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Significance of arachidonic acid in ocular infections and inflammation

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Abstract

Innate immune responses in the cornea mainly play an important role to mobilize multiple interrelated pathways of corneal lipid, which involve in inflammatory corneal diseases. Signaling lipid mediators derived from arachidonic acid (AA) control cell proliferation, apoptosis, metabolism, and migration, are known as eicosanoids, phosphoinositides, sphingolipids, and fatty acids. Emerging evidences have highlighted the implication of lipid mediators in both injury and repair mechanisms in the cornea. Recently, the role of AA and its metabolites to induce proinflammatory mediators and inflammatory cell infiltration in the pathogen-infected cornea and to cause severe keratitis have been revealed. In this review, we focus on the novel roles of AA downstream signaling in the corneal inflammatory diseases and also the biological relevance of AA signaling in the therapeutic strategies for targeting sight-threatening diseases.

Keywords

Innate immune; Arachidonic acid; Lipid; Cornea; Inflammation; cPLA_{2α}; *Acanthamoeba*; Keratitis; MIP-133; TLRs

Introduction

Arachidonic acid (AA, C₂₀H₃₂O₂) is an omega-6 (n-6) polyunsaturated fatty acid (PUFA), esterified on the *sn*-2 acyl position of phospholipids (especially, phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositides) in the outer and intracellular membranes of mammalian cells^[1]. Arachidonic acid serves as the precursor for the bioactive lipid mediators, the eicosanoids. The eicosanoid biosynthesis in mammalian cells is induced by the activation of phospholipase A₂ (PLA₂) and the release of AA from membrane phospholipids upon stimulation by growth factors, cytokines or mechanical trauma^[1, 2]. Arachidonic acid is subsequently transformed to eicosanoids by cyclooxygenase (COX, which produces prostaglandins [PGs] and thromboxane [TX]), lipoxygenase (LOX, that

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Conflict of Interests

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leads to the formation of leukotriens [LTs]), and the cytochrome P450 monooxygenases (CYP P450, which generates hydroxyeicosatetraenoic acid [HETEs] and epoxyeicosatetraenoic acid [EETs]) pathways ^[1, 2] (Fig. 1).

Eicosanoids (PGs, LTs, and TX) production is considerably increased during inflammation. They interact to the specific G protein-coupled receptors (GPCRs) and trigger their biological effects ^[1]. Eicosanoids are naturally acknowledged for their capacity to provoke biological responses, vascular homeostasis, protection of the gastric mucosa and platelet aggregation, regulation of immunopathological processes ranging from inflammatory responses to chronic tissue remodeling, asthma, rheumatoid arthritis, cancer, and autoimmune disorders ^[3]. Metabolites of AA are well known in several biological processes which are important for the physiological responses and the pathological processes of the cell ^[4–11] (Fig. 2).

Eicosanoids regulate many important aspects of immunity, including pathogen recognition through the extracellular and the intracellular receptors (Fc, mannose or Toll-like receptors [TLRs]), antibody formation, cytokine production, cell proliferation, differentiation, migration, and antigen presentation ^[3]. Cells of the innate immune system, such as dendritic cells (DCs), macrophages, and polymorphonuclear neutrophils (PMNs) produce eicosanoids which act at nanomolar concentrations on target cells ^[3]. Immune cells trigger their effects in an autocrine and paracrine fashion, and affect the function of neighboring cells. It has been documented that eicosanoids and their specific receptors cooperate with other important signaling molecules, mainly cytokines and chemokines, and have an important role in regulating physiological processes in both homeostatic and inflammatory conditions ^[3]. Biosynthetic pathways of eicosanoid production are clinically relevance because their derivatives are associated in the pathogenesis of several immune related disorders. Thus, numerous therapeutic approaches based on eicosanoids and their receptors are being used, and several are on the horizon; however, pharmacological inhibition of eicosanoid biosynthesis might simultaneously be beneficial and harmful ^[3].

We will discuss the emphasized properties of AA and its derived eicosanoids, and their increasing role in disease pathogenesis, focusing their connection in inflammatory corneal diseases.

Role of innate immune system in the recruitment of arachidonic acid signaling

Involvement of innate immunity in the activation of signaling pathways of eicosanoids' production in cells is not completely understood; however, it is clear that the calcium-dependent cytosolic group IVA PLA_{2α} (cPLA_{2α}) is an important enzyme in AA mobilization, and that, depending on cell type and activation conditions, regulatory cross-talk mechanisms exist between cPLA_{2α}, and Ca²⁺-dependent secreted PLA₂ (sPLA₂), and Ca²⁺-independent cytosolic PLA₂ (iPLA₂) enzymes present in the cells ^[12]. Numerous agents that exert effects on cells through innate immune receptors provoke a series of signals which lead to increased PLA₂ activity ^[12]. The PLA₂ signaling mechanism developed over the years for major immunoinflammation (Fig. 3). This schematic presentation contemplates

a scenario where the concerted role of two distinct PLA₂s lead to a complete AA signaling. cPLA_{2α} seems to be dominant over sPLA₂ action in AA release; however, sPLA₂ may primarily act to amplify the inflammatory response by providing additional signals for full activation of cPLA_{2α}, the reader is kindly referred to reference [12] for the depth review on the subject. Recently, we investigated the cPLA_{2α} induced AA signaling in the pathogenesis of *Acanthamoeba* keratitis. This signaling is effectively attenuated by cPLA_{2α} specific inhibitors which therapeutically mitigate corneal inflammatory responses *in vivo* Chinese hamster in *Acanthamoeba* infection [13, 14] (Fig. 4).

Toll-like receptors (TLRs) are important tool of innate immune system of cells and constituents of the first line of defense against invading pathogens. TLRs recognize a broad range of pathogens via their specific family members based on amino acid sequences, including TLR1-TLR10 (all related to human); TLR11-TLR13 (all related to mice) [15, 16]. Out of all, only TLR3 and TLR9 families express exclusively in endosomal compartments and are recognized as intracellular receptors [17]. Recognition of invading pathogens by TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9 in various cell types and AA mobilization via the stimulation of cPLA_{2α} and sPLA₂ signaling, have been extensively studied [18–37]. We investigated the recognition of TLR4 by pathogenic but not non-pathogenic species of *Acanthamoeba* in human corneal epithelial (HCE) cells and Chinese hamster corneal epithelial (HCORN) cells *in vitro* and *in vivo* Chinese hamster corneas [38]. This recognition induces inflammatory responses in cornea and demonstrates TLR4 signaling in the pathogenesis of *Acanthamoeba* keratitis (Fig. 5). This study was not focused to investigate the involvement of AA downstream signaling activated through TLR4 recognition by *Acanthamoeba* trophozoites; however, it provoked further study to explore TLR4 induced AA signaling in corneal inflammation by pathogenic species of *Acanthamoeba* which supported by the previous investigations [30–32, 35] that TLR4 regulates cPLA_{2α}-AA downstream signaling in inflammation.

Arachidonic acid in cornea

Most of the available evidence about AA metabolites in the eye arises from studies on the conjunctiva, uvea, and intraocular pressure (IOP) [39–42]. However, Bito et al. [41] and Taylor et al. [43] explored the AA metabolites formation in isolated corneal epithelial and corneal fibroblast cells. Bazan et al. [44] demonstrated that in healthy cornea, prostaglandin E₂ (PGE₂) is the chief cyclooxygenase product produced in the stroma, while thromboxane B₂ (TXB₂) predominated in the endothelium and epithelium; moreover, the main lipoxygenase product, mono-hydroxyeicosatetraenoic acid (mono-HETE) and 12-HETE, were detected in the epithelium and in the stroma, respectively. While lipoxygenase products could not be detected in control endothelium. In contrast of healthy cornea, membrane lipid in epithelium and endothelium decreased after injury, these decreases were observed in phosphatidylinositol, phosphatidylcholine, and phosphatidylethanolamine. However, at the same time, cyclooxygenase reaction products, particularly prostaglandin F_{2α} (PGF_{2α}) was increased in the epithelium. Moreover, they observed that prostaglandins (6-keto-PGF_{1α}, TxB₂, PGF_{2α}, and PGE₂) levels in the stroma and in the epithelium; while PGF_{2α}, PGE₂, and PGD₂ were increased in endothelium after injury. Increased lipoxygenase products were observed in the endothelium, stroma, and epithelium after wounding. Thus, Bazan et al. [44]

first time demonstrated that the metabolism of AA is altered by cryogenic injury and resulting changes differ in the corneal epithelium, stroma, and endothelium.

