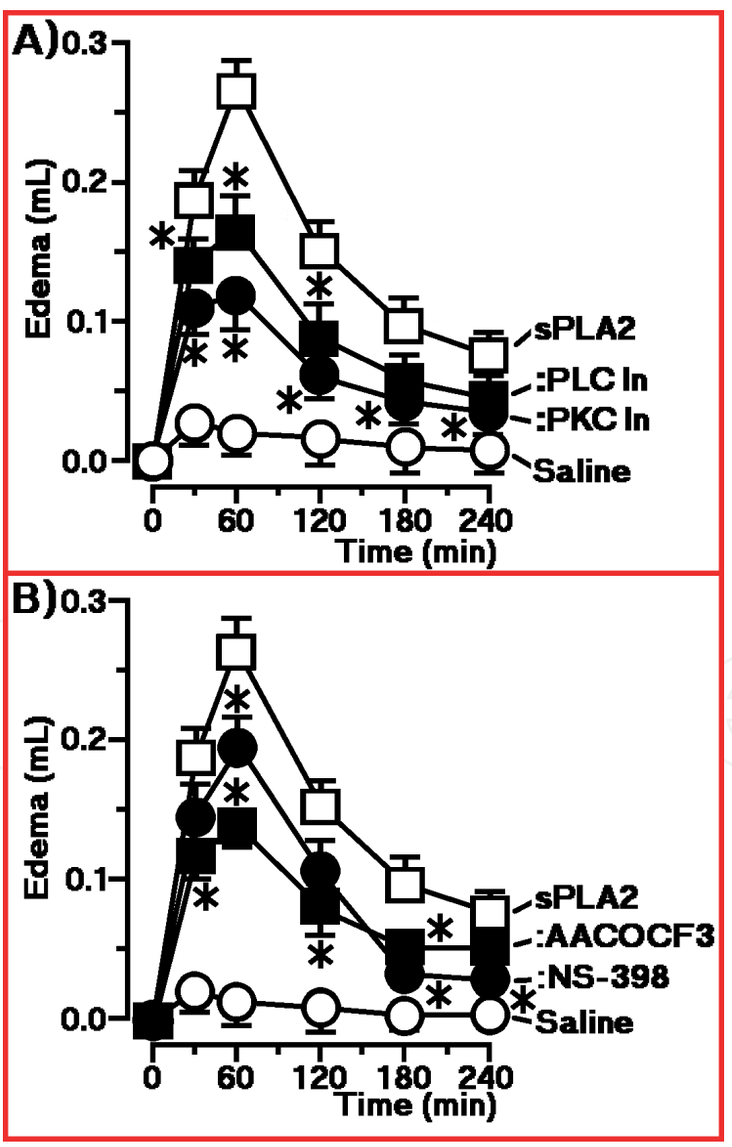

Figure 3.
Values of edema induced by sPLA2 of Cdt at the adjusted concentration of 10 μg/site (n = 5). (A) The effect of the inhibitor of PKC (PKC inhibitor 30′) and inhibitor of PLC (PLC inhibitor 30′). In (B), we evaluated the edema induced by sPLA2 in the presence of a specific inhibitor of cPLA2 (AACOCF3) and inhibitor of COX2 (NS-398).

the phosphorylation of several proteins [14, 39–43]. Thus, the sPLA2 of *Crotalus durissus terrificus* venom may induce an increase in AA metabolism through the interaction of Cdt sPLA2 with G-protein coupled cellular receptors, which activate PLC, generating PUFAs and AA. **Figures 2** and **3** present evidence of interconnections and pathways that generate AA, PLC, and PKC, with cPLA2 and COX2 revealing a possible route of signaling and mobilization of AA, and which could include PUFA release from membrane phospholipids. **Figure 2** also shows that the edema induced by sPLA2 involves the presence of ROS and lipid peroxidation, and that the AA produced can be oxidized to generate MDA as one of the byproducts [39, 44–46]. The results shown in **Figures 2** and **3** suggest that increased cellular oxidative stress and AA mobilization happen intensely and quickly. In this work, we have shown a possible mechanism of edema action induced by sPLA2 from *Crotalus durissus terrificus*, suggesting that the enzymatic activity of Cdt sPLA2 may participate in the inflammatory process, but this activity could also involve the presence of cellular receptors. sPLA2 induces two mechanisms. One mechanism increases oxidative stress, especially in the form of hydrogen peroxide, which leads to increased MDA concentrations; thus, increased oxidative stress has a relevant role in the course of edema. On the other hand, edema induced by sPLA2 also involves a PLC signaling pathway, which mobilizes IP3 (and intracellular calcium) and DAG. These two compounds potentiate the PKC signaling pathway and can lead to a significant increase of cPLA2 through cPLA2 phosphorylation, and this results in enhanced AA metabolism via COX2, an enzyme that could be a second important point in the control of induced inflammation by sPLA2 from *Crotalus durissus terrificus*.

5. "To be or not to be" enzymatically active important for Cdt sPLA2 inflammation

A great question that arises for characterizing the pharmacological and biological activity of Cdt sPLA2 is the importance of the enzymatic activity of sPLA2. For many years, several studies concluded that all biological, physiological, pharmacological, and pathological activity depended on the enzymatic activity of sPLA2, and this remained unanimous until the 1990s. In 1984, the structure and function of the basic sPLA2 of *Agkistrodon piscivorus* were elucidated, leading to the first structural characterization of basic Lys49 sPLA2 [47]. This enzyme also exhibits a moderate enzymatic activity on membrane phospholipids [47]. Subsequently, several works with purified Lys49 basic sPLA2 from snake (*Bothrops sp.*) were able to induce several pharmacological activities, such as pronounced edema, myonecrosis, oxidative stress, nephrotoxicity, insulin degranulation, and anticoagulant activity [17, 48–52]. In the case of the sPLA2 from Cdt, it was observed that its enzymatic activity can be practically abolished through treatment with certain compounds. Numerous natural compounds have the potential to downregulate or modulate the PLA2 activities, as well as other enzymes involved in AA metabolism, including cPLA2 or enzymes involved in prostaglandin metabolization [52–56]. One of the most abundant polyphenols in the human diet, 5-caffeoylquinic acid (5CQA), exerts potent anti-inflammatory, antibacterial, and antioxidant activities. The anti-inflammatory activity of 5CQA may involve multiple mechanisms of action, including the inhibition of the production and secretion of chemical mediators involved in the inflammatory process.

