



# Annexins as potential targets in ocular diseases

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**Annexins (AnxAs) are Ca<sup>2+</sup>/phospholipid-binding proteins extensively studied and generally involved in several diseases. Although evidence exists regarding the distribution of AnxAs in the visual system, their exact roles and the exact cell types of the eye where these proteins are expressed are not well-understood. AnxAs have pro-resolving roles in infectious, autoimmune, degenerative, fibrotic and angiogenic conditions, making them an important target in ocular tissue homeostasis. This review summarizes the current knowledge on the distribution and function of AnxA1–8 isoforms under normal and pathological conditions in the visual system, as well as perspectives for ophthalmologic treatments, including the potential use of the AnxA1 recombinant and/or its mimetic peptide Ac<sub>2–26</sub>.**

**Keywords:** Annexins; Inflammation; Angiogenesis; Eye; Retina; Therapeutic target

## Introduction

Annexins (AnxAs) are a protein superfamily composed of 12 isoforms in vertebrates, with structural similarities and phospholipid-binding properties in a Ca<sup>2+</sup>-dependent manner.<sup>1–3</sup> Structurally, AnxAs comprise two domains: a small N-terminal region, which varies in length and composition, and a central domain formed by four-to-eight repeats of a highly conserved 70–80 amino acid sequence (Fig. 1). The N-terminal domain is unique for each member of the superfamily, being responsible for the specific activities and functions of AnxAs, and contains sites for post-translational processes, such as phosphorylation, glycosylation and proteolysis<sup>1–3</sup>.

In particular, AnxA1 demonstrates clinical relevance and has been explored for over two decades, standing out as a prognostic biomarker for different diseases and recommended in clinical settings.<sup>4–7</sup> Advances in research on AnxA proteins using transgenic animal models have strengthened a translational potential for the development of annexin-based therapeutical strategies<sup>8</sup>.

The number of people affected by common causes of vision loss increases substantially as the population grows and ages.<sup>9</sup> Although previous reviews provide information about AnxAs in cancer, inflammation, angiogenesis and tissue repair,<sup>8,10–13</sup> we are not aware of studies addressing the roles of these proteins in the visual system. Although there are 12 isoforms of AnxAs,

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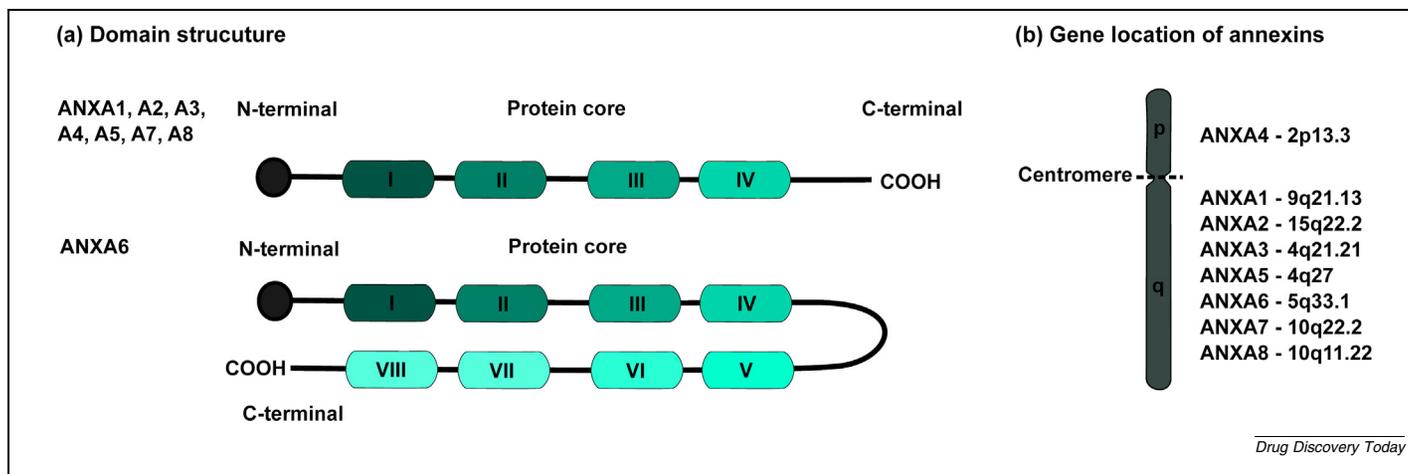


FIGURE 1

Annexin structure and gene location. **(a)** AnxAs comprise two domains: a small N-terminal region, specific for each member, and a central domain formed by four (ANXA1–5, A7 and A8) to eight (ANXA6) repeats of a sequence of 70–80 amino acids, highly conserved. **(b)** Human ANXA1–8 genes are spread throughout the genome on chromosomes 2, 4, 5, 9, 10 and 15.

only AnxA1–8 have been described in the visual system.<sup>14–18</sup> The presence of these AnxAs in the ocular tissue suggests viable roles for these proteins in the eye physiology, leveraging possibilities for more studies exploring innovative therapies in the treatment of ocular inflammation.