Hurst et al. [45] further demonstrated that (12S)-HETE, but not (12R)-HETE, is accumulated in the corneal all layers (epithelial, stromal, and endothelial) in response to alkali injury and cryogenic injury. These findings support Bazan et al. [44] studies and reflecting the possibility of activation of 12-lipoxygenase. Gupta et al. [46] showed that lipoxygenase activity is a significant factor in regulating corneal epithelial wound healing in the rat, by influencing epithelial cell migration. Also, Nakamura et al. [11] demonstrated 5-lipoxygenase involvement in corneal epithelial migration. Stoltz et al. [47] showed that corneal epithelial hypoxia in response to contact lens wear outcomes in the time-dependent formation of NADPH-CYP P450-dependent arachidonate metabolites, (12R)-HETE and (12R)-HETrE. Biologically (12R)-HETE blocks Na^+/K^+ -ATPase, augments corneal thickness, and diminishes IOP, while (12R)-HETrE causes vasodilation, neutrophil chemoattraction, and angiogenesis. However, origin of 12-HETE and 12-HETrE had a debate as they arise from the fact that both lipoxygenase and CYP P450 have the ability to synthesize these two compounds from AA. Whereas lipoxygenase produces only the (S) enantiomer, CYP P450 has the capacity to synthesize a racemic mixture with the (R) enantiomer predominating in the corneal epithelium of certain species (e.g., porcine) as well as in hepatic tissues, ciliary epithelium, and inflamed epidermis [47]. Moreover, cyclooxygenase or lipoxygenase inhibitors (indomethacin, diclofenac, and BW755C) were unaffected on the formation of 12-HETE and 12-HETrE while their formation is blocked by CYP P450 enzyme blockers such as SKF-525A, carbon monoxide (CO), and clotrimazole [47]. For the detailed study of CYP P450 in AA metabolism, reader requested for the review reference [48].

Recently, cyclooxygenase-2 (COX-2) expression was revealed in all the corneal layers, epithelium, stromal cells, and endothelium in keratitis dog eyes [49]. Takahira et al. [50] showed that the application of AA blocked the inactivating voltage-gated K^+ current and increased the noisy, sustained K^+ current in the rabbit corneal epithelium and revealed an inactivating voltage-gated K^+ channel in the corneal epithelium. Arachidonic acid metabolism is essential for the functional activity of cornea. Thus, further investigations are needed to explore the biochemical roles of AA metabolites in the biology of cornea.

Cytosolic Ca^{2+} -dependent $\text{PLA}_{2\alpha}$ -induced arachidonic acid signaling in corneal epithelial cell-derived proinflammatory mediators

Cytosolic Ca^{2+} -dependent $\text{PLA}_{2\alpha}$ (c $\text{PLA}_{2\alpha}$) out of 5 sub-groups, α - ζ [51–53], has been investigated comprehensively because it is the only PLA_2 that shows specificity for hydrolysis of *sn*-2 arachidonic acid (AA) from membrane phospholipids for eicosanoids biosynthesis in response to diverse extracellular stimuli, [54, 55] and is controlled by phosphorylation and an intracellular calcium ($[\text{Ca}^{2+}]_i$) increase [51]. Phosphorylation of c $\text{PLA}_{2\alpha}$ by MAPKs is required for c $\text{PLA}_{2\alpha}$ -induced AA production in stimulated cells [54, 55]. Several studies revealed the twofold role of PLA_2 s in ocular diseases, which may be associated to their enzymatic activities or to regulatory roles comprising signaling and protein-protein interactions [56]. We observed the functional activity of c $\text{PLA}_{2\alpha}$ *in vitro* HCE cells and HCORN cells, and *in vivo* Chinese hamster corneas which induces

pathogenesis of *Acanthamoeba* keratitis [13, 14]. We investigated that cPLA_{2α} enzyme activity at both gene expression and protein production level is significantly upregulated by *Acanthamoeba-cytopathic* protein (MIP-133) in HCE and HCORN cells [13, 14].

MIP-133 is a mannose-induced 133 kDa serine protease of *Acanthamoeba* which secretes upon interaction of *Acanthamoeba* with a mannosylated protein on corneal epithelial cells. Enzyme activity of cPLA_{2α} induced by MIP-133 is significantly inhibited by specific inhibitors (AACOCF3 and MAFP) of cPLA_{2α} [13, 14] (Fig. 6A). We confirmed the MIP-133 induced upregulation of cPLA_{2α} by inhibition with chicken anti-MIP-133 antiserum [13]. Further to explore if cPLA_{2α} is involved in MIP-133-induced AA secretion from corneal epithelial cells, we observed the effect of cPLA_{2α} inhibitors (AACOCF3 and MAFP) on AA secretion. Results demonstrated that AACOCF3 and MAFP significantly diminished AA secretion stimulated by MIP-133 from HCE cells [13] (Fig. 6B). Our findings suggested that cPLA_{2α} pathway is involved in AA release from corneal epithelial cells stimulated with MIP-133 [13]. Moreover, we observed that functional activity of cPLA_{2α}-induced AA release signaling in corneal epithelial cell-mediated proinflammatory mediators. In addition, *P. aeruginosa* challenged upregulation of proinflammatory cytokines/chemokines (IL-8, TNFα, IL-1β, and IL-6) gene expression have been revealed in HCE cell lines [57]. We demonstrated that the pretreatment of HCE and HCORN cells with MAFP and AACOCF3 inhibits protein production of cytokines/chemokines (IL-8, IL-1β, IL-6, IFN-γ, and CXCL2) [13, 14] (Fig. 7 and 8).

Results of our study suggested that activation of cPLA_{2α}-AA pathway stimulated by MIP-133 is responsible for the upregulation of proinflammatory cytokines/chemokines in cornea [13, 14]. Thus, these findings indicate a novel pathogenic mechanism of *Acanthamoeba* keratitis mediated via cPLA_{2α}-AA pathway.

Arachidonic acid in the accumulation of inflammatory cells

The cornea is a nonvascular that is significantly dissimilar from other tissues of eye in regard to numerous function and biological response to a wide variety of extracellular stimuli [58]. Cytosolic Ca²⁺-dependent PLA_{2α} activation may have both valuable and damaging inflammatory effects on the cornea, depending on the efficacy and period of the host inflammatory response. Cytosolic Ca²⁺-dependent PLA_{2α} may involve in the critical balance between eliminate the microorganism to generate a successful inflammatory response in cornea and in corneal scarring and blindness by inducing an excessive inflammatory response [58]. Leukocyte recruitment is characteristic of corneal inflammation, and these cells are known to be a source of cyclooxygenase (COX) and lipoxygenase (LOX) products. In corneal stimulation, polymorphonuclear leukocytes (PMNs) and lymphocytes can enter the corneal stroma from the vascular space within hours. These cells may become resident in a seemingly healed corneal epithelium. Mediators released by the injured corneal epithelium may provide a chemotactic signal to vascular PMNs. COX and LOX products were increased over control levels in the three corneal layers, especially in the corneal stroma, suggesting a persistence of PMNs associated with inflammation. PMNs and macrophages have been recognized in the stroma after a viral infection in the cornea. PMNs

were also found in rabbit corneal stroma following complete reepithelialization after mechanical denudation [59–63].

We showed that MIP-133 interacts with the corneal epithelial cells, and this interaction stimulates a rapid immune response by the production of cytokines/chemokines (IL-8, IL-6, IL-1 β , and IFN- γ) which can recruit an effective host response to corneal infections [13]. Histologic features of the Chinese hamster corneas infected with *Acanthamoeba castellanii* and treated with cPLA_{2 α} inhibitors (AACOCF3 and CAY 10650) confirmed the efficacy of cPLA_{2 α} inhibitors treatment. The results indicated that inflammatory responses in the BSA-treated group were significantly more severe than either AACOCF3 or CAY10650-treated or untreated Chinese hamsters [14] (Fig. 9A). Treatment with the AACOCF3 and CAY10650 had an intense effect on the severity of keratitis (Fig. 9B). Moreover, Chinese hamsters treated with AACOCF3 had significantly less severe keratitis as compared with CAY10650 group. Observation of clinical severity of disease indicated that a cPLA_{2 α} inhibitor can be used as a therapeutic target in *Acanthamoeba* keratitis [14].

It has been revealed that corneal lesions are severely infiltrated with neutrophils in *Acanthamoeba* and *Pseudomonas aeruginosa* keratitis [64, 65]. Neutrophils are infiltrated to the cornea in response to the chemokine, CXCL2, which is secreted from corneal cells during infection [66, 67]. We have demonstrated that corneal infections with *Acanthamoeba* trophozoites stimulate CXCL2 production, and promotes the neutrophils infiltration to the infected cornea [38, 68]. We also showed that blockers of cPLA_{2 α} significantly inhibited CXCL2 production from HCORN cells [14]. The increased production of CXCL2 and its inhibition by AACOCF3 and MAFP, suggested that cPLA_{2 α} plays an important role in controlling chemokine production. Moreover, cPLA₂ signaling has been shown to be involved in the expression of CXCL2 through cPLA_{2 α} -12/15-LOX pathway [69]. CXCL2 has a chemotactic effect on neutrophils and stimulates neutrophil infiltration [68]. In addition, macrophages and neutrophils are the first-line of defense in active *Acanthamoeba* keratitis infections; elimination of these cell types drastically changes the result of *Acanthamoeba* keratitis [68]. However, the signaling pathways involved in attracting neutrophils and macrophages into the infected cornea in *Acanthamoeba* keratitis are not completely understood.