In **Figure 3A**, we show the effect of 5CQA on edema induced by purified sPLA2 from Cdt. When incubated with sPLA2, 5CQA forms a stable molecular complex and may interact with the catalytic site of the protein and strongly decrease its enzymatic activity, changing the secondary structure and leading to the virtual abolishment of sPLA2 enzymatic activity. The edematogenic assay performed with

native sPLA2 and 5CQA incubated with sPLA2 clearly showed that edema induced by sPLA2:5CQA was not abolished, but significantly diminished (**Figure 4A**). Thus, in part, the anti-inflammatory effect of 5CQA probably involves the downregulation of pharmacological and enzymatic activity of sPLA2 [57, 58]. In **Figure 3B**, we show the effect of p-bromophenacyl bromide (p-BPB) and umbelliferone (7-HOC) on edema induced by sPLA2. These data reveal that previous treatment with sPLA2/7-HOC highly decreased the proinflammatory effect induced by sPLA2 purified from Cdt, whereas previous treatment with p-BPB abolished this effect.

Unlike flavonoids, both compounds 7-HOC and p-BPB chemically react with the structure of sPLA2 and form highly stable molecular complexes, both inducing large structural modifications that lead to the virtual abolishment of the enzymatic activity of sPLA2. However, the edematogenic experiments conducted with both compounds incubated with sPLA2 did not abolish the proinflammatory effect induced by the protein, as shown in **Figure 3B**. Thus, in this case, comparison between the results from pharmacological assays suggests that the abolishment of enzymatic activity did not suppress or inhibit the pharmacological effect of sPLA2. This paradox between enzymatic activity and pharmacological effect suggests that at least one more complex pharmacological mechanism is involved in the enzymatic activity, which is independent of the enzymatic activity only. These facts suggest the existence of a distinct pharmacological site, as already proposed by [10, 20].

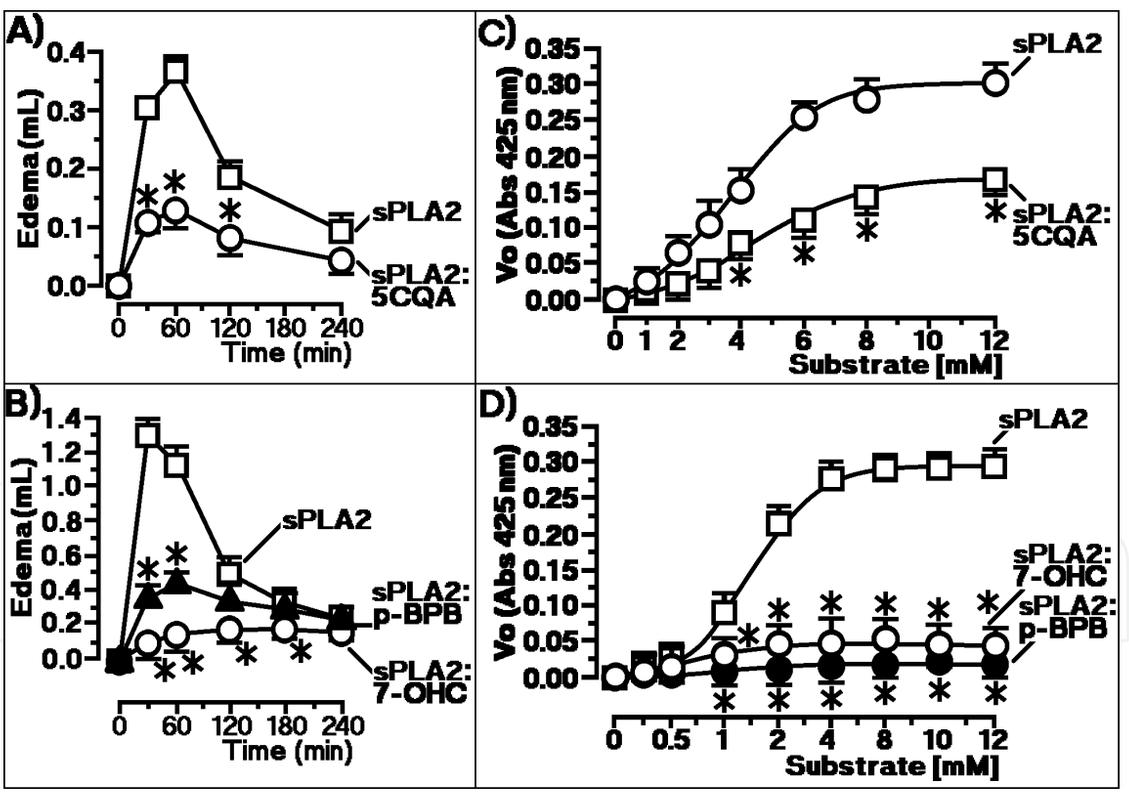

Figure 4.
*In (A), we show paw edema induced after the injection of sPLA2 and sPLA2:5CQA (10 μg/paw) into the right paw of Swiss mice. Measurements were performed after 30, 60, 120, 180, and 240 min, and statistical differences were observed with sPLA2 incubated with 5CQA. In (B), we show enzymatic activity analyzed using 4N3OBA as a substrate, then monitored at a wavelength of 425 nm. In this condition, we examined the effect of the substrate on the enzymatic activity of the native and 5CQA-pretreated sPLA2 (sPLA2:5CQA). Chemical treatment of sPLA2 with 5CQA shifts both the Km and Vmax of the native sPLA2. In (C), we show the mouse paw edema induced by untreated sPLA2 and sPLA2 treated with umbelliferone (sPLA2:7-HOC) or with p-bromophenacyl bromide (sPLA2:p-BPB). Doses of 10 μg/paw were used. Observations were conducted at intervals of 30, 60, 90, 120, and 180 min. (D) Results of enzymatic kinetic analysis of untreated (sPLA2) and 7-HOC- or p-BPB-treated sPLA2 (sPLAs:7-HOC) using 4N3OBA as substrate. sPLA2 Vmax; sPLA2:7-HOC Vmax. For the enzymatic assay results in (B) and (D), each point represents the mean ± SEM of n = 12 and *p < 0.05, and in (A) and (C), each point represents the mean ± SEM of five experiments and *p < 0.05.*

The authors performed several mutagenesis experiments besides those analyzing its catalytic site; there is another pharmacological site located in the calcium binding loop, and the presence of a second pharmacological site has also been considered by [8, 59, 60]. Thus, the enzymatic activity of sPLA2 from Cdt is not crucial for its pharmacological effect and involves other molecular regions, which are collectively designated as pharmacological sites [51, 61]. Some studies performed with sPLA2 from *Crotalus durissus ssp.* showed that the calcium binding loop is involved in the pharmacological activity [57], and others performed by [52] showed that regions close to the active site of sPLA2 could also be involved. According to [54], the C-terminal region could also participate in the interaction with pharmacological receptors. Even so, the crucial and commonly raised point is that the decreased enzymatic activity of Cdt sPLA2 is not accompanied by a proportional decrease in the proinflammatory activity of this enzymatic toxin, as shown by treatment of Cdt sPLA2 with p-BPB (**Figure 4**).