This review summarizes the current knowledge on the distribution and function of AnxA1–8 isoforms under normal and pathological conditions in the visual system, as well as their possible roles in physiological regulation of the ocular structures. In addition, we summarize the main studies on AnxA participation in ocular surface diseases, glaucoma, uveitis and retinopathies. Understanding the biological functions of AnxAs in the eye can contribute to future therapeutic approaches, which could mitigate vision loss and other impairments in visual function.

### AnxAs in the eye

The roles of some AnxAs have been investigated in the visual system,<sup>15–18</sup> leaving room to explore the promising therapeutic potential that these proteins might have in ocular diseases. Some AnxAs have been studied in infectious and autoimmune diseases.<sup>3,8,13</sup> Taking into consideration that ocular diseases can present several etiologies, further investigations about the roles of AnxAs in the eye can bring advances to the ophthalmology field, because the eye is an organ that can be exposed to several microorganisms, can be affected by particular autoimmune diseases and several idiopathic pathologies. Hence, a better understanding of the function of AnxAs in the eye could lead to uncovering, at least in part, mechanisms of diseases within the visual system. The following sections explore the findings about the distribution and physiological function of AnxAs in the eye, under normal and pathological conditions (Fig. 2).

### Cornea

AnxAs are widely expressed in different epithelia (e.g., intestinal epithelium, epidermis, bladder epithelium).<sup>8,19–22</sup> In the corneal epithelium, AnxA1, AnxA2 and AnxA8 have prominent roles. These AnxAs can work in the regulation of the corneal stroma

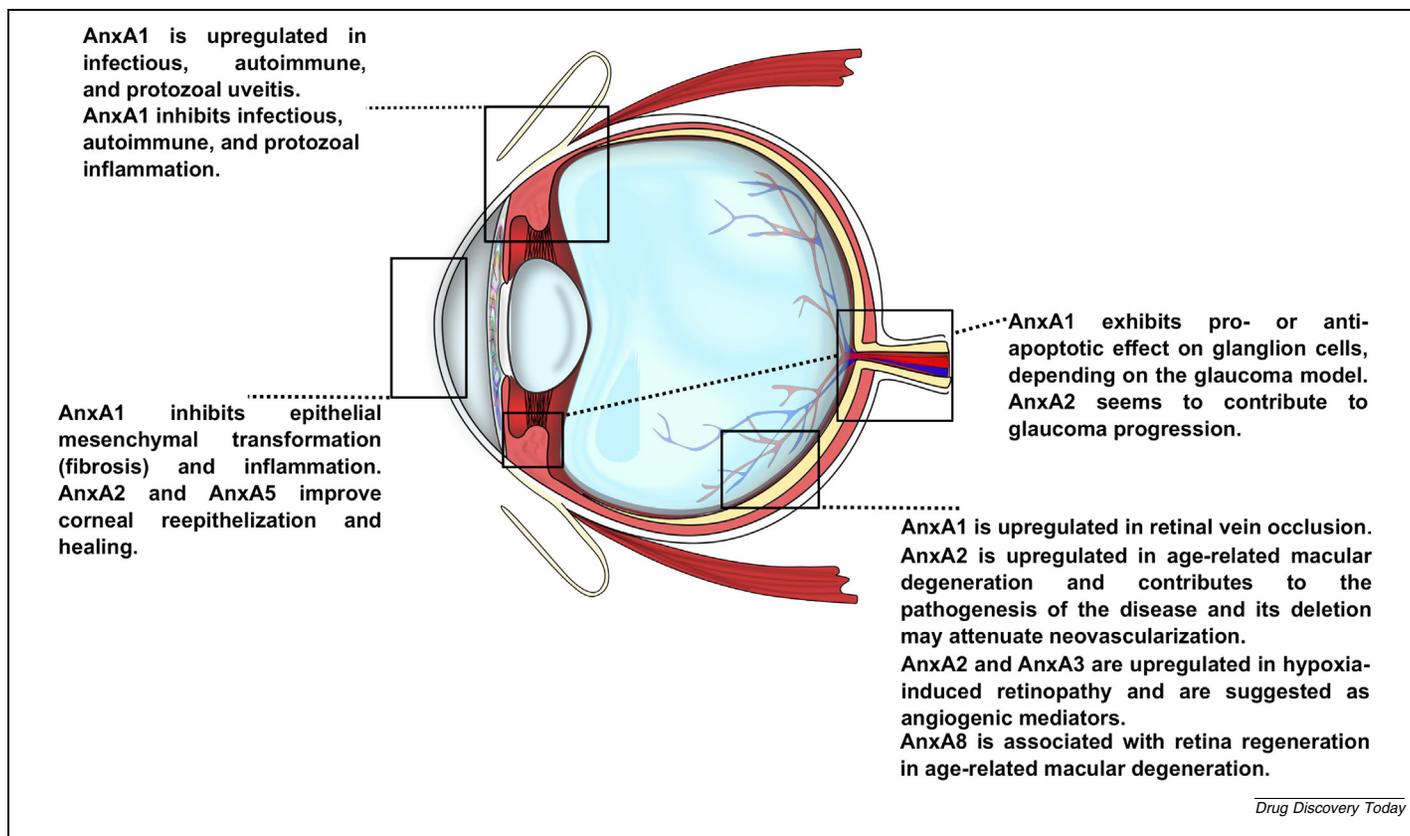
and epithelium remodeling through structural organization and participation in the synthesis of the extracellular matrix.<sup>19,23,24</sup> Under physiological conditions, AnxA1 and AnxA8 are expressed in human and mouse corneal epithelia.<sup>25–28</sup> AnxA1 and AnxA8 mRNA expression has also been detected in the endothelium of normal human corneas assessed by microarray analysis and serial analysis of gene expression (SAGE).<sup>29</sup> ANXA2 gene expression has been demonstrated in isolated corneal epithelial cells and keratocytes.<sup>30</sup> By contrast, AnxA8 is overexpressed in myofibroblasts compared to isolated corneal keratocytes and fibroblasts.<sup>28</sup>

