Adiponectin: A potential candidate for treating fibrosis in posterior segment of the eye

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A B S T R A C T

Fibrosis in ocular tissues causes severe visual deterioration and blindness in patients with glaucoma, cataract, age related macular degeneration (AMD) and diabetic retinopathy (DR). Currently available anti-fibrotic agents exhibit undesirous cytotoxic effects and thus prove ineffective to treat post-surgical fibrosis. Accordingly, there is a need to develop efficient and novel anti-fibrotic agents. Adiponectin (APN), an adipokine from adipocytes is increased in the aqueous and vitreous humor of the patients with micro-angiopathy and chronic inflammation. Furthermore, it is reported to be elevated in the subretinal fluid, vitreous and epiretinal membrane of patients with AMD, proliferative vitreoretinopathy (PVR) and proliferative diabetic retinopathy (PDR) respectively.

Since APN has anti-angiogenic activity and reduces VEGF levels, we hypothesize that APN might regulate the angiogenic and inflammatory milieu of the eye. Furthermore, it is reported to be elevated in the subretinal fluid, vitreous and epiretinal membrane of patients with AMD, proliferative vitreoretinopathy (PVR) and proliferative diabetic retinopathy (PDR) respectively.

Background

Wound healing mechanism following a primary injury as part of the reparative process includes fibrogenesis. When the deposition of extracellular matrix (ECM) and collagen exceeds the need for remodeling the damaged tissue, it leads to the formation of pathological fibrovascular tissue [1–3]. All ocular tissues are susceptible to fibrosis and it is one of the critical factors in the pathogenesis of many vision-threatening ocular diseases such as glaucoma, cataract, AMD, PVR and PDR. While proper visual axis and cellular phototransduction is necessary for accurate sight, fibrosis can destabilize the visual axis upon injury or inflammation to the eye [4].

The anterior segment of the eye, especially cornea is prone to infection, inflammation and external injury resulting from accidents or chemical burns. Eventually, healing process is initiated by regenerative corneal epithelial cells, however when the process is dysregulated, it leads to fibrotic scar formation and vision loss [5]. Fibrotic scarring and encapsulation are more common occurrences as post-operative complication following glaucoma filtration surgery due to aberrant wound healing process at the site of aqueous humor (AH) drainage. Despite the fact that the surgery is performed to decrease the resistance of AH outflow caused by excessive deposition of ECM components at the trabecular meshwork, surgical failure due to pathological fibrosis could exaggerate the disease pathogenesis [6]. Currently available anti-fibrotic agents, 5-fluorouracil and mitomycin C, both exhibit adverse effects and thus prove ineffective to treat post-surgical fibrosis in glaucoma [7].

In case of posterior segment of the eye, sight threatening retinal and choroidal diseases such as AMD, PVR and PDR are characterized by abnormal vitreoretinal or subretinal fibrosis which can induce retinal detachment and blindness [8]. For instance, in the preclinical stage of diabetic retinopathy (PCDR), hyperpermeability of blood retinal barrier generates a hypoxic environment leading to infiltration of many pro-angiogenic and inflammatory molecules. Chronic inflammation activates myofibroblasts that originate from residual fibroblast cells or from epi- or endo-mesenchymal transition. Further, excessive deposition of ECM by myofibroblast during fibrosis causes retinal tear and its detachment leading to vision loss [4,9–12]. In AMD, deposition of hard exudate in the macula region can damage the photoreceptor and retinal pigment epithelial cells resulting in loss of their function [13,14]. Furthermore, post-operative retinal surgery and idiopathic conditions can cause deposition of fibrovascular membrane on the macula region (epiretinal membrane) resulting in pre-retinal fibrosis [12]. Anti-vascular endothelial growth factor (anti-VEGF) treatment has also been
identified to induce pro-fibrotic risk factors in patients with AMD and PDR causing sub-foveal fibrosis [15–17]. In fact, a recent report indicates that optimal duration between intra-vitreal bevacizumab (IVB) and vitrectomy needs to be established as longer pre-incubation period with the drug can cause vitreous hemorrhage and fibrosis [18]. Although multiple mechanisms have been shown to cause fibrosis, so far there is no successful treatment or approved antifibrotic agents available to treat pathological fibrosis. Apparently, the major unmet drug target in most of the ocular diseases remains to be fibrosis.