6. Analysis of peroxiredoxins during edema induced by sPLA2 from *Crotalus durissus terrificus*

Oxidative stress is implicated in numerous proinflammatory responses in mammalian cells. H_2O_2 is known to trigger the release and metabolism of AA in various cell types, but the mechanisms involved appear to diverge profoundly from one cell to another. Thus, mobilization of AA in response to oxidative stress appears to be a very complex process involving potentially multiple enzymes and pathways. Studies reveal that the pathological actions induced by sPLA2 from snake venom involve the induction of significant increases in proinflammatory mediators that may also induce a significant rise in reactive oxygen species levels, which can effectively lead to the establishment of numerous events. Thus, the decrease or control of the concentration of these reactive oxygen species may contribute to the decrease of several pathological actions induced by the A2 secretory phospholipase venom. This is evidenced in some studies, such as those that used plant extracts with antioxidant action. The increase in the cellular oxidative process resulting from the mobilization of AA is, in short, associated with the mobilization of H_2O_2 [62–64]; however, this event is not known to be the case for the sPLA2 found in several snake venoms. Some studies show that there is a direct cause and effect relationship between the increased expression of several calcium-dependent PLA2 isoforms and the increased concentration of hydrogen peroxide. Besides, this mechanism involves the presence of G-protein-bound cellular receptors and the consequent protein kinase activation. In addition, much data support the possible existence of cross talk between cPLA2 and sPLA2 while eliciting a full AA release response [63, 65, 66]. During the action of secretory and cytosolic A2 phospholipases, a large amount of AA is produced, which can be considered one of the major components that may be reduced via enzymatic peroxidation to prostaglandins, leukotrienes, thromboxanes, and other cyclooxygenase-, lipoxygenase-, or cytochrome P-450-derived products. Thus, during the process of oxidative stress, AA and other bioactive lipids can be converted into lipid hydroperoxide (LOOH). LOOHs are the primary products of lipid peroxidation, which are relatively stable and long lasting compared to other ROS. Among the many different aldehydes, which can be formed as secondary products during lipid peroxidation, MDA appears to be the most mutagenic [36, 56, 67].

The most accepted paradigm is that oxidative stress initiates a chain reaction of lipid peroxidation, which can be reduced by the presence of tocopherol (e.g., vitamin E) or some other chain-breaking antioxidant. However, several

studies have shown that these antioxidants do not neutralize the oxidized phospholipids that were formed prior to the application of these compounds. Thus, lipid peroxidation is not spontaneously reversible, and enzymatic pathways that return lipids to their reduced states have been described. On the other hand, several authors showed that peroxiredoxins (Prxs), particularly Prx 6, play an essential role in the reduction of H_2O_2 and short hydroperoxides; besides, they can directly reduce phospholipid hydroperoxides. Prxs are thiol-dependent peroxidases that catalyze the reduction of a wide variety of hydroperoxides, and the catalytic activity is provided by the presence of a highly conserved catalytic cysteine residue, whose oxidation by hydroperoxide generates sulfenic acid (Cys-SOH). The Prx reduction mechanism involving Cys-SOH is a matter of debate, with glutaredoxin 2 (GRX2), thioredoxin 3 (Trx3), thioredoxin reductase 2 (Trr2), and ascorbate being proposed as possible reducers [68–70]. Several other studies revealed that, during oxidative stress, several Prxs are overexpressed, which can be used as a sensor of oxidative stress in several cells [71–73]. Thus, Prxs represent a group of antioxidant proteins able to decompose several types of hydroperoxides at rates of $10^{5–8}$ M/second. These enzymes utilize a cysteine residue, which, after the peroxide decomposition, oxidizes (CP-SOH), forming a disulfide bond with a second cysteine, which is reduced by the enzymes thioredoxin (Trx) and thioredoxin reductase (TrxR). In addition, several drugs have been characterized as peroxiredoxin inhibitors, and their use has been helpful in unraveling the physiological and biological roles of certain peroxiredoxins. Among these Prx inhibitors, the best known is adenanthin (Adn), which inhibits Prxs I, Prx II, and other thiol-dependent antioxidant enzymes [74, 75]. Another commercial drug is MJ33, which is described as a potent inhibitor of Prx 6, an extremely essential enzyme for regulating oxidative stress, inflammation, and NADPH oxidase (NOX)2 activation [76]. In addition, conoidin A (ConA) is characterized as a potent inhibitor of peroxiredoxin II, an antioxidant enzyme that acts in the intracellular signaling and defense against oxidative stress [77]. Enzyme inhibition is one of the ways in which enzyme activity is regulated experimentally and naturally.

In the case of the pharmacological tests, inoculation of 5 μg sPLA2 purified from the total venom of *Crotalus durissus terrificus* induced an inflammatory reaction, revealing a typical acute edema with a peak at 60 min (**Figure 5**). To assess the effects of inhibitors, MJ33, ConA, and Adn were injected intraperitoneally (2 μg/g mice) 30 min prior to administration of PLA2 isolated from Cdt venom. As shown in **Figure 5A**, MJ33 showed insignificant anti-inflammatory activity that was only observed along with the edema peak. **Figure 5B** shows the effect of ConA administrated before sPLA2, revealing insignificant inhibition of edema. Although both MJ33 and ConA are essential Prx inhibitors, they display some limitations, as found with MJ33, which is a specific inhibitor of Prx 6. Prx 6 is a complex Prx, exhibiting its maximal antioxidant activity only at acidic pH values [78].

Prx 6 shows calcium-independent phospholipase A2 enzyme activity that is also maximal at acidic pH [79]. The determination of its functional and enzymatic properties was recently elucidated. The low MJ33 inhibitory effect observed in our study could have been due to the presence of a calcium-independent PLA2 domain. Some studies showed that Prx 2 appear to be an essential negative regulator of LPS-induced inflammatory signaling through modulation of ROS synthesis via NADPH oxidase activities; therefore, Prx 2 is crucial for the prevention of excessive host responses to microbial products [80]. Although ConA shows the ability to covalently inhibit Prx 2 activity, the results presented in **Figure 5B** suggest that Prx 2 does not play a relevant role in reducing edema induced by Cdt sPLA2. On the other hand, LPS stimulates monocytes/macrophages through Toll-like receptor