### Uvea

In the uvea, the AnxA1 protein has been found in the epithelia of the iris, body and ciliary processes in rats.<sup>26,31</sup> AnxA2 has also been detected in this layer, mainly expressed in the protein-rich fraction of the cytoskeleton derived from trabecular meshwork cells, as well as human and pig ciliary muscle cells.<sup>32</sup>

### Neuroretina

In the mouse retina, high levels of AnxA1 mRNA have been detected, followed by AnxA5 and AnxA6 and, to a lesser extent, AnxA2, AnxA3, AnxA4, AnxA7 and AnxA8. Compared with neurons, Müller and microglia cells have higher levels of these eight AnxA isoforms.<sup>14</sup> Müller–microglia cell interaction appears to be a bidirectional mode of communication that helps shape the overall response to retinal damage.<sup>33,34</sup> These two cell types play an important part in the defense against retinal pathogens because they can act as phagocytes.<sup>33,34</sup> Annexins also have important roles on other cells, such as phagocytic cells, especially macrophages, whereby AnxA1 can regulate the activity of the cytoskeleton and the phagocytosis process, and the lack of AnxA1 compromises the phagocytic function of these cells.<sup>35</sup> Although this phenomenon has not been observed in cells such as microglia, astrocytes or Müller cells in the neuroretina, further studies are needed to better elucidate this matter.

**FIGURE 2**

Role of AnxAs in eye diseases. Schematic diagram showing what is known about AnxAs in injury and infectious, autoimmune and degenerative conditions of the eye.

Some AnxAs are important for neuronal development in the encephalon and synaptic structure. In neurons, AnxA6 and AnxA7 participate in the maturation process,<sup>36,37</sup> whereas AnxA1 regulates the formation of dendritic spines, which are important postsynaptic structures for signal transduction in the neuronal circuit.<sup>38</sup> In the neuronal development of the retina, however, there is no evidence related to AnxA actions; therefore, it is paramount to further explore this and clarify whether AnxAs participate in neuroretina development.

#### Retinal pigmented epithelium

In general, AnxAs participate in membrane structure, organization and membrane trafficking because of their affinity to membrane phospholipids.<sup>1,2</sup> Phagocytosis in the outer segment of photoreceptors is mediated by retinal pigment epithelium (RPE) cells – a fundamental event for retinal maintenance.<sup>39</sup> In this context, the depletion of AnxA2 with RNA interference (RNAi) in human RPE cells (ARPE-19 cell line) impairs phagocytosis in the photoreceptor outer segments, with accumulation of these segments on the apical surface of the RPE and delayed phagosome transport. These alterations have also been observed in the retinas of AnxA2-knockout mice.<sup>17</sup> By contrast, diurnal clearance phagocytosis by the RPE is a conserved process, mediated by  $\alpha\beta 5$  integrin receptors linked to AnxA5 and AnxA6 that share a binding motif.<sup>15</sup> In this context, AnxA5 instead of AnxA6 has been proposed to contribute to  $\alpha\beta 5$ -integrin-dependent phagocytosis and the phagocytic activity of RPE *in vitro* and *in vivo*.<sup>15</sup>