**Functions of adiponectin**

Adiponectin (APN) is a 30 kDa adipocytokine primarily secreted by adipose tissue and is abundantly found in circulation (in microgram levels) [19–21]. APN exists as multimers of various molecular weights like trimeric form or Low Molecular Weight (LMW), Middle Molecular Weight (MMW), High Molecular Weight (HMW) form and cleaved form of trimer as globular APN (gAPN). Increased APN exerts protective effect on vascular endothelial cells against oxidative stress by regulating nitric oxide synthesis [22]. It is also reported to possess anti-angiogenic and anti-proliferative capabilities as demonstrated in bovine capillary endothelial cells and in porcine aortic endothelial cells [23]. Chronic increase in APN levels associated with PDR is believed to be a protective mechanism of APN with its anti-angiogenic property. Intriguingly, peptide derived from globular APN and recombinant APN both inhibited the laser induced choroidal neovascularization in mice [24]. In addition, APN plays an essential role in energy homeostasis and is considered to be a clinical biomarker for various diseases like cardiac dysfunction, pulmonary diseases, chronic kidney diseases and diabetic retinopathy [25–28].

APN reduces liver, cardiac and pulmonary fibrosis [29–31] via reduction of transforming growth factor (TGF-β1), connective tissue growth factor (CTGF) and induction of tissue inhibitor of matrix metalloproteinases (TIMP1). Agonist peptide against Adipo R1, the receptor for APN significantly reduced the proliferation of hepatic stellate and NIH 3T3 cells through reduction in the expression of alpha smooth muscle actin (α-SMA), collagen type 1 alpha 1 (COL1A1) and TGF β1 [32]. Although there are strong reports for APN being anti-fibrotic and is proposed to be a therapeutic target for liver and pulmonary fibrosis [29,33], interestingly, APN plays a contrary role in renal fibrosis [34]. Understanding the exact molecular mechanism of wound healing process by APN could make it an attractive therapeutic candidate for treating ocular fibrosis.

**Hypothesis**

Recent findings suggest that the adipokine, APN displays significant regulatory role in ocular diseases. At the same time, there are contradictory reports on the involvement of APN in fibrosis. APN plays anti-fibrotic role in liver, cardiac and pulmonary fibrosis whereas it is shown to accelerate renal fibrosis. However, there are no studies on the association of APN to ocular fibrosis. Based on the literature support and our experimental data, we hypothesize that the anti-angiogenic APN could modulate the angio-fibrotic switch and skew the balance towards profibrotic condition during late stages of PVR and PDR. This effect could be tissue and cell type specific and might be driven by the disease context. In addition, various forms of APN could activate respective signaling pathways responsible either for suppression or activation of pathological fibrosis. Thus, APN might have a pleiotropic effect and the system cues may govern its pro- or anti-fibrotic nature.

**Rationale for the hypothesis**

The following evidences support our notion that APN could be profibrotic in the posterior segment of the eye. Initially, we reported the expression of APN and its receptors Adipor1 and R2 in human ocular tissues [35]. Further, we observed elevated levels of APN in the vitreous of patients with PDR. Remarkably, PDR patients who underwent LASER treatment showed decreased Vascular Endothelial Growth Factor (VEGF) levels whereas APN was 3-fold higher [36]. This negative correlation between APN and VEGF reinstated the anti-angiogenic activity of APN and pointed out that APN might regulate VEGF levels. Subsequently, our experiments confirmed that APN treatment in retinal pigment epithelial cells significantly decreased VEGF expression [37]. In line with our report, Bora et al demonstrated that mice treated with APN peptide inhibited VEGF expression in choroidal neovascularization model [38]. Since the angio-fibrotic switch in PVR and PDR largely depends on the delicate balance between angiogenic and fibrotic molecules [39], we propose that high levels of APN in PDR could initially play an anti-angiogenic role and after laser treatment or with anti-VEGF, APN might turn the switch towards profibrotic condition.