It has been demonstrated that in the stroma, the lipoxygenase pathway resulted in an increased accumulation of 5- and 12-lipoxygenase products, which are known to be potent chemotactic agents for PMNs; these substances may play an imperative role in the initiation of PMNs migration in corneal stroma. Macrophages also appear to have a significant capacity to metabolize AA for the production of HETEs and leukotrienes, primarily 12-HETE. Stromal prostaglandins also increased early, but they reached maximal values after the injury. In acute phase of injury, TXB₂ and 6-keto-PGF_{1 α} are produced during inflammation. Migration of PMNs is the major source of TXB₂, whereas the increase in PGE₂ and 6-keto-PGF_{1 α} suggests that other cells, as well, contribute to the synthesis of these products [70]

Recently in a clinical study of patients with bacterial keratitis, fungal keratitis, and *Acanthamoeba* keratitis showed a profound diminishment of the sub-basal corneal nerve

plexus with increased corneal dendritic cells (DCs). DCs are the most potent antigen-presenting cells (APCs) of the body and have been demonstrated to be critical for the initiation of adaptive immune responses and for maintenance of peripheral tolerance. DCs, as well, represent the principal immune guards to the foreign world in the cornea and ocular surface [71]. Although numerous studies have comprehensively demonstrated the implication of AA metabolites in DCs activation, migration, and function [72–76]. DCs biology in cornea and involvement of lipid mediators in the DCs' functions in ocular diseases need to be elucidated.

Recently, Biswas et al. [77] showed important findings of ocular infection with herpes simplex virus (HSV) which results in a blinding immunoinflammatory stromal keratitis lesion. They demonstrated that the inflammatory milieu and angiogenic stimuli started early after infection play a vital role in HSV-stimulated ocular lesions. COX-2-induced prostanoid synthesis played an important role in participant of this environment and the COX-2 inhibitors abrogate the cascade of events that culminate in stromal keratitis [77].

Thus, existing literatures highlighted the importance of AA in corneal immunobiology in numerous corneal inflammatory diseases; however, this knowledge is only the beginnings, not the end. Implications of AA-derived metabolites and their inhibitors in the cornea would attract the researchers to understand the pathogenesis of corneal diseases and to design the valuable approach for disease management.

Arachidonic acid (AA) in apoptosis

The term “apoptosis” was first introduced by Kerr and his colleagues in 1972 [78]. Apoptosis occurs via two major pathways - i) the extrinsic pathway and ii) the intrinsic pathway [79]. i) The extrinsic pathway of apoptosis is started upon the binding of an extracellular ligand to transmembrane death receptors of the tumor necrosis factor (TNF) super family. This interaction induces the assembly of the death-inducing signaling complex which then triggers initiator caspases to activate the enzymatic pathway and induces apoptotic cell death. ii) The intrinsic pathway (also known as mitochondrial pathway) is stimulated by the stimuli that lead to the permeabilization of mitochondrial outer membrane and then secrete the critical protein, cytochrome c (cyt c), from the mitochondrial intermembrane space to the cytosol to initiate and regulate caspase activation [79]. Thus, the mitochondria exhibit an important role in the mechanism of apoptosis. AA-derived eicosanoids, PGs, TX, LTs, EETs, and HETEs are the important group of lipid mediators that play a vital role in several physiological and pathophysiological processes [79].

Arachidonic acid is emerged as an imperative player in the apoptosis signaling. Nonsteroidal anti-inflammatory drugs (NSAID), which are among the few agents that can effectively prevent neoplasias [80], stimulate apoptosis via increased intracellular levels of AA, and their effects could mimic through the addition of exogenous AA [81]. It has been shown that the levels of intracellular AA could be modulated through over expression of COX-2 and fatty acyl-CoA ligase. Apoptosis was diminished by the stimulation of either enzyme, which also protected from the killing effects of AA [82]. Stimulation of apoptosis by the AA involved activation of caspase-3, a signaling mechanism that is amplified by the secretion of

mitochondrial cytochrome c [83] and Smac/DIABLO [84]. Most of these eicosanoids generated from LOX, COXs, and CYP P450 pathways regulate apoptosis in the context of tumorigenesis and cancer although these eicosanoids, as well, participate in the apoptosis in non-cancer tissues [85, 86]. The PLA₂ reaction is the principal step through which AA is synthesized from phospholipids [87]. It has also been shown that AA-selective Ca²⁺-dependent cPLA₂ is essential for the cytotoxic action of TNF α , which triggers mitochondrial permeability transition pore opening by the activation of hydrolysis of phospholipid and then AA signals apoptosis via a mitochondrial effect that can be amplified by inhibition of LOX and COX [88].

Recently, we demonstrated the implication of cPLA_{2 α} and AA role in corneal inflammation and apoptosis of corneal epithelial cells in *Acanthamoeba castellanii* infection [13, 14]. *Acanthamoeba* trophozoites' secreted cytopathic protein, MIP-133, was known to be potent at triggering a caspase-3-dependent apoptosis signaling in corneal epithelial cells *in vitro* [64, 89, 90]. We have revealed that specific inhibitors of cPLA_{2 α} enzymes (AACOCF3, MAFP, and CAY10650) and chicken anti-MIP-133 antiserum inhibit MIP-133 induced activation of cPLA_{2 α} activity and attenuate MIP-133-induced apoptosis of corneal epithelial cells [13, 14] (Fig. 10). Also, we showed that MIP-133 induced apoptosis in corneal epithelial cells of Fas receptor-deficient mice (*lpr/lpr*) is Fas receptor-independent [13]. MIP-133 interacts with phospholipids on HCE and HCORN cells to stimulate cPLA_{2 α} , AA release, and utilizes this cPLA_{2 α} pathway to provoke apoptosis of corneal epithelial cells [13, 14] (Fig. 11). Our findings [13, 14] are largely in concurrence with those of Kirschnek and Gulbins^[91], who demonstrated an important role for PLA₂ in *Pseudomonas aeruginosa*-stimulated apoptosis of human lung fibroblast cell line and conjunctiva epithelial cell line. They observed that AA production by activation of cPLA₂ during *P. aeruginosa* infection exhibits an important role in the induction of host cell apoptosis [91].

Thus, *Acanthamoeba* induces apoptosis on contact-dependent mechanism to corneal epithelium which starts from MIP-133 interaction to cell membrane phospholipids of corneal epithelial cells and leads to the induction of cPLA₂→AA pathway in sequential pathogenesis of *Acanthamoeba* keratitis [13, 14].

Future prospects

Arachidonic acid-derived metabolites has been an intensive area of lipid research for many decades. Therapeutic interventions based on the conventional AA cascades have become routine practices, such as COX inhibitors and LOX inhibitors in several diseases. The emerging evidence of AA-derived eicosanoids in corneal biology reviewed here suggests an important role of AA in modulating inflammatory cascades and apoptosis, may provide fruitful and stimulating avenue for exploring the novel approaches into the biological activities of AA-derived metabolites, which may be led to potential therapeutic interventions in the cure of corneal inflammation and infection. The complex corneal biology and the multifaceted nature of the AA metabolic pathways and complex pathogenic property of microbial pathogens might be a challenge to target efficient therapeutic remedies. Thus, researchers need to pay more attention to develop effective, safe, and “on target” pharmacological intervention to cure microbial corneal diseases.