#### Role of AnxAs in eye diseases

##### Ocular surface diseases

**Injuries to the ocular surface.** Either physical or chemical trauma or even severe infections can result in permanent damage on the cornea, leading to clouding and loss of visual acuity.<sup>40</sup> Corneal injury by epithelial scarification is widely used for wound healing analysis.<sup>41</sup> In an epithelial scraping model in Wistar rats, the translocation of AnxA2 protein expression from the cytoplasm to a more peripheral or extracellular position in the corneal epithelium has been correlated with cell proliferation, suggesting a protective role of this protein in tissue repair.<sup>30</sup> AnxA2 has a powerful function in extracellular matrix remodeling because it can bind to collagen fibrils and promote cell–cell adhesion, preventing fibrosis.<sup>42</sup> The fibrinolytic system is crucial for wound healing through its ability to remodel the temporary matrix. In a dose-dependent fashion, treatment with recombinant human AnxA5 stimulates the release of urokinase-type plasminogen activator and migration of rabbit corneal epithelial cells (RCEs) *in vitro*. In addition, *in vivo* treatment with recombinant AnxA5-containing eyedrops improved healing in two corneal wound models.<sup>43</sup>

Transforming growth factor (TGF) $\beta$  induces the epithelial–mesenchymal transition of corneal cells, increasing extracellular matrix synthesis, a process related to corneal fibrosis.<sup>41</sup> *In vitro* studies using an immortalized human corneal keratocyte line (HTK) have shown that treatment with the AnxA1 mimetic peptide Ac<sub>2–26</sub> inhibited TGF $\beta$ -induced transdifferentiation of HTK

cells into myofibroblasts by reducing  $\alpha$ -SMA and pro-fibrotic factors (fibronectin, collagen I and IV).<sup>24</sup> Peptide effects were abrogated when WRW4, an antagonist of the formylated peptide receptor type 2 (FPR2), was added. Furthermore, Ac<sub>2-26</sub> attenuated scar development in the cornea and inflammation by reducing neutrophil migration, as well as reducing tumor necrosis factor (TNF) $\alpha$  and interleukin (IL)-1 $\beta$  levels in mice subjected to mechanical injury.<sup>24</sup> Taken together, the data show that Ac<sub>2-26</sub> could be a promising therapeutic strategy for the treatment of corneal scars, owing to its antifibrotic and anti-inflammatory potential.

**Conjunctivitis.** Conjunctivitis occurs as a result of different etiological agents, whether viral, bacterial, fungal or allergic. It is a common eye disorder worldwide, being the most frequent reason for medical consultations in general and ophthalmology clinics.<sup>44</sup> Clinically, conjunctivitis is characterized by inflammation, occasionally followed by swelling in the conjunctiva, engorgement of blood vessels, secretion production and pain. Our group showed that administration of Ac<sub>2-26</sub> was sufficient to attenuate ovalbumin-induced conjunctivitis in mice, reducing the release of IL-2, IL-4, IL-10, IL-13, eotaxin and RANTES in the eye and lymph nodes compared with untreated mice.<sup>25</sup> In addition, AnxA1<sup>-/-</sup> animals exhibited an exacerbated allergic profile compared with the wild-type animals.<sup>25</sup> The anti-allergic effects of AnxA1 in the conjunctivitis model are mediated by FPR2.<sup>45</sup> The Ac<sub>2-26</sub> peptide inhibits mast cell degranulation *in vivo* and *in vitro*, and this effect can be blocked by the addition of FPR pan-antagonist Boc-2.<sup>45,46</sup> Taken together, these studies demonstrate an important role for AnxA1 in the regulation of allergic inflammatory responses.

### Glaucoma

Glaucoma is a neuropathic optic disease that leads to the degeneration of retinal ganglion cells (RGCs) by increased intraocular pressure (IOP), leading to irreversible blindness.<sup>47,48</sup> It can be classified in two types: open-angle glaucoma, which causes resistance to drainage of aqueous humor into the trabecular meshwork; and closed-angle glaucoma, characterized by obstruction of the drainage channel owing to the position of the iris.<sup>47</sup> This condition affects ~ 70 million people, whose risk factors include age, genetic predisposition, ethnicity, myopia, diabetes and the use of drugs that increase IOP.<sup>48</sup> Secondary glaucoma can result from corticosteroid use, inflammation and tumors, and evidence suggests that autoimmune processes can be involved<sup>47,48</sup>.