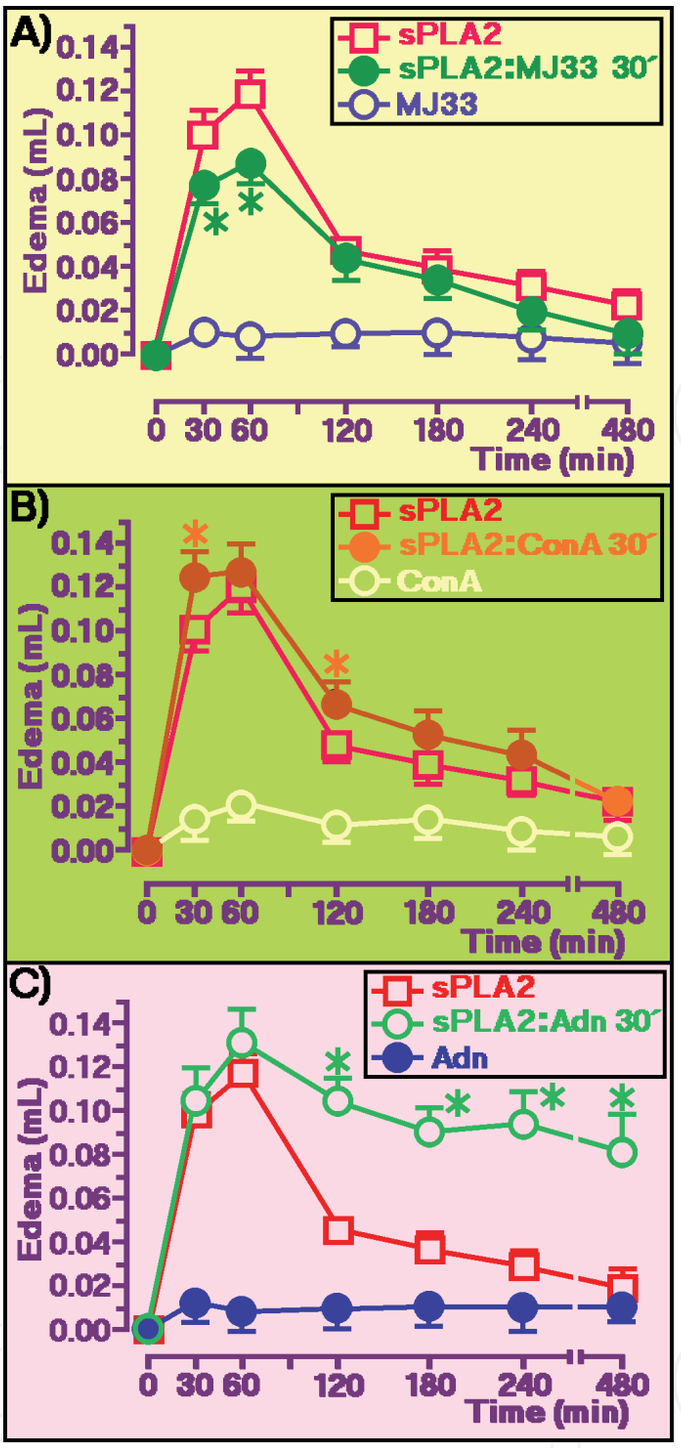

Figure 5.
*In (A), we show paw edema induced after the injection of sPLA2 and sPLA2:MJ33 (5 μg/paw) into the right paw of Swiss mice. Measurements were performed after 30, 60, 120, 180, 240 and 480 min, and statistical differences were observed with sPLA2 applied after MJ33 injection 30 minutes before sPLA2 injection. In (B), we show paw edema induced after the injection of sPLA2 and sPLA2:ConA (5 μg/paw) into the right paw of Swiss mice. Measurements were performed after 30, 60, 120, 180, 240 and 480 min, and statistical differences were observed with sPLA2 incubated with ConA (conoidin A) applied 30 minutes before sPLA2. In (C), we evaluate the effect of sPLA2 in comparison with adenanthin (Adn) previously applied 30 min before sPLA2. Each point represents the mean ± SEM of five experiments and *p < 0.05.*

4 (TLR4), resulting in a series of signaling activation events, which potentiate the production of inflammatory mediators, such as IL-6 and TNF-α [81, 82]. The results presented in **Figure 5C** clearly show that thiol-dependent antioxidant enzymes play an essential role in edema control and recovery induced by sPLA2 purified from

Cdt, and, similar to ConA and MJ33, these enzymes did not exhibit an inhibition or decrease of the edema peaks that occur at 60 min. **Figure 5C** also reveals that the edematogenic effect induced by sPLA2 diminished after 60–90 min, and the hind paw volume returned to its normal volume after 240 min. However, in animals treated with Adn 30 min before the sPLA2 injection, the edematogenic effect persisted for even 8 h after the experiment.

7. Conclusion

During inflammation (edema), induced by purified sPLA2, arachidonic acid generation and its metabolization by COX2 during the edema play crucial roles during this pharmacological event. Arachidonic acid can be mobilized by the catalytic activity of sPLA2 from *Crotalus durissus terrificus* (or other sources) or by activation of cytosolic PLA2. The enzymatic activity of secretory PLA2 (sPLA2) was not crucial for this initial mobilization, and the presence of sPLA2 receptors plays a crucial role in the mobilization of high amounts of arachidonic acid (AA). The classic AA production pathway, which basically involves cPLA2 modulation, also involves the interaction of a more complex pathway that includes the activation of PLC, producing IP3 and DAG. In turn, IP3 and DAG activate PKC, stimulating a strong increase of AA by cPLA2 [1, 83, 84]. However, AA is also mobilized by two other distinct pathways. One involves PLC activation, which has an essential role in AA production by DAG lipase and MAG lipase. In this pathway, catalysis leads to diacylglycerol hydrolysis, releasing a free fatty acid and monoacylglycerol as 2-acyl glycerol, which is converted to AA by MAG lipase action [85–87].

Another pathway that is initiated during AA mobilization involves the release of platelet aggregation factor (PAF)—another subproduct of the enzymatic hydrolysis of membrane phospholipids that cross through the cell membrane—and its specific receptor (PAF receptor or PAF-R) leads to the stimulation of PLC by G-protein [83, 88]. Thus, it is possible that sPLA2 from snake venom, such as venom from *Crotalus durissus terrificus*, mobilizes AA by three different pathways, and AA oxidative metabolism is a key factor that induces increased ROS and oxidative stress during edema. In addition, there are several studies that show AA production is an important way to increase the generation of hydrogen peroxide during inflammation. Thus, it is possible that the action of sPLA2 also increases cell oxidative stress and AA metabolism, culminating in the production of PGE2 and MDA [36]. All this occurs through the interaction of sPLA2 with its receptors to modulate the activity and function of cPLA2 and iPLA2, inducing a significant increase in AA metabolism and COX2 expression, a fact that contributes to the production of free radicals (**Figure 6**) [45, 89–95].