Our conception is further justified by the study from Ricker et al., who proposed APN as an independent biomarker for PVR, which involves inflammatory and aberrant wound healing response after reattachment surgery for Rhegmatous Retinal Detachment (RRD) [40]. Notably, significantly increased APN levels have been found in subretinal fluids of patients with PVR. Apart from retinal fluids, APN was found to be 2 fold increased in gliotic retina wherein gliosis (fibrosis in the retina) occurs due to release of many pro-inflammatory cytokines during PVR [41]. Furthermore, when full length APN was treated in human adult retinal pigment epithelial cells (ARPE-19) the mRNA expression of pro-fibrotic markers, TGFβ 1 & 2, Col1A1 and α-SMA were increased (unpublished data). In support of our hypothesis, APN knockout mice with chronic kidney disease shows reduced expression of collagen I and fibronectin. In addition, recombinant APN increased the deposition of ECM and expression of α-SMA in monocytes indicating critical role of APN in monocytes to fibroblast differentiation in renal fibrosis [34]. Similarly, in synovial fibroblasts, APN promoted the expression of proinflammatory cytokines [42]. Moreover, APN was also elevated in cerebrospinal fluid of patients with multiple sclerosis and in synovial fluid of patients with rheumatoid arthritis [43]. Thus, APN seems to be positively associated with various fibrotic conditions. Accordingly, the existing literature support and our experimental data collectively corroborate that APN could play a profibrotic role in the retinal diseases.

To further substantiate our hypothesis, future studies can evaluate the correlation between APN levels and pro-anti-fibrotic molecules in the vitreous humor of patients at different clinical stages of PVR and PDR. Since APN shows cell type specific modulation of fibrotic activity, APN conditional knockout animal will be an excellent model to define its role in each tissue and cell type. Similarly, the association between TGFβ, VEGF, TIMP, Matrix metalloproteinases (MMP) and CTGF expression could be analyzed in APN peptide treated animal model during wound healing to delineate the exact molecular mechanism of APN mediated fibrosis.

**Conclusion**

We conclude on the basis of available evidence that APN could play a pro-fibrotic role in the retinal diseases. Among the various inflammatory molecules implicated in retinal fibrosis, APN is found to be increased both in patients with PVR and PDR. But the association of APN and inflammatory markers in different stages of retinal diseases and fibrosis is not yet clear. APN being an important cytokine, its level in local and systemic circulation makes it a potential biomarker for many disorders [44]. Taken together, we postulate that APN may be involved in the progression of retinal fibrosis, by maintaining significant levels in the developing groups of PVR and in late stages of PDR. The spatio-temporal regulation of APN interaction may determine its pro-fibrotic or anti-fibrotic activity. Many critical factors like the tissue or cell type, the disease context and related cellular microenvironment could influence the role of APN in fibrosis (see Fig. 1). For
instance, the cytokine interleukin-6 (IL-6) displays both pro- and anti-inflammatory properties. Induction of IL-6 classic signaling is anti-inflammatory while the trans-signaling is pro-inflammatory [45]. In line with this, the ratio of different multimeric forms (LMW, MMW, HMW and gAPN) of APN and their preferred receptor interaction could initiate either pro- or anti-fibrotic activity. Thus, APN could be a potential candidate for treating angiogenesis in the beginning stages of PDR, however, eventually at the later stage APN might turn pro-fibrotic in the posterior segment. Accordingly, novel therapeutic modalities could be designed to target APN for the treatment of vitreoretinal diseases.

Conflict of interest

None.

Disclosure

None.

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References


Fig. 1. Possible role of APN in ocular fibrosis: Increased level of APN is found in vitreous and sub-retinal fluids of patients with PDR and PVR respectively, whereas plasma APN is decreased. APN is known for its anti-angiogenic role and decreases VEGF expression. It also protects endothelial cell damage by increasing nitric oxide production. However significant levels of APN in late stages of PDR and PVR involving fibrosis is unclear. Likewise, anti-VEGF drug treatment induces fibrovascular membrane, which can cause trabecular retinal detachment leading to loss of vision. We suppose that APN might play an important role in the regulation of angio-fibrotic switch in ocular fibrosis by decreasing VEGF and skewing the balance towards fibrosis. Since APN shows anti-fibrotic effect in liver, cardiac and pulmonary fibrosis and pro-fibrotic effect in renal fibrosis, we speculate that the role of APN as a pro- or anti-fibrotic largely depends on the cell type, pathological milieu and its receptor interaction. Abbreviations: PDR – Proliferative Diabetic Retinopathy, PVR – Proliferative vitreoretinopathy, VEGF – Vascular Endothelial Growth Factor, CTGF – Connective Tissue Growth Factor, Interleukin-13 (IL-13), APN – Adiponectin, TIMP–1 – Tissue Inhibitor of Matrix metalloproteinase, TGF – Transforming Growth Factor-β.