Considering that AnxA2 composes the cytoskeleton of the trabecular meshwork (TM) and ciliary muscle cells, and that any structural change in the cytoskeleton can hinder the drainage of the aqueous humor, this protein might be involved in the progression of glaucoma.<sup>32</sup> In fact, TM cells transfected with cochlin (present in the TM of glaucoma patients) and AnxA2 exhibit a change in morphology, becoming fusiform with multiple thin, filopod-like projections.<sup>49</sup> Transfection of cochlin increases the secretion of AnxA2 by TM.<sup>49</sup> In addition, TM cells treated with the soluble membrane protein CD44 (sCD44), levels of which are increased in the aqueous humor of glaucoma patients, show increased phosphorylation of AnxA2, which might contribute to the formation of the cross-linked actin network, as suggested by its co-localization with actin filaments.<sup>50</sup> Proteomic analysis of

the sclera from patients with open-angle glaucoma showed an increase in the expression of AnxA2.<sup>51</sup>

Serum-deprived ganglion cells undergo apoptosis, similar to the process that occurs in glaucoma. Treatment with Ac<sub>2-26</sub>-derived AnxA1 peptide showed a protective effect on RGCs in a dose-dependent manner; and this phenomenon was observed by reducing the expression of the apoptosis regulators caspase 3 and Bcl-2-associated X (Bax) protein and increasing the pro-survival B-cell lymphoma 2 (Bcl-2) protein.<sup>52</sup> *In vivo*, the induction of RGC cell death by ischemia–reperfusion (IR) injury caused an increase in the expression and nuclear translocation of AnxA1.<sup>53</sup> In the same study, simulating the IR injury *in vitro*, the expression of IL-1 $\beta$  increased, which was induced by the nuclear translocation of AnxA1 and its interaction with the p65 subunit of nuclear factor (NF)- $\kappa$ B. The observed differences in the pro- and anti-apoptotic effects of AnxA1 in RGCs could be associated with different models.<sup>53</sup> Furthermore, in an animal model of IR-induced glaucoma, ABCA1 expression in the ganglion cell layer decreased, reducing AnxA1 translocation to the cell membrane and its secretion, thus favoring inflammation and apoptosis.<sup>54</sup>

Unlike AnxA1, which is suggested as a possible treatment for glaucoma, AnxA5 is considered a good apoptotic marker in ganglion cells, which can be used for glaucomatous degeneration screening.<sup>55</sup> In addition, AnxA5-associated liposomes have been used to deliver topical drugs to the retina, which has proven to be a huge challenge in the clinic owing to its anatomical barriers.<sup>56</sup>

### Uveitis

Uveitis is defined as the inflammation of the vascular uveal tract of the eye, including the iris, ciliary body and choroid; however, underlying structures are also affected.<sup>57</sup> Clinically, uveitis is classified according to the anatomical portion of the eye that it affects (e.g., anterior, intermediate, posterior or panuveitis). The prevalence and phenotypic expression of different types of uveitis depend on age, gender, race, geographic distribution, environmental factors, genetics and social habits.<sup>58</sup> This condition can also be categorized according to the etiological agent, which can be infectious or noninfectious.<sup>57</sup> The first can be triggered by bacterial, viral, fungal and parasitic infections, whereas the latter can be of idiopathic or autoimmune etiology.<sup>57,59</sup>

**Infectious uveitis.** AnxA1 is a strong candidate for the treatment of infectious uveitis owing to its potent anti-inflammatory properties. Our group showed that AnxA1 levels were increased in the corneal epithelium, iris, ciliary body and retina in a rodent model of endotoxin-induced uveitis (EIU).<sup>26</sup> AnxA1 is essential for the homeostasis of the inflammatory process, regulating leukocyte diapedesis and preventing mast cell activation.<sup>60–62</sup> In addition, AnxA1 and its mimetic peptide Ac<sub>2-26</sub> downregulated ocular inflammation in *in vivo* models of EIU.<sup>26</sup> The anti-inflammatory activity of AnxA1 occurs after the phosphorylation of serine in its polypeptide chain and promotes a decrease in the inflammatory infiltrate and proinflammatory cytokines with topical and systemic treatment with Ac<sub>2-26</sub> or intravitreally with human recombinant AnxA1 (hrAnxA1).<sup>63</sup> *In vitro*, treatment with Ac<sub>2-26</sub> peptide in LPS-challenged ARPE-19 cells reduced IL-6 and IL-8 release and cell proliferation and increased cell migration.<sup>26,64</sup> In addition, a reduction occurred in the gene

expression of connective tissue growth factor (CTGF) and lecithin retinol acyltransferase (LRAT), which were correlated with different degrees of fibrosis and retinal atrophy.<sup>64</sup>