Acknowledgments

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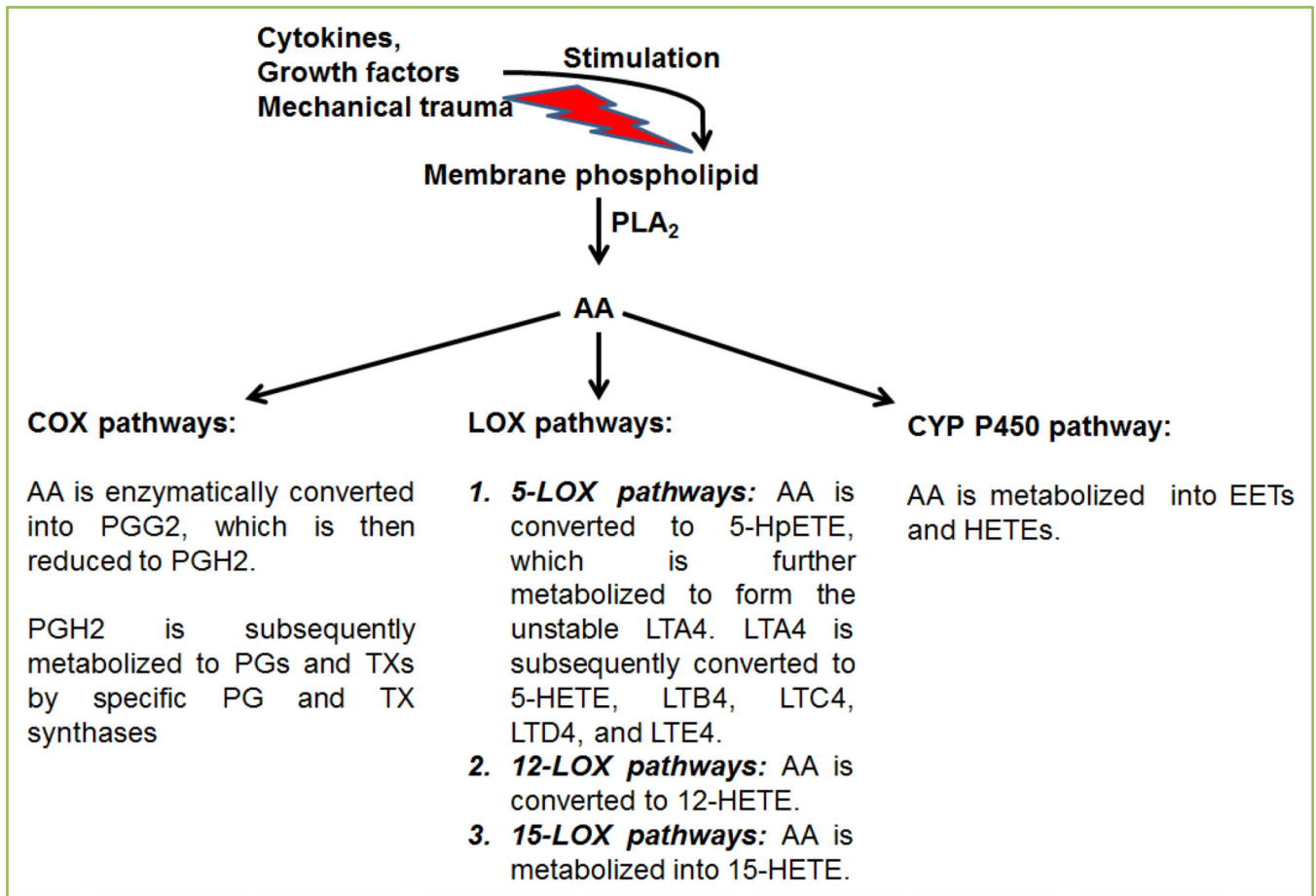


Figure 1. Enzymatic pathways for the synthesis of eicosanoids derived from arachidonic acid (AA)

Arachidonic acid is synthesized by the enzymatic activity of phospholipase A₂ (PLA₂) from cellular membrane upon stimulation by cytokines, growth factors or mechanical trauma. Arachidonic acid is then converted to eicosanoids via the three major pathways: the COX, the LOX, and the cytochrome P450 monooxygenase pathways.

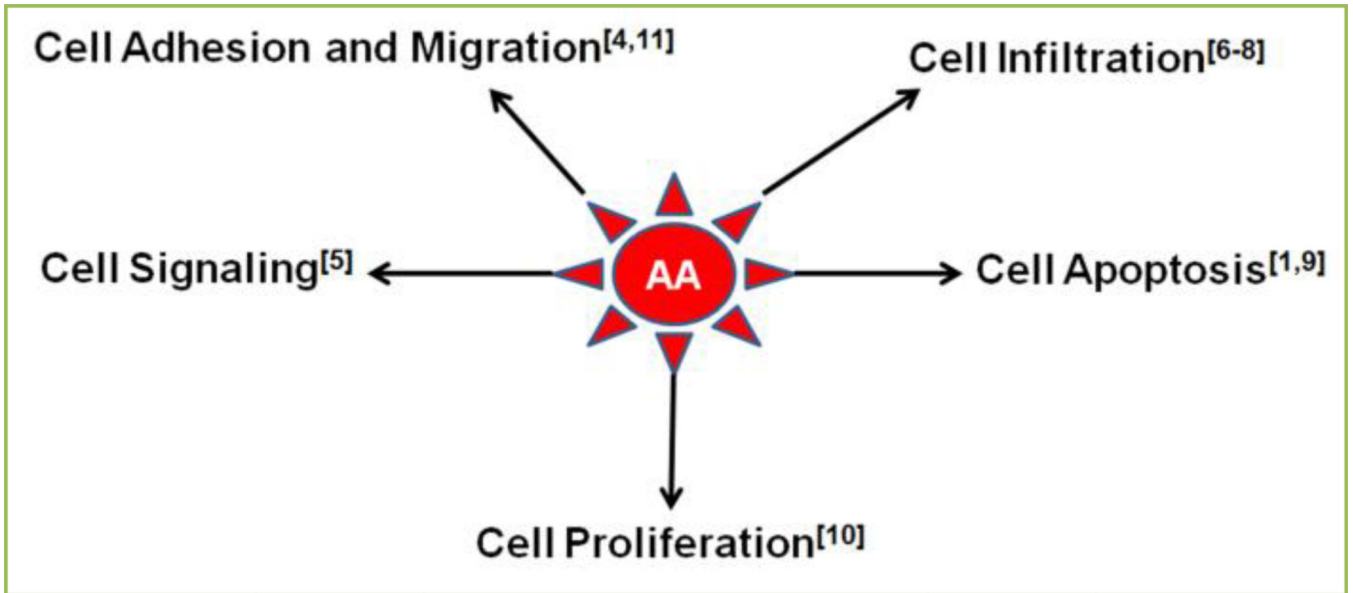


Figure 2.
Important roles of arachidonic acid (AA) in a variety of cellular functions.

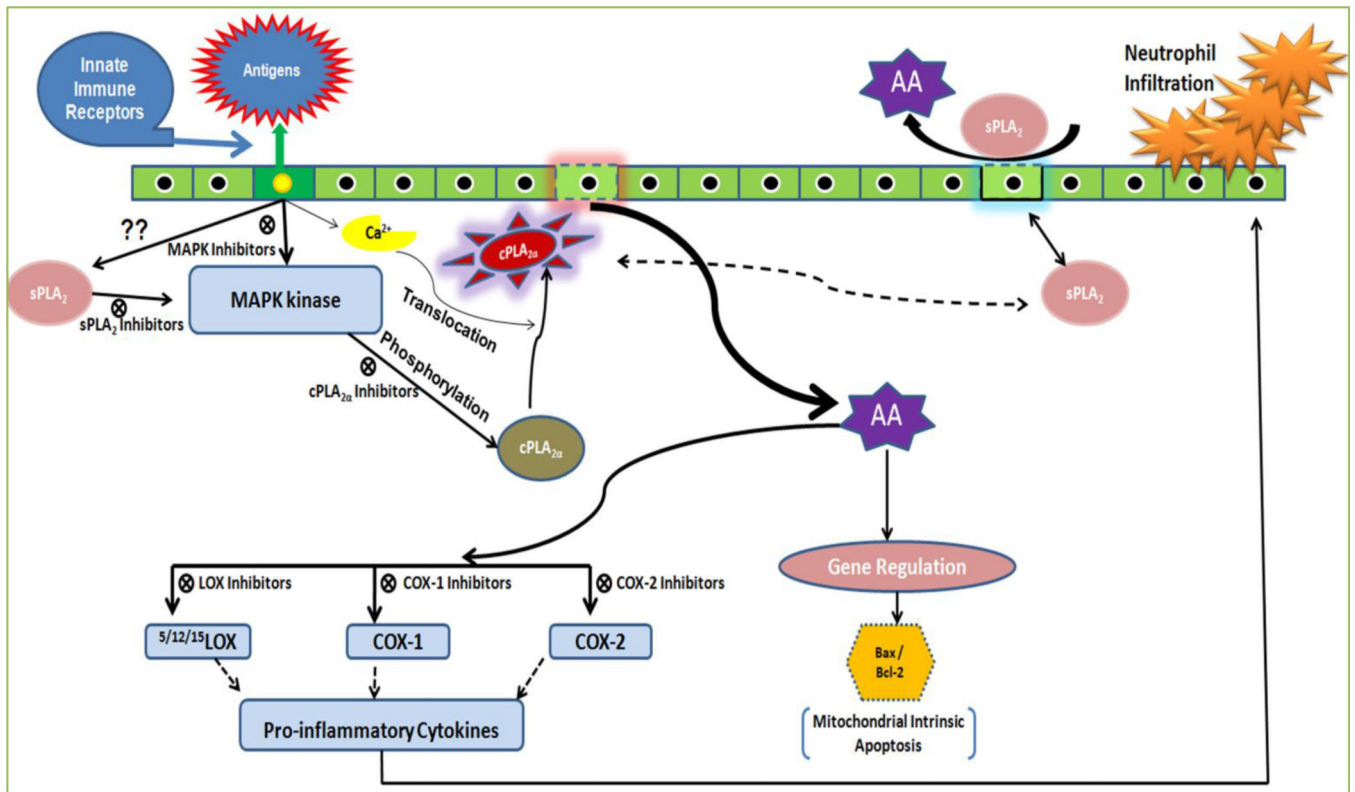


Figure 3. Arachidonic acid (AA) signal transduction is activated by the phosphorylation of cPLA₂α during the recognition of inflammation or antigen via innate immune receptors. Inhibitors of various cascades therapeutically may play an important role in disease management.