Several studies have shown that arachidonic acid produced by the action of sPLA2 and cPLA2 can activate NADPH oxidase (NOX) enzymes and induce a significant increase in hydrogen peroxide, which gains entry to the intracellular environment through aquaporins and has a predominant role in increasing cellular oxidative stress [91–96]. This would explain the importance of thiol-dependent antioxidant enzymes playing key roles in the control of edema induced by *Crotalus durissus terrificus* sPLA2. On other side, the inflammation (edema) induced by sPLA2 involves the mobilization of arachidonic acid and hydrogen peroxide, and both are the main elements involved in the inflammatory process. The data compiled in this work suggest that oxidative stress is integral in the progression and maintenance of inflammation (edema) induced by sPLA2 from

Crotalus durissus terrificus. Furthermore, our results show that *Crotalus durissus terrificus* sPLA2-induced edema is strongly regulated by thiol-dependent enzymes, and that adenanthin (Adn) was able to neutralize this control and the inflammatory process (edema).

On the other hand, several articles have reported that natural antioxidant compounds, such as flavonoids and related substances, when given prior to sPLA2 injection, have significant anti-inflammatory activities. This probably stems from the ability of many of these compounds to partially inhibit the enzymatic and pharmacological activities of sPLA2 from *Crotalus durissus terrificus*, as well as from their strong antioxidant activities [53–56, 97]. Thus, the search for new natural compounds with anti-inflammatory properties remains an important area of research.

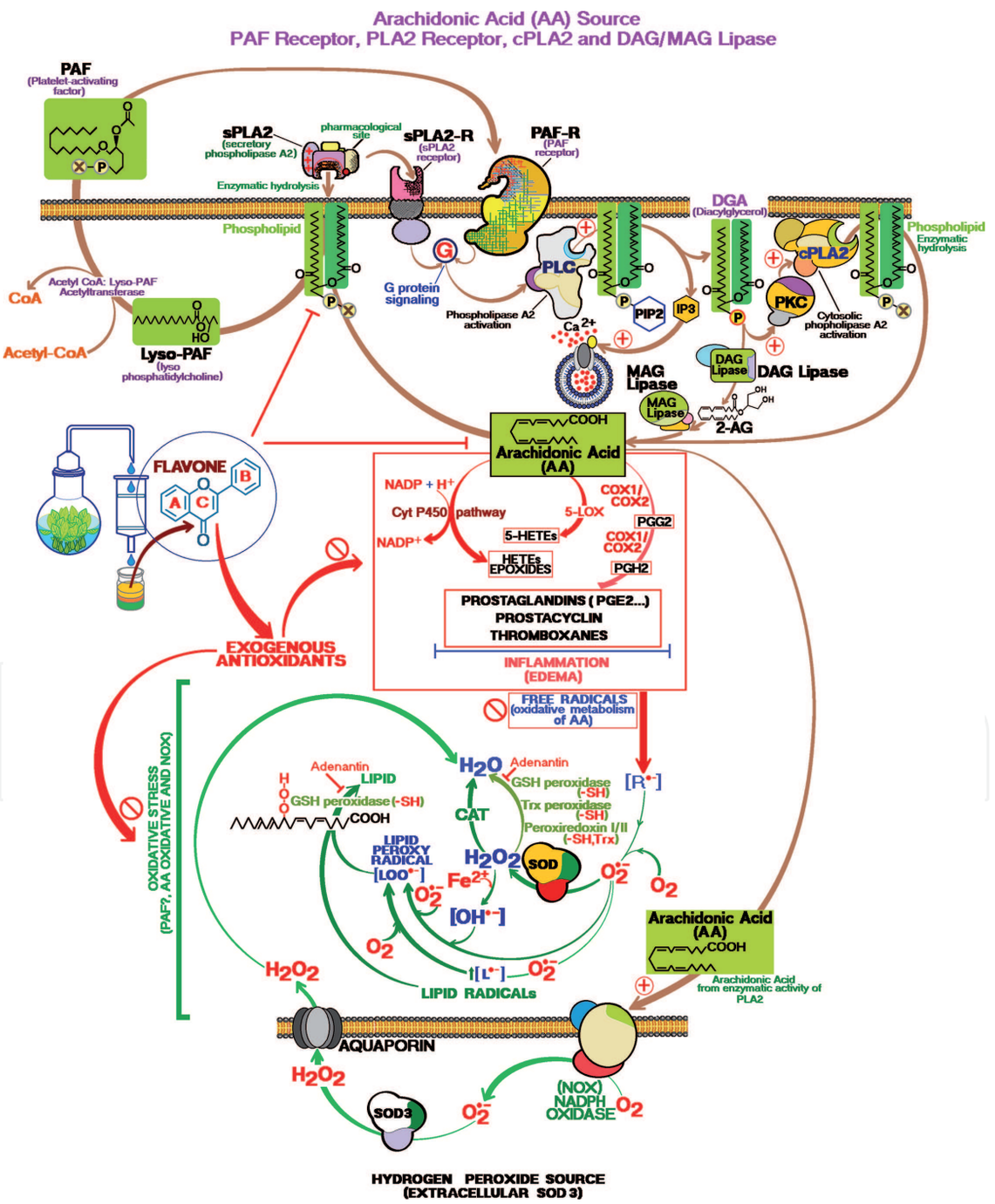

Figure 6.
Summary of possible inflammation mechanism of Cdt sPLA2 action during the inflammatory process.

Acknowledgements

The present project had the financial support of the FAPESP process no: 2017 /20291-0 on the responsibility of Professor M.H. Toyama and the resources coming from the FAPESP process no: 2017/19942-7, to CNPq and UNESP.

Conflict of interest

The authors have no conflict of interests to declare.

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References

[1] Hanna VS, Hafez EAA. Synopsis of arachidonic acid metabolism: A review. Journal of Advanced Research. 2018;**11**:23-32

[2] Liu C et al. Arachidonic acid metabolism pathway is not only dominant in metabolic modulation but associated with phenotypic variation after acute hypoxia exposure. Frontiers in Physiology. 2018;**9**:236

[3] Hosford D, Braquet P. Platelet-activating factor (PAF), P. J. B. T.-E. of I. E. Delves, 2nd ed. Oxford: Elsevier, 1998, pp. 1971-1973.