**Autoimmune uveitis.** Autoimmune uveitis is a noninfectious inflammatory disease that also involves increased endogenous levels of AnxA1 in human and murine retinas.<sup>63,65</sup> In an experimental autoimmune uveitis (UAE) model, hrAnxA1 was used for intravitreal treatment in mice, and it suppressed the clinical signs of the disease and reduced the influx of leukocytes into the eye.<sup>63</sup> Another study using rhAnxA1 (ip) demonstrated an attenuation of the self-reactive CD4<sup>+</sup> cell proliferation and EAU regression, along with a reduced expression of the proinflammatory cytokines IL-17, interferon (IFN)- $\gamma$  and IL-6. In addition, AnxA1<sup>-/-</sup> mice were shown to exhibit severe retinal inflammation during UAE, characterized by an increased number of monocytes and macrophages, neutrophils and CD4<sup>+</sup> IL-17<sup>+</sup> cells, associated with loss of visual function compared with wild-type animals.<sup>65</sup> A threefold reduction occurred in the AnxA1 serum levels of uveitis patients compared with control subjects, which was prominent in patients with clinically active uveitis.<sup>65</sup> By contrast, patients with inactive disease and in presumed remission had a nonsignificant increase in AnxA1 levels.<sup>65</sup> These data suggest AnxA1 as a potential therapeutic target for noninfectious uveitis.

**Toxoplasmic retinochoroiditis.** Toxoplasmosis is one of the most common causes of posterior uveitis worldwide, with a risk of infection and subsequent ocular involvement that varies geographically.<sup>66</sup> Ocular toxoplasmosis is almost always accompanied by vitritis and choroiditis caused by intraocular *Toxoplasma gondii* infection. Depending on the location of the retina, this condition can cause visual impairment. In active ocular toxoplasmosis, the choroid exhibits vascular changes, hemorrhage, inflammatory infiltrates and edema, which can cause optic neuritis.<sup>66</sup> Intravitreal *T. gondii* infection in a murine model evoked an intense inflammatory response characterized by accumulation of leukocytes in ocular tissues and morphological changes in the retina. Furthermore, immunohistochemical analyses showed that *T. gondii* infection positively modulated the expression of AnxA1 in neutrophils and RPE cells involved in the inflammatory response, reinforcing it as a candidate for therapy in ocular toxoplasmosis.<sup>67</sup>

### Retinopathies

**Age-related macular degeneration.** Age-related macular degeneration (AMD) is a common, multifactorial, chronic and progressive degenerative disorder of the macula that affects older individuals. This condition often causes loss of central vision owing to abnormalities in the photoreceptors, RPE, Bruch's membrane or choroid, which can lead to geographic atrophy or neovascularization.<sup>68</sup> Globally, AMD ranks third as a cause of blindness.<sup>68</sup> Choroidal neovascularization, which occurs in patients with AMD, is simulated in mice by using laser-induced rupture of Bruch's membrane.<sup>69</sup>

AnxA2 is closely related to retinopathy.<sup>70</sup> In AMD and the ischemic retina in mice a prominent expression of AnxA2 has been shown by immunohistochemistry in newly formed vessels in the retina and choroid, indicating that this protein is expressed in endothelial cells that participate in neovascularization.<sup>71,72</sup> Furthermore, in mice and primates (*Macaca fascicularis*)

with AMD, it has been suggested that AnxA2 could be related to the pathogenesis of the disease owing to the accumulation of AnxA2 in drusen, which are protein deposits in the retina affected by AMD, and formation of anti-AnxA2 autoantibodies.<sup>73,74</sup> In addition, AnxA8 has been recommended as a regulator of Wnt signaling and as a determinant of the RPE phenotype, implying a new approach to regenerative medicine using RPE cells derived from stem cells to treat AMD.<sup>18,75</sup>

**Vascular and ischemic retinopathies.** Retinopathy of prematurity (ROP) and retinal vein occlusions (RVO) affect individuals in different age groups and commonly lead to vision loss through retinal degeneration and the formation of fibrotic membranes.<sup>76–80</sup> ROP is a retinal vasoproliferative disorder that affects premature babies.<sup>77</sup> Advances in healthcare have increased the survival rate, but the numbers of ROP cases continue to increase all over the world. In 2010, an estimated 184 700 premature babies had ROP, of whom 20 000 developed blindness or severe visual impairment.<sup>79</sup> RVO is a condition characterized by obstruction and dilation of the veins owing to increased blood pressure, causing hemorrhage and leading to different degrees of ischemia.<sup>80</sup> In 2015, the global prevalence of RVO was ~28.06 million.<sup>76</sup>