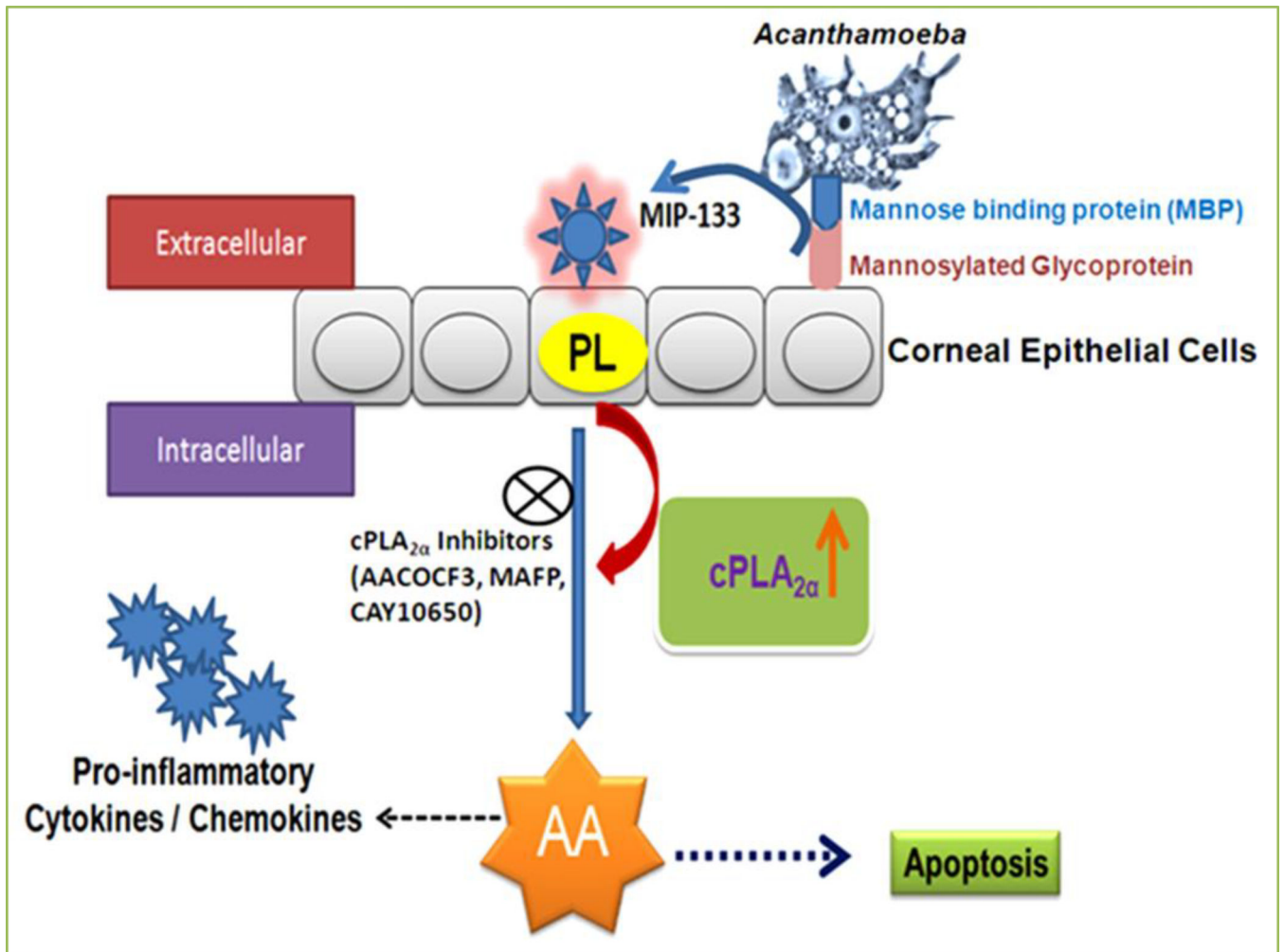


Figure 4. Role of *Acanthamoeba* cytopathic protein, MIP-133, in the pathogenesis of *Acanthamoeba* keratitis

Acanthamoeba binds to the corneal surface by mannose binding protein (MBP). This binding of *Acanthamoeba* to corneal epithelial cells induces release of the mannose-induced 133 kDa protease (MIP-133). MIP-133 interacts with phospholipids on plasma membrane of human corneal epithelial (HCE) cells and Chinese hamster corneal epithelial (HCORN) cells, and activates cytosolic phospholipase A_{2α} (cPLA_{2α}). cPLA_{2α} is involved in apoptosis, arachidonic acid (AA) release, and activation of proinflammatory cytokines/chemokines from HCE and HCORN cells. cPLA_{2α} inhibitors (AACOCF3, MAFP, and CAY10650) may be a therapeutic target in *Acanthamoeba* keratitis [13,14].

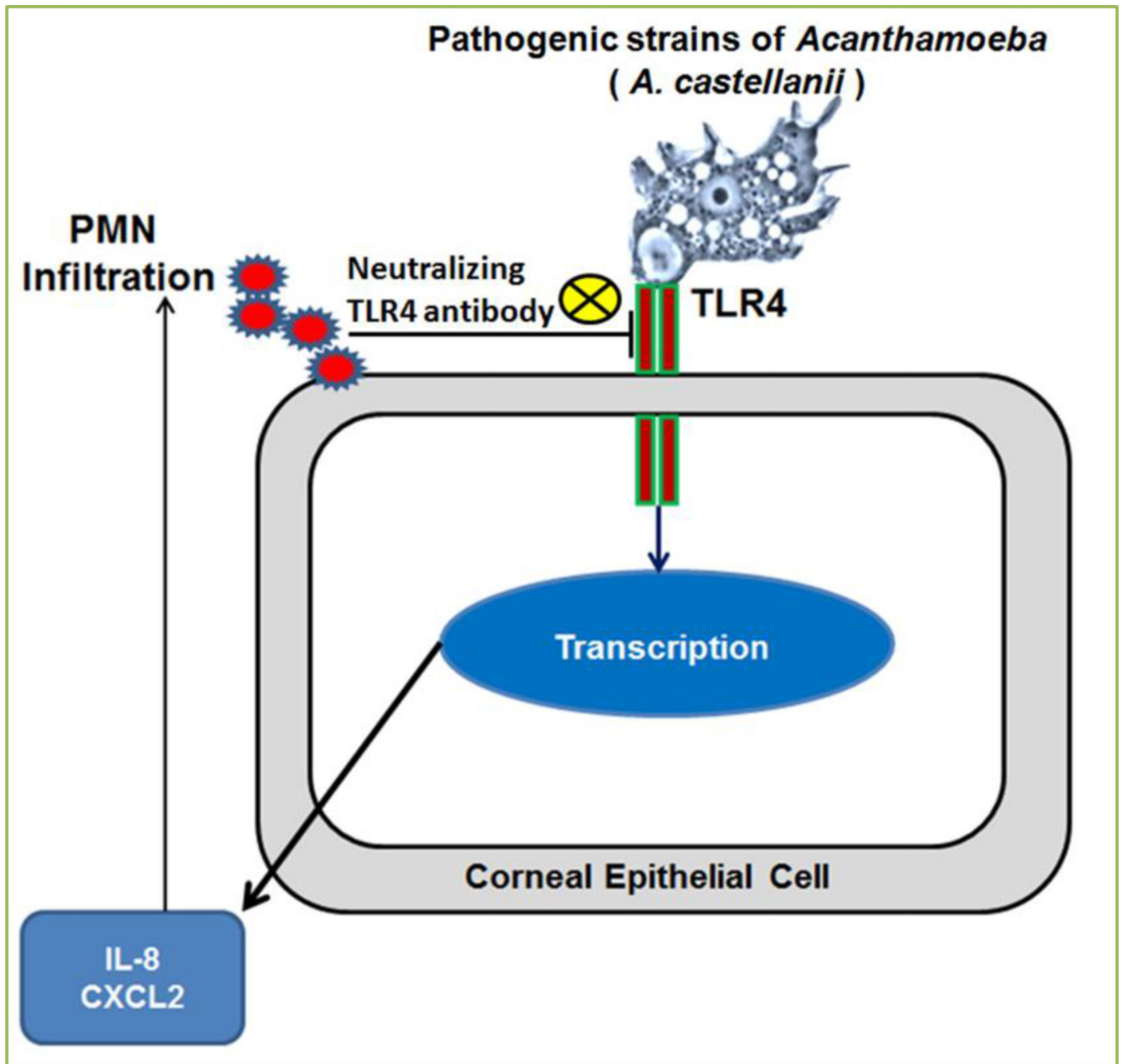


Figure 5. Pathogenesis of *Acanthamoeba* keratitis is induced by the activation of TLR4 pathway Recognition of TLR4 by pathogenic *Acanthamoeba* Spp., *A. castellanii*, induces secretion of proinflammatory cytokines from corneal epithelial cells and increases infiltration of inflammatory cells in cornea [38].