[4] da Silva IA Jr, Chammas R, Lepique AP, Jancar S. Platelet-activating factor (PAF) receptor as a promising target for cancer cell repopulation after radiotherapy. Oncogene. 2017;**6**:e296

[5] Kasperska-Zajac A, Brzoza Z, Rogala B. Platelet-activating factor (PAF): A review of its role in asthma and clinical efficacy of PAF antagonists in the disease therapy. Recent Patents on Inflammation & Allergy Drug Discovery. 2008;**2**(1):72-76

[6] Xu B et al. Effects of platelet-activating factor and its differential regulation by androgens and steroid hormones in prostate cancers. British Journal of Cancer. 2013;**109**(5):1279-1286

[7] Murakami M, Taketomi Y, Miki Y, Sato H, Hirabayashi T, Yamamoto K. Recent progress in phospholipase A2 research: From cells to animals to humans. Progress in Lipid Research. 2011;**50**(2):152-192

[8] Murakami M, Sato H, Miki Y, Yamamoto K, Taketomi Y. A new era of secreted phospholipase A 2. Journal of Lipid Research. 2015;**56**(7):1248-1261

[9] Yarla NS, Bishayee A, Vadlakonda L, Chintala R, Duddukuri GR, Reddanna P, et al. Phospholipase A2 isoforms as novel targets for prevention and treatment of inflammatory and oncologic diseases. Current Drug Targets. 2016;**17**(16):1940-1962

[10] Murakami M, Lambeau G. Emerging roles of secreted phospholipase A2 enzymes: An update. Biochimie. 2013;**95**(1):43-50

[11] Pompeia C, Cury-Boaventura MF, Curi R. Arachidonic acid triggers an oxidative burst in leukocytes. Brazilian Journal of Medical and Biological Research. 2003;**36**(scielo):1549-1560

[12] Covey TM, Edes K, Fitzpatrick FA. Akt activation by arachidonic acid metabolism occurs via oxidation and inactivation of PTEN tumor suppressor. Oncogene. 2007;**26**:5784

[13] Chen J-K, Capdevila J, Harris RC. Cytochrome P450 Epoxygenase metabolism of arachidonic acid inhibits apoptosis. Molecular and Cellular Biology. 2001;**21**(18):6322-6331

[14] Balboa MA, Balsinde J. Oxidative stress and arachidonic acid mobilization. Biochimica et Biophysica Acta—Molecular and Cell Biology of Lipids. 2006;**1761**(4):385-391

[15] Yagami T et al., Human group IIA secretory phospholipase A2 induces neuronal cell death via apoptosis. Molecular Pharmacology. 2002;**61**(1):114-126

[16] Elena C, Seila F-F, Vincenza N, Angeles A, Pedro BJ, Gianfrancesco G. Group IIA secretory phospholipase A2 (GIIA) mediates apoptotic death during NMDA receptor activation in rat primary cortical neurons. Journal of Neurochemistry. 2010;**112**(6):1574-1583

[17] Fagundes FHR, Aparicio R, dos Santos ML, Filho EBSD, Oliveira SCB,

Toyama DO, et al. A catalytically inactive Lys49 PLA2 isoform from *Bothrops jararacussu* venom that stimulates insulin secretion in pancreatic beta cells. Protein & Peptide Letters. 2011;**18**(11):1133-1139

[18] Ximenes RM et al. Harpalycin 2 inhibits the enzymatic and platelet aggregation activities of PrTX-III, a D49 phospholipase A (2) from *Bothrops pirajai* venom. BMC Complementary and Alternative Medicine. 2012;**12**:139

[19] Murakami M, Taketomi Y. Secreted phospholipase A_2 and mast cells. Allergology International. 2015;**64**(1):4-10

[20] Lambeau G, Gelb MH. Biochemistry and physiology of mammalian secreted phospholipases A2. Annual Review of Biochemistry. 2008;**77**(1):495-520

[21] Farooqui AA, Horrocks LA. Phospholipase A_2-generated lipid mediators in the brain: The good, the bad, and the ugly. Neuroscience. 2006;**12**(3):245-260

[22] Halliwell B, Gutteridge JMC. Reactive species in disease: Friends or foes? In: Free Radicals in Biology and Medicine. 5th ed. Oxford: Oxford University Press; 2015

[23] Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Molecular Cancer. 2013;**12**:86

[24] Kalisperati P et al. Inflammation, DNA damage, helicobacter pylori and gastric tumorigenesis. Frontiers in Genetics. 2017;**8**:20

[25] Szabó C. Hydrogen sulphide and its therapeutic potential. Nature Reviews. Drug Discovery. 2007;**6**:917

[26] Orient A, Donkó Á, Szabó A, Leto TL, Geiszt M. Novel sources of reactive oxygen species in the human body. Nephrology, Dialysis, Transplantation. 2007;**22**(5):1281-1288

[27] Lawrence T. The nuclear factor NF-κB pathway in inflammation. Cold Spring Harbor Perspectives in Biology. 2009;**1**(6):a001651

[28] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radical Biology & Medicine. 2010;**49**(11):1603-1616

[29] Ong ZY et al. Pro-inflammatory cytokines play a key role in the development of radiotherapy-induced gastrointestinal mucositis. Radiation Oncology. 2010;**5**(1):22

[30] Knoops B, Argyropoulou V, Becker S, Ferté L, Kuznetsova O. Multiple roles of peroxiredoxins in inflammation. Molecules and Cells. 2016;**39**(1):60-64

[31] Barros LO, Silva SV, Almeida FC, Silva ECB, Carneiro GF, Guerra MMP. Efeito da adição de glutationa peroxidase e cisteína ao diluidor de congelação do sêmen equino. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2013;**65**(scielo):430-438

[32] Ribas V, García-Ruiz C, Fernández-Checa JC. Glutathione and mitochondria. Frontiers in Pharmacology. 2014;**5**:151

[33] Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. Circulation Research. 2015;**116**(3):531-549

[34] Meléndez-Martínez D et al. Rattlesnake *Crotalus molossus nigrescens* venom induces oxidative stress on human erythrocytes. Journal of Venomous Animals and Toxins including Tropical Diseases. 2017;**23**:24

[35] Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on

malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, Metabolism, and Cardiovascular Diseases. 2005;**15**(4):316-328

[36] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity. 2014;**2014**:360438

[37] Özyürek M, Bektaşoğlu B, Güçlü K, Güngör N, Apak R. A novel hydrogen peroxide scavenging assay of phenolics and flavonoids using cupric reducing antioxidant capacity (CUPRAC) methodology. Journal of Food Composition and Analysis. 2010;**23**(7):689-698

[38] Lee I-T, Lin C-C, Lin W-N, Wu W-L, Hsiao L-D, Yang C-M. Lung inflammation caused by adenosine-5′-triphosphate is mediated via Ca^{2+}/PKCs-dependent COX-2/PGE2 induction. The International Journal of Biochemistry & Cell Biology. 2013;**45**(8):1657-1668

[39] Fonteh AN, Atsumi G, LaPorte T, Chilton FH. Secretory phospholipase A2 receptor-mediated activation of cytosolic phospholipase A2 in murine bone marrow-derived mast cells. Journal of Immunology. 2000;**165**(5):2773-2782

[40] Reséndiz JC, Kroll MH, Lassila R. Protease-activated receptor-induced Akt activation—Regulation and possible function. Journal of Thrombosis and Haemostasis. 2007;**5**(12):2484-2493