In the context of ischemic and vascular retinopathies, evidence suggests that AnxAs are involved in the regulation of events that occur in these clinical conditions, such as neovascularization. Proteomic analysis of retinas from pigs with RVO showed increased expression of AnxA1, mainly in ganglion cells, and this effect was associated with a possible anti-inflammatory response against RVO.<sup>81</sup> Using the same approach, in an oxygen-induced retinopathy (OIR) rat model, an increase in the expression of AnxA3 was observed, which was related to the hypoxia-inducible factor (HIF)1 and vascular endothelial growth factor (VEGF). Considering that these factors are closely linked to the development of OIR, AnxA3 was recommended as a potential angiogenic mediator.<sup>82</sup> Another study using an OIR mouse model demonstrated that the ANXA2 gene showed high expression in the retina, indicating its potential regulatory effect on neovascularization.<sup>83</sup>

In a murine model of hypoxia-induced retinopathy, increases in the expression of mRNA and AnxA2 protein in the retina were observed; and this change was mainly present in retinal endothelial cells.<sup>84</sup> In RF/6A chorioretinal endothelial cells, cytokines and growth factors related to angiogenesis, such as TNF- $\alpha$ , IL-1 $\beta$  and especially VEGF, can induce the expression of AnxA2.<sup>84</sup> In IOR, induction with adenoviral vectors that express AnxA2 increased neovascular tufts, whereas the use of siRNA for AnxA2 attenuated neovascularization.<sup>84</sup>

The induction of AnxA2 expression in hypoxic situations seems to be directly regulated by the transcription factor HIF-1. The expression of AnxA2 on the cell surface is increased under hypoxia, which promotes plasminogen activation and endothelial cell migration, favoring neovascularization.<sup>85</sup> The relationship between AnxA2 and plasminogen activation is an important pathway in promoting angiogenesis. For instance, an increase occurred in intracellular and cell surface AnxA2 in isolated retinal microvasculature endothelial cells (RMVECs) exposed to hypoxia. Simultaneously, the level of the AnxA2-tPA complex (ANXA2-tPA) increased on the cell surface, which favored the production of plasmin.<sup>86</sup> The increased tPA activity

under hypoxic conditions can be reversed by the action of the hexapeptide LCKLSL, which binds to the N-terminal portion of AnxA2 in the tPA-binding region.<sup>86</sup> Interestingly, this peptide that binds to AnxA2 is also an inhibitor of angiogenesis *in vitro* and *in vivo*.<sup>86</sup>

Overexpression of the AnxA2 receptor (AX2R) can inhibit neovascularization *in vitro* via Krüppel-like transcription factor 2, as well as the cell cycle, keeping the endothelial cells in the S/G2 phases.<sup>87</sup> Evidence shows that the receptor can be stimulated by other factors and activates pathways that inhibit neovascularization.<sup>87</sup> Although AnxA2 favors the neovascularization process, it can have protective effects on human retinal endothelial cells (HRECs) *in vitro*, because cells subjected to serum and oxygen deprivation increase the expression of AnxA2 via HIF-1. This is related to the promotion of autophagy and increased survival of these cells.<sup>88</sup>

### Concluding remarks and challenges

This review summarizes, for the first time, the findings regarding distribution of AnxAs in the visual system and presents the possible participation of these proteins as pro-resolving agents that have been shown to be very effective in limiting inflammation in a diverse range of tissues, and have now been explored in the normal physiological function of the eye. Furthermore, we discuss here the importance of AnxA1–8 (because the other isoforms have not been fully explored in the literature) in the pro-

gression of ophthalmic pathologies and we highlight therapeutic perspectives in different pathological contexts of the eye (Fig. 2; Table 1).

Based on the studies reviewed here, we suggest that, although the AnxA1–8 isoforms were the main proteins investigated with possible actions in the eye, AnxAs could have a broader involvement in the development and maintenance of visual system homeostasis. This is an interesting issue to be explored in future research because little is known about the role of AnxAs in eye development.

AnxA knockout animals are very important tools for ophthalmologic investigation owing to their susceptibility to eye affections, demonstrating exacerbation of this condition in models of autoimmune uveitis, allergic conjunctivitis, choroidal neovascularization and retinal hypoxia.<sup>25,65,72,85</sup> Researchers frequently use mimetic peptides or AnxA recombinant proteins as a therapeutic perspective in *in vivo* and *in vitro* models, especially AnxA1 (Table 1). Moreover, recombinant AnxA5 protein and intraocular AnxA2 RNAi have been used in ocular diseases.<sup>43,84</sup> Nevertheless, except for AnxA1, therapeutic investigation of most AnxAs is scarce.