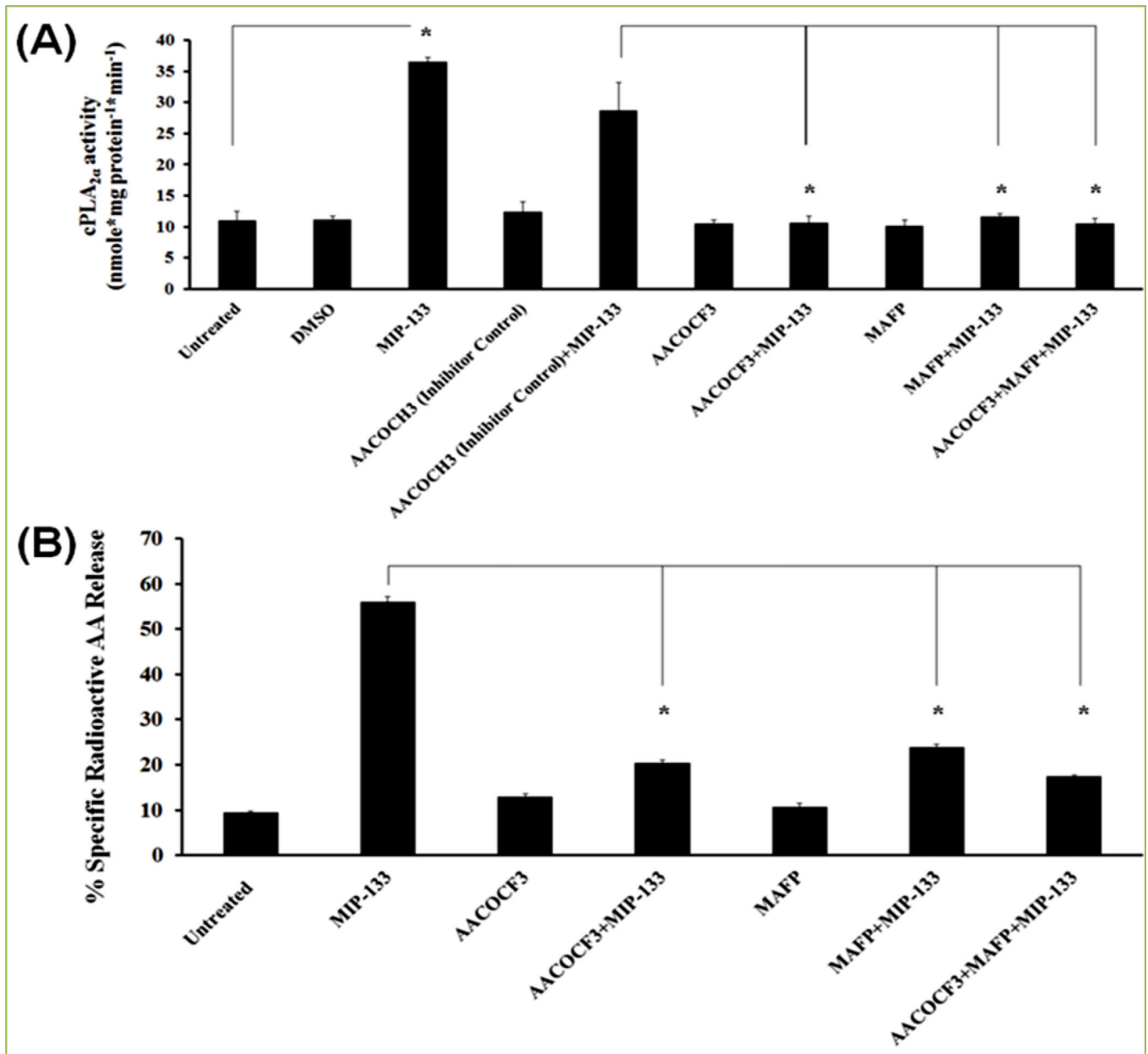


Figure 6. Effect of cPLA_{2α} inhibitors (AACOCF3 and MAFP) on MIP-133 induced cPLA_{2α} enzyme activity in HCE cells and arachidonic acid (AA) release from HCE cells
HCE cells were preincubated for 1 hour with cPLA_{2α} inhibitors (10 μM MAFP or 20 μM AACOCF3) or an inactive negative control (20 μM AACOCH3), and then incubated with or without 15 μg/mL MIP-133 for 24 hours. Induction of cPLA_{2α} enzyme activity was examined using a cPLA₂ assay kit (A). AA production by MIP-133 treatment was determined by liquid scintillation counting (B). The data are mean±SEM of three independent experiments. *Asterisk* indicates *P* value < 0.05 by unpaired Student's *t*-test. Reprinted from Tripathi et al. [13] with permission from copyright holder the Association for Research in Vision and Ophthalmology (ARVO).

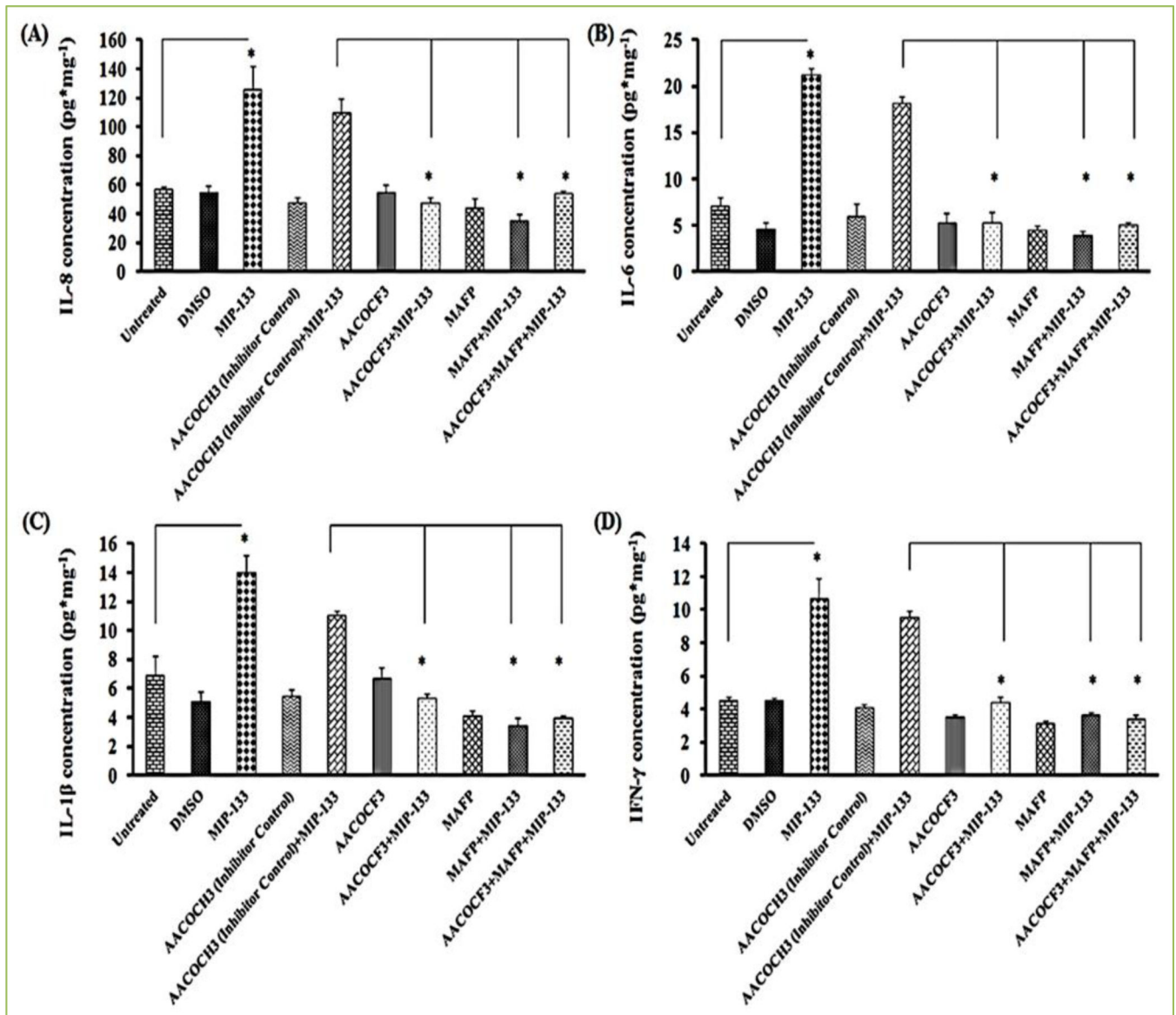


Figure 7. MIP-133 induces the up-regulation of proinflammatory cytokines (IL-8 (A), IL-6 (B), IL-1 β (C), and IFN- γ (D) protein expression) is blocked by cPLA $_{2\alpha}$ inhibitors in HCE cells Inhibition of cPLA $_{2\alpha}$ involved pre-incubating HCE cells for 1 hour with cPLA $_{2\alpha}$ inhibitors (10 μM MAFP or 20 μM AACOCF3) or an inactive control (20 μM AACOCH3), and then incubated with or without 15 $\mu\text{g}/\text{ml}$ of MIP-133 for 24 hours. Cytokine production was examined by ELISA. The data are mean \pm SEM of three independent experiments. *Asterisk* indicates P value < 0.05 by unpaired Student's t -test. AACOCF3, MAFP, AACOCH3, and DMSO without MIP-133 did not show significant effect on CXCL2 production when compared to untreated cells. The inactive control compound AACOCH3 did not block IL-8, IL-6, IL-1 β , and IFN- γ protein production induced by MIP-133. Reprinted from Tripathi et al.^[13] with permission from copyright holder the Association for Research in Vision and Ophthalmology (ARVO).