[41] Holinstat M et al. Protease-activated receptor Signaling in platelets activates cytosolic phospholipase a(2) (α) differently for cyclooxygenase-1 and 12-lipoxygenase catalysis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;**31**(2):435-442

[42] Carrim N et al. Thrombin-induced reactive oxygen species generation in platelets: A novel role for protease-activated receptor 4 and GPIbα. Redox Biology. 2015;**6**:640-647

[43] Duvernay MT, Matafonov A, Lindsley CW, Hamm HE. Platelet lipidomic profiling: Novel insight into cytosolic phospholipase A2α activity and its role in human platelet activation. Biochemistry. 2015;**54**(36):5578-5588

[44] Sun GY et al. Role of cytosolic phospholipase A(2) in oxidative and inflammatory signaling pathways in different cell types in the central nervous system. Molecular Neurobiology. 2014;**50**(1):6-14

[45] Quach ND, Arnold RD, Cummings BS. Secretory phospholipase A(2) enzymes as pharmacological targets for treatment of disease. Biochemical Pharmacology. 2014;**90**(4):338-348

[46] Soichiro T et al. C-type lectin-like domain and fibronectin-like type II domain of phospholipase A2 receptor 1 modulate binding and migratory responses to collagen. FEBS Letters. 2015;**589**(7):829-835

[47] Chen Y-C, Maraganore JM, Reardon I, Heinrikson RL. Characterization of the structure and function of three phospholipases A2 from the venom of Agkistrodon halys pallas. Toxicon. 1987;**25**(4):401-409

[48] Beghini DG, Toyama MH, Hyslop S, Sodek LC, Novello JC, Marangoni S. Enzymatic characterization of a novel phospholipase A2 from *Crotalus durissus cascavella* rattlesnake (maracambóia) venom. Protein Journal. 2000;**19**(8):679-684

[49] Lee W-H et al. Crystallization and preliminary X-ray diffraction studies of piratoxin II, a phospholipase A2 isolated from the venom of *Bothrops pirajai*. Acta Crystallographica. Section D, Biological Crystallography. 1998;**54**(6 II):1229-1230

[50] Hernandez-Oliveira S, Toyama MH, Toyama DO, Marangoni S, Hyslop S, Rodrigues-Simioni L. Biochemical, pharmacological and structural characterization of a new PLA2 from *Crotalus durissus terrificus* (South American Rattlesnake) venom. Protein Journal. 2005;**24**(4):233-242

[51] Dos Santos ML, Fagundes FHR, Teixeira BRF, Toyama MH, Aparicio R. Purification and preliminary crystallographic analysis of a new Lys49-PLA2 from *B. jararacussu*. International Journal of Molecular Sciences. 2008;**9**(5):736-750

[52] Ximenes RM et al. Inhibition of neurotoxic secretory phospholipases A2 enzymatic, edematogenic, and myotoxic activities by harpalycin 2, an isoflavone isolated from Harpalyce brasiliana Benth. Evidence-Based Complementary and Alternative Medicine. 2012;**12**:139

[53] Iglesias CV et al. Effects of morin on snake venom phospholipase A2 (PLA2). Toxicon. 2005;**46**(7):751-758

[54] Fonseca FV et al. Effect of the synthetic coumarin, ethyl 2-oxo-2H-chromene-3-carboxylate, on activity of *Crotalus durissus ruruima* sPLA2 as well as on edema and platelet aggregation induced by this factor. Toxicon. 2010;**55**(8):1527-1530

[55] Belchor MN et al. Evaluation of rhamnetin as an inhibitor of the pharmacological effect of secretory phospholipase A2. Molecules. 2017;**22**(9):1441

[56] Tamayose CI et al. Non-clinical studies for evaluation of 8-C-rhamnosyl apigenin purified from *Peperomia obtusifolia* against acute edema. International Journal of Molecular Sciences. 2017;**18**(9):1972

[57] Toyama DO, Ferreira MJP, Romoff P, Fávero OA, Gaeta HH, Toyama MH. Effect of chlorogenic acid (5-caffeoylquinic acid) isolated from *Baccharis oxyodonta* on the structure and pharmacological activities of secretory phospholipase A2 from *Crotalus durissus terrificus*. BioMed Research International. 2014;**2014**:1-10

[58] Toyama DDO et al. An evaluation of 3-rhamnosylquercetin, a glycosylated form of quercetin, against the myotoxic and edematogenic effects of sPLA2 from *Crotalus durissus terrificus*. BioMed Research International. 2014;**2014**(341270):11

[59] Chioato L, Ward RJ. Mapping structural determinants of biological activities in snake venom phospholipases A2 by sequence analysis and site directed mutagenesis. Toxicon. 2003;**42**(8):869-883

[60] Dennis EA, Cao J, Hsu Y-H, Magrioti V, Kokotos G. Phospholipase A2 enzymes: Physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chemical Reviews. 2011;**111**(10):6130-6185

[61] Toyama DO, Marangoni S, Diz-Filho EBS, Oliveira SCB, Toyama MH. Effect of umbelliferone (7-hydroxycoumarin, 7-HOC) on the enzymatic, edematogenic and necrotic activities of secretory phospholipase A2 (sPLA2) isolated from *Crotalus durissus collilineatus* venom. Toxicon. 2009;**53**(4):417-426

[62] Balboa MA, Balsinde J. Involvement of calcium-independent phospholipase A2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. The Journal of Biological Chemistry. 2002;**277**(43):40384-40389

[63] Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventre JV. Cross-talk between cytosolic phospholipase A2α (cPLA2α) and secretory phospholipase A2 (sPLA2) in

hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2α activity that is responsible for arachidonic acid release. The Journal of Biological Chemistry. 2003;**278**(26):24153-24163

[64] Sun GY, Shelat PB, Jensen MB, He Y, Sun AY, Simonyi A. Phospholipases A2 and inflammatory responses in the central nervous system. Neuromolecular Medicine. 2010;**12**(2):133-148

[65] Martínez J, Moreno JJ. Role of Ca^{2+}-independent phospholipase A2 on arachidonic acid release induced by reactive oxygen species. Archives of Biochemistry and Biophysics. 2001;**392**(2):257-262

[66] Adibhatla RM, Hatcher JF. Phospholipase a(2), reactive oxygen species, and lipid peroxidation In CNS pathologies. BMB Reports. 2008;**41**(8):560-567

[67] Catalá A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chemistry and Physics of Lipids. 2009;**157**(1):1-11

[68] Wood ZA, Schröder E, Robin Harris J, Poole LB. Structure, mechanism and regulation of peroxiredoxins. Trends in Biochemical Sciences. 2003;**28**(1):32-40