Among annexins, AnxA1 is a potent regulator of the infiltration of specific cells of the immune system,<sup>16,31</sup> as well as angiogenesis, degeneration, fibrosis<sup>89</sup> and infection,<sup>67</sup> which are relevant processes in the different pathology of the ocular tissues. Studies have identified mast cell stabilizers to promote cellular

TABLE 1

#### Summary of studies using AnxAs as pharmacological treatments in eye diseases.

| AnxA                                  | Condition                            | Organism  | Treatment  | Effects  | Refs  |
|---------------------------------------|--------------------------------------|---|--|--|-------|
| <i>Injuries to the ocular surface</i> |                                      |   |  |  |       |
| AnxA1                                 | Mechanical corneal injury            | C57bl/6 mouse   | Ac <sub>2-26</sub> (1 mg/kg): eye drops 4-times daily for 7 days   | Alleviates the development of corneal scarring and inflammation response   | 24    |
| AnxA5                                 | Corneal epithelial wound healing     | Japanese white rabbit                                   | hrAnxA5 (30 or 100 µg/ml): eye drops every hour after removal of the corneal epithelium with iodine vapor<br>hrAnxA5 (300 µg/ml): eye drops 4-times within 8 h after wounding through surgical model | Promotion of corneal epithelial wound healing  | 43    |
| <i>Uveitis</i>                        |                                      |   |  |  |       |
| AnxA1                                 | EIU                                  | Wistar rats   | Ac <sub>2-26</sub> (1 mg/kg):ip (15 min after LPS)sc (2 h after LPS injection)<br>eye drops (4, 8 and 12 h after LPS   | Treatments decreased leukocyte influx in ocular tissues; reduced TNF-α, IL-1β, IL-6 and NO levels; inhibited COX-2 expression in eye during EIU                | 16,31 |
| AnxA1                                 | EIU                                  | C57bl/6 and B10.  | hrAnxA1 (0.5 µg): ivi  | Suppression of acute and chronic uveitis in mice through reduction of neutrophil influx  | 63    |
| AnxA1                                 | EAU                                  | RIII mice   | hrAnxA1 (1 µg): ip once a day for 7 days   | Absence of AnxA1 exacerbates the inflammatory response during EAU  | 65    |
| AnxA1                                 | EAU                                  | C57bl/6 (wild-type and AnxA1 null mice) B10. RIII mouse | hrAnxA1 (1 µg): ip once a day for 7 days   | hrAnxA1 restrained autoreactive CD4 <sup>+</sup> cell proliferation, reduced expression of pro-inflammatory cytokines IL-17, IFN-γ and IL-6 and attenuated EAU |       |
| <i>Retinopathy</i>                    |                                      |   |  |  |       |
| AnxA2                                 | Hypoxia-induced ischemic retinopathy | C57bl/6 mouse   | Ad AnxA2 or Si AnxA2 (1 µg): ivi   | Ad-AnxA2-treated mice showed a marked increase in multiple neovascular tufts in retina, whereas Si-AnxA2-treated animals decreased neovascularization          | 84    |

Abbreviations: Ac<sub>2-26</sub>, AnxA1-mimetic peptide; Ad AnxA2, adenoviral vectors that express mouse AnxA2; EAU, experimental autoimmune uveitis; EIU, endotoxin-induced uveitis; hr, human recombinant; ip, intraperitoneally; ivi, intravitreal injection; iv, intravenously; LPS, lipopolysaccharide; NO, nitric oxide; sc, subcutaneously; Si AnxA2, small interfering RNA of mouse AnxA2.

AnxA1 release, limiting mast cell degranulation and allergen-mediated allergic reactions, which is extremely relevant in the treatment of ocular allergies.<sup>90</sup> Similarly, AnxA1 has been shown to be effective in limiting inflammation in a diverse range of experimental studies, including osteoarthritis, myocardial IR injury, stroke, multiple sclerosis, sepsis, among others.<sup>91</sup> Altogether, studies indicate that these glucocorticoid-inducible proteins are not only helping to shed some light on the resolution phase of inflammation research but are also paving the way for exciting perspectives in drug discovery, pursuing annexin-based therapeutic strategies in ophthalmology and several other areas.

Some limitation should, however, be mentioned regarding choices of route of administration in the experimental tests – most studies use intraperitoneal and oral treatments, whereas we suggest that other routes of administration such as topical and intravitreal treatments should be explored to optimize future treatments. Moreover, to continue moving this research forward, the translational application of AnxAs should be further investigated reaching clinical trials.

### Conflicts of Interest

The authors have no conflicts of interest to declare.

### CRedit authorship contribution statement

**Rafael André da Silva:** Conceptualization, Investigation, Visualization, Writing – original draft. **Vinicius Moraes de**

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### Data availability

No data was used for the research described in the article.

### Acknowledgments

Work in our laboratories is funded by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). RAS, and VMPR were supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Finance code 001) scholarships. LPDSF was supported by the FAPESP scholarship (2021/00270-4).

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