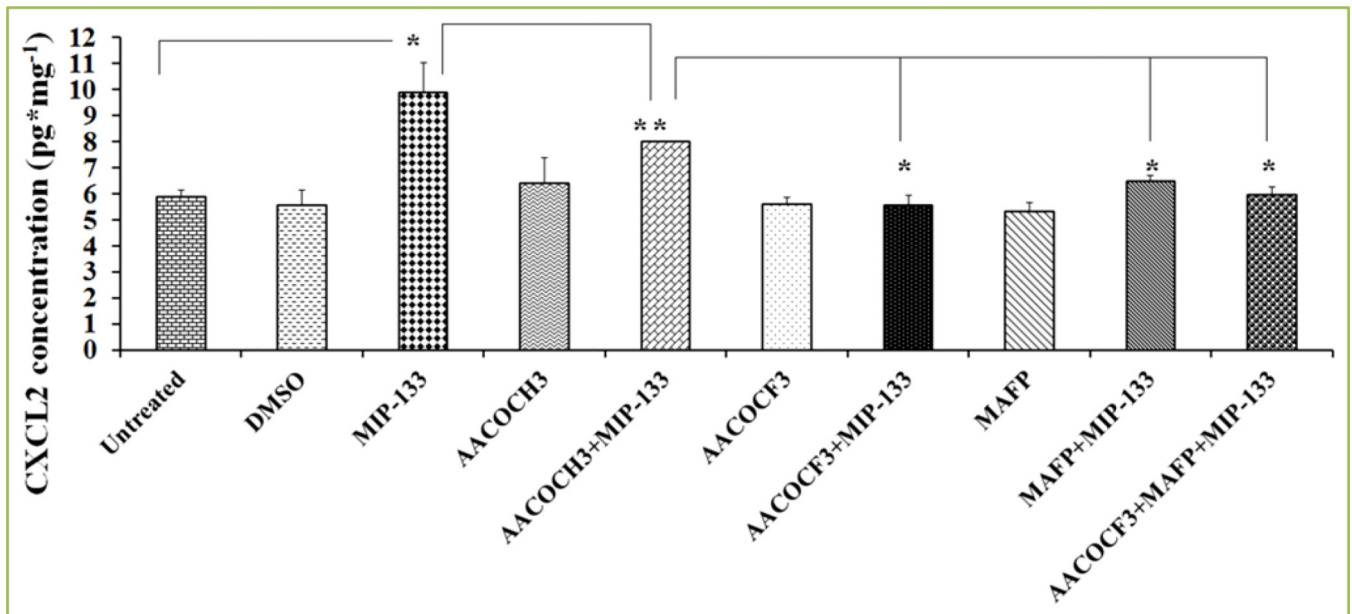


Figure 8. MIP-133 induces the up-regulation of chemokine CXCL2, and cPLA_{2α} inhibitors diminish CXCL2 production in HCORN cells

Inhibition of cPLA_{2α} involved pre-incubating HCORN cells for 1 hour with cPLA_{2α} inhibitors (20 μM AACOCF3 or 10 μM MAFP) or an inactive control compound (20 μM AACOCH3) or DMSO (5 μL solvent of AACOCF3, MAFP, and AACOCH3), and then incubated with or without 15 mg/ml of MIP-133 for 24 hours. CXCL2 production was examined by ELISA. The data shown represent mean±SEM of three independent experiments (**P*<0.05). *P* values were obtained by unpaired Student's *t*-test. AACOCF3, MAFP, AACOCH3, and DMSO without MIP-133 did not show significant effect on CXCL2 production when compared to untreated cells. The inactive control compound AACOCH3 did not block CXCL2 production induced by MIP-133. Reprinted from Tripathi et al.^[14] with permission from Elsevier.

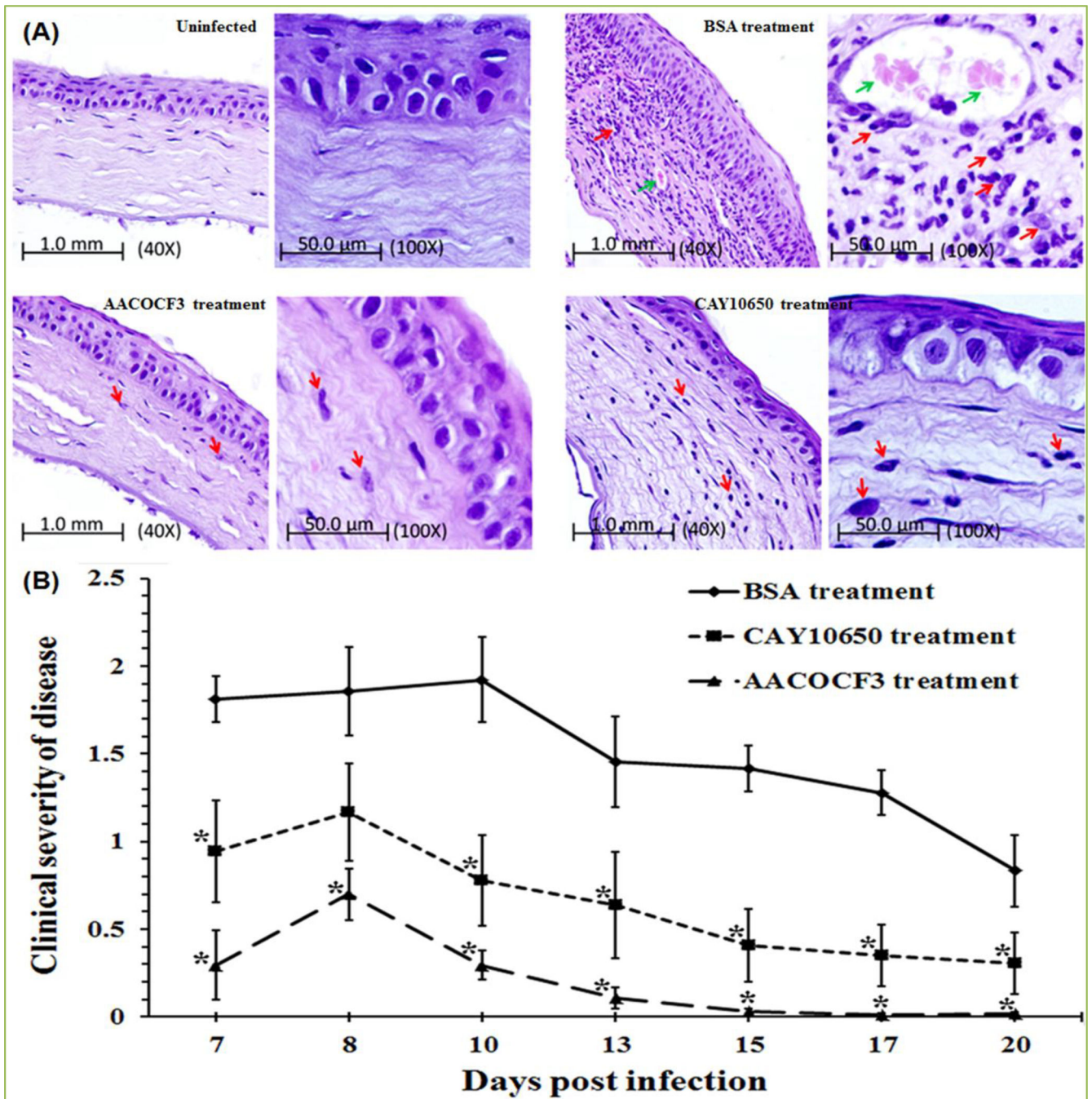


Figure 9. Therapeutic role of cPLA_{2α} inhibitors *in vivo* Chinese hamsters
 cPLA_{2α} inhibitors (AACOCF3 and CAY10650) 50 μg/5 μl was injected under the contact lens of an infected cornea three times a day for 6 days and topically from 7 to 20 days post-infection. As a control infected animals were treated with 50 μg/5μl of BSA. Lenses were removed 7 days post-infection. (A) Histopathological examination of infected cornea treated with cPLA_{2α} inhibitor. Photomicrograph of corneas from a Chinese hamster infected with *Acanthamoeba* trophozoites-laden contact lenses. Animals were anesthetized and sacrificed 15 days after application of cPLA_{2α} inhibitors. **Uninfected group:** Cornea of control animal;

BSA treatment group: Cornea from infected animal, the corneal stroma of this group contained marked lamellar connective tissue disruption, extensive stroma swelling and neovascularized; Severe PMN cells and lymphocytes infiltration were present; **AACOFC3 treatment group:** Cornea from infected animal treated with AACOCF3 had very mild inflammation. Which is similar to the corneal section from control, untreated animals; **CAY10650 treatment group:** Cornea from animals treated with CAY10650 had very mild inflammation and few PMN cells infiltration. PMN cells (small arrows), and blood vessels (thick arrows) were present in the corneal stroma. Few inflammatory cells were also observed under the corneal endothelium from control, BSA treated animals. **(B)** cPLA_{2α} inhibitor mitigates the pathogenesis of *Acanthamoeba* keratitis *in vivo*. Corneas were scored for clinical severity for the period indicated. Each graph line represents the mean±SEM of clinical severity of nine animals for the observed time points. The results shown are representative of three separate experiments. *Asterisk* indicates *P* values < 0.05 compared with infected untreated group and were analyzed by the Mann-Whitney's *U* test. Reprinted from Tripathi et al. ^[14] with permission from Elsevier.