[69] Cordray P, Doyle K, Edes K, Moos PJ, Fitzpatrick FA. Oxidation of 2-Cys-peroxiredoxins by arachidonic acid peroxide metabolites of lipoxygenases and Cyclooxygenase-2. The Journal of Biological Chemistry. 2007;**282**(45):32623-32629

[70] Rhee SG, Woo HA. Multiple functions of peroxiredoxins: Peroxidases, sensors and regulators of the intracellular messenger H_2O_2, and protein chaperones. Antioxidants & Redox Signaling. 2010;**15**(3):781-794

[71] Lee D, Moawad AR, Morielli T, Fernandez MC, O'Flaherty C. Peroxiredoxins prevent oxidative stress during human sperm capacitation. Molecular Human Reproduction. 2017;**23**(2):106-115

[72] Fisher AB. Peroxiredoxin 6 in the repair of peroxidized cell membranes and cell signaling. Archives of Biochemistry and Biophysics. 2017;**617**(Supplement C):68-83

[73] Fisher AB, Vasquez-Medina JP, Dodia C, Sorokina EM, Tao J-Q , Feinstein SI. Peroxiredoxin 6 phospholipid hydroperoxidase activity in the repair of peroxidized cell membranes. Redox Biology. 2018;**14**:41-46

[74] Soethoudt M, Peskin AV, Dickerhof N, Paton LN, Pace PE, Winterbourn CC. Interaction of adenanthin with glutathione and thiol enzymes: Selectivity for thioredoxin reductase and inhibition of peroxiredoxin recycling. Free Radical Biology & Medicine. 2014;**77**:331-339

[75] Siernicka M et al. Adenanthin, a new inhibitor of thiol-dependent antioxidant enzymes, impairs the effector functions of human natural killer cells. Immunology. 2015;**146**(1):173-183

[76] Lee I, Dodia C, Chatterjee S, Feinstein SI, Fisher AB. Therapeutic efficacy of MJ33, a novel inhibitor of phospholipase A2 (PLA2) of peroxiredoxin 6 (Prdx6), in LPS-induced acute lung injury (ALI). FASEB Journal. 2013;**27**(1_supplement):1107.11

[77] Haraldsen JD et al. Identification of conoidin A as a covalent inhibitor of peroxiredoxin II. Organic & Biomolecular Chemistry. 2009;**7**:3040-3048

[78] Chang C-H, Lo W-Y, Lee T-H. The antioxidant peroxiredoxin 6 (Prdx6)

exhibits different profiles in the livers of seawater- and fresh water-acclimated milkfish, Chanos chanos, upon hypothermal challenge. Frontiers in Physiology. 2016;7:580

[79] Manevich Y, Reddy KS, Shuvaeva T, Feinstein SI, Fisher AB. Structure and phospholipase function of peroxiredoxin 6: Identification of the catalytic triad and its role in phospholipid substrate binding. Journal of Lipid Research. 2007;**48**(10):2306-2318

[80] Yang C-S et al. Roles of peroxiredoxin II in the regulation of proinflammatory responses to LPS and protection against endotoxin-induced lethal shock. The Journal of Experimental Medicine. 2007;**204**(3):583-594

[81] Fang H et al. Lipopolysaccharide-induced macrophage inflammatory response is regulated by SHIP. Journal of Immunology. 2004;**173**(1):360-366

[82] Hoareau L et al. Signaling pathways involved in LPS induced TNFalpha production in human adipocytes. Journal of Inflammation (London). 2010;7:1

[83] Tang X, Edwards EM, Holmes BB, Falck JR, Campbell WB. Role of phospholipase C and diacylglyceride lipase pathway in arachidonic acid release and acetylcholine-induced vascular relaxation in rabbit aorta. American Journal of Physiology-Heart and Circulatory Physiology. 2006;**290**(1):H37-H45

[84] Reisenberg M, Singh PK, Williams G, Doherty P. The diacylglycerol lipases: Structure, regulation and roles in and beyond endocannabinoid signalling. Philosophical Tansactions of the Royal Society of London. Series B, Biological Sciences. 2012;**367**(1607):3264-3275

[85] Labar G, Wouters J, Lambert DM. A review on the monoacylglycerol lipase: At the Interface between fat and endocannabinoid signalling. Current Medicinal Chemistry. 2010;**17**(24):2588-2607

[86] Chang JW et al. Remarkably selective inhibitors of monoacylglycerol lipase bearing a reactive group that is bioisosteric with endocannabinoid substrates. Chemistry & Biology. 2012;**19**(5):579-588

[87] Grabner GF, Zimmermann R, Schicho R, Taschler U. Monoglyceride lipase as a drug target: At the crossroads of arachidonic acid metabolism and endocannabinoid signaling. Pharmacology & Therapeutics. 2017;**175**:35-46

[88] Soliman ML, Ohm JE, Rosenberger TA. Acetate reduces PGE(2) release and modulates phospholipase and cyclooxygenase levels in neuroglia stimulated with lipopolysaccharide. Lipids. 2013;**48**(7):651-662

[89] Rupprecht G, Scholz K, Beck K-F, Geiger H, Pfeilschifter J, Kaszkin M. Cross-talk between group IIA-phospholipase A(2) and inducible NO-synthase in rat renal mesangial cells. British Journal of Pharmacology. 1999;**127**(1):51-56

[90] Patel MI et al. Cytosolic phospholipase a(2)-α: A potential therapeutic target for prostate cancer. Clinical Cancer Research. 2008;**14**(24):8070-8079

[91] Drummond GR, Selemidis S, Griendling KK, Sobey CG. Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. Nature Reviews. Drug Discovery. 2011;**10**(6):453-471

[92] Nisimoto Y, Diebold BA, Constentino-Gomes D, Lambeth JD. Nox4: A hydrogen peroxide-generating oxygen sensor. Biochemistry. 2014;**53**(31):5111-5120

[93] Vieceli Dalla Sega F et al. Specific aquaporins facilitate Nox-produced hydrogen peroxide transport through plasma membrane in leukaemia cells. Biochimica et Biophysica Acta—Molecular and Cell Research. 2014;**1843**(4):806-814

[94] Montezano AC, Touyz RM. Reactive oxygen species, vascular Noxs, and hypertension: Focus on translational and clinical research. Antioxidants & Redox Signaling. 2014;**20**(1):164-182

[95] Lennicke C, Rahn J, Lichtenfels R, Wessjohann LA, Seliger B. Hydrogen peroxide—Production, fate and role in redox signaling of tumor cells. Cell Communication and Signaling: CCS. 2015;**13**:39

[96] Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. Redox Biology. 2017;**11**:613-619

[97] Ximenes RM et al. Harpalycin 2 Inhibits the Enzymatic and Platelet Aggregation Activities of PrTX-III, a D49 Phospholipase A. BMC Complementary and Alternative Medicine. 2012;**12**:139