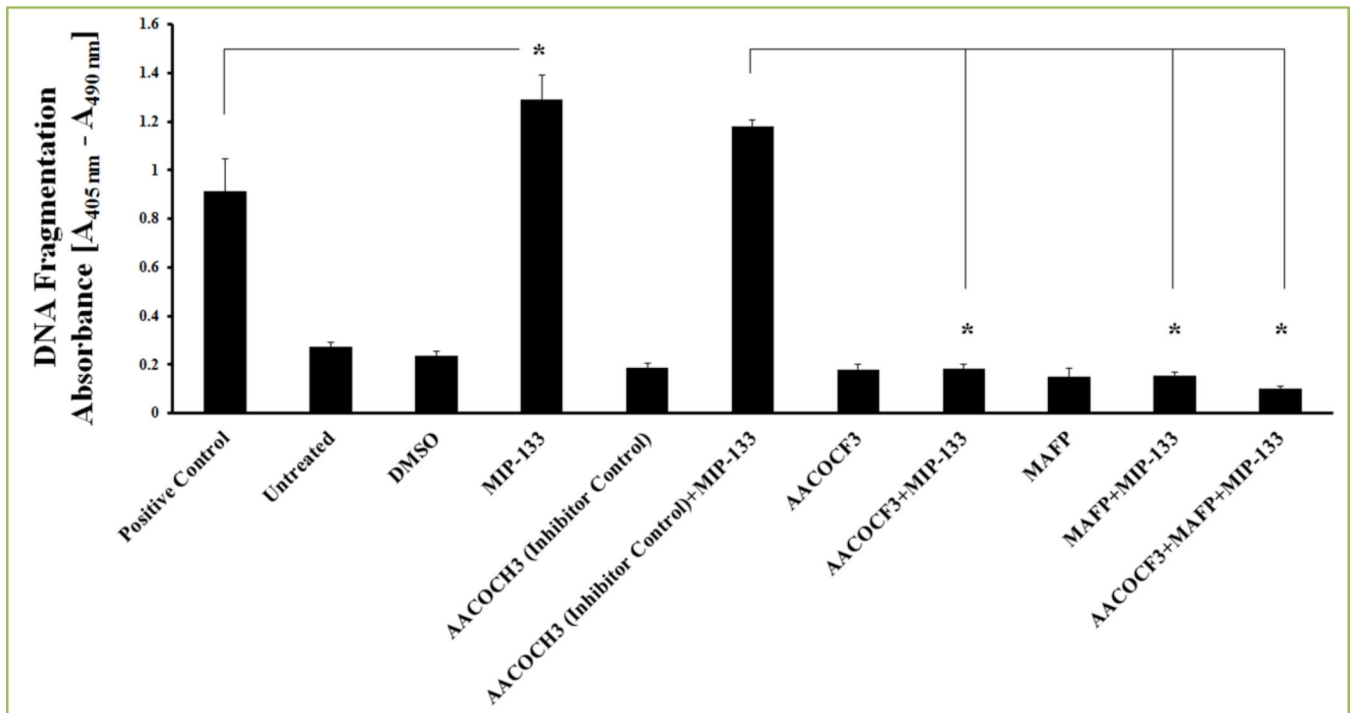


Figure 10. MIP-133 induced apoptosis is blocked by cPLA_{2α} inhibitors in HCE cells
HCE cells were pre-incubated for 1 hour with cPLA_{2α} inhibitors (10μM MAFP or 20μM AACOCF3) or an inactive negative control (20μM AACOCH3), and then incubated with or without 15 μg/ml MIP-133 for 24 hours. Apoptosis was examined by Cell Death Detection ELISA^{plus}. MIP-133-induced apoptosis was confirmed by a positive control provided in the apoptosis kit. The data are mean ± SEM of three independent experiments. *Asterisk* indicates *P* value < 0.05 and were obtained by unpaired Student's *t*-test. Reprinted from Tripathi et al. [13] with permission from copyright holder the Association for Research in Vision and Ophthalmology (ARVO).

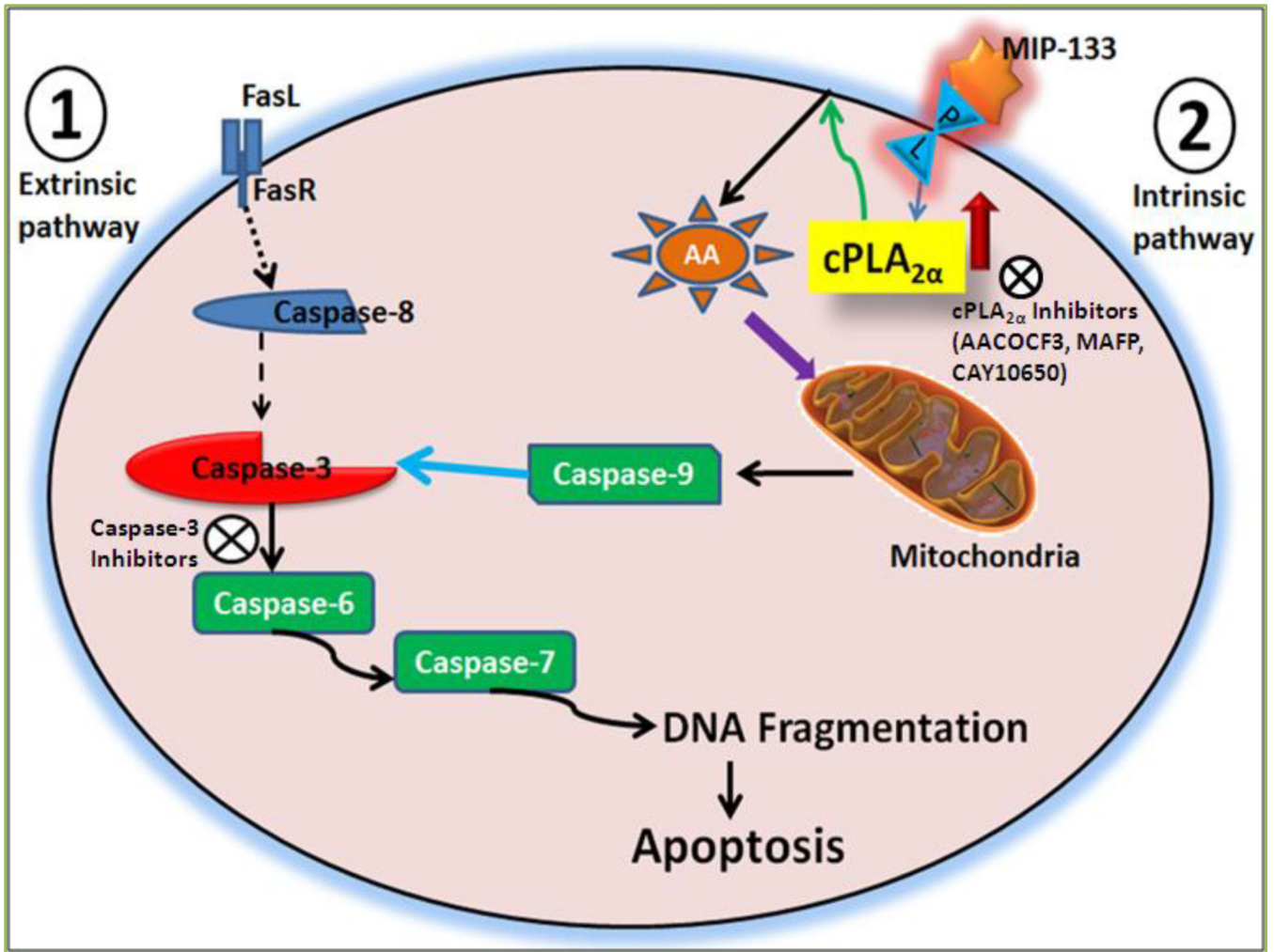


Figure 11. MIP-133 induces apoptosis of corneal epithelial cells through intrinsic apoptotic pathway^[13,14].