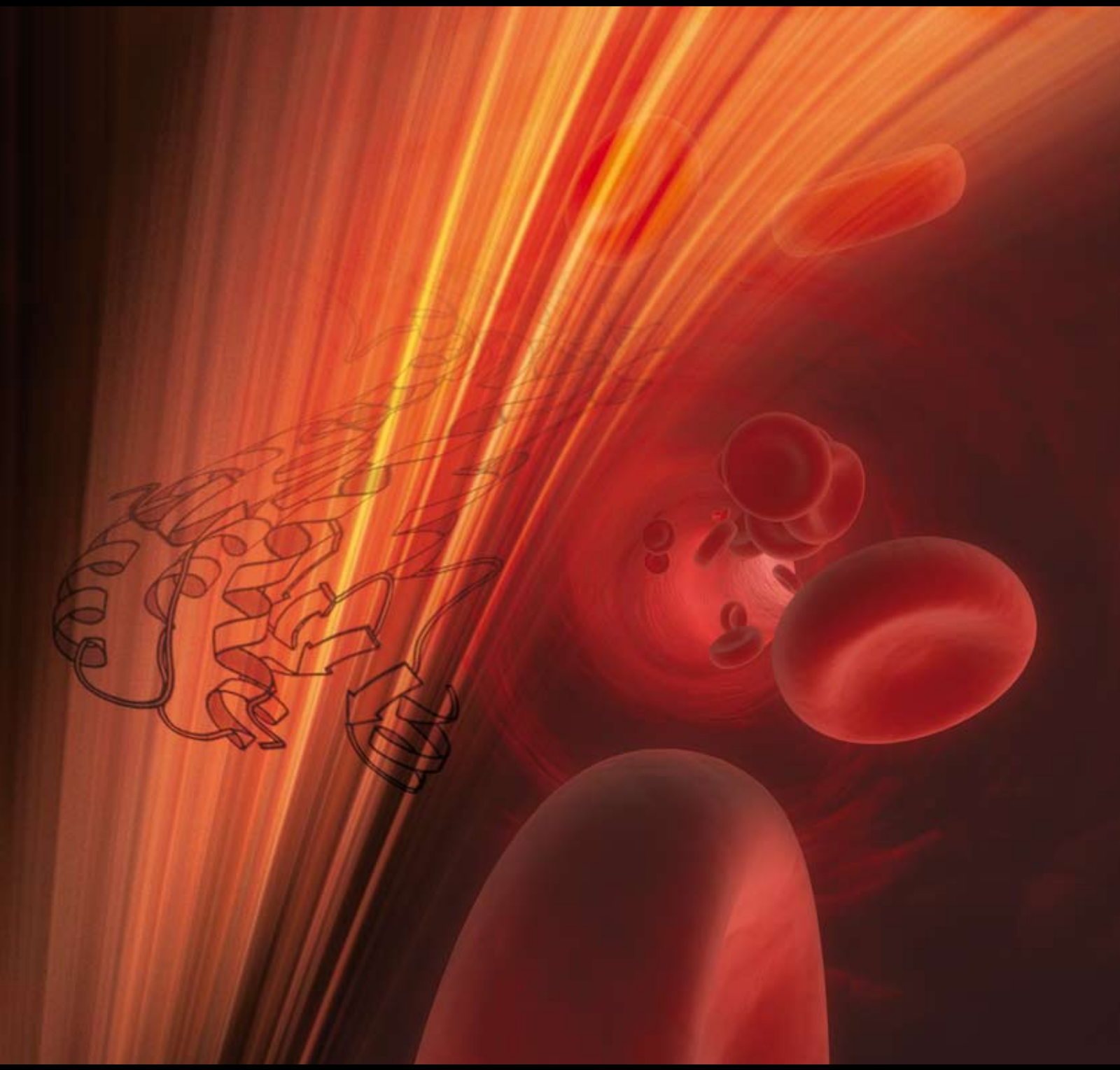
PPAR Research

PPARs in Eye Biology and Disease

Guest Editor: Suofu Qin and Roy S. Chuck



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Editorial
PPARs in Eye Biology and Disease

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Welcome to this special issue of PPAR research dedicated to "PPARs in Eye Biology and Disease." PPARs are well known to regulate the expression of genes involved in lipid and glucose metabolism. Very recently, these transcription factors have been demonstrated to modulate proliferative, inflammatory, and oxidative stress responses, including those that happen in the eye. We have collected a comprehensive group of review papers that are focused on discussing the relationships of PPARs with choroidal neovascularization, inflammation, and redox balance, as well as perspective therapeutic potentials of PPAR modulators in eye diseases.

Angiogenesis is an important element of normal development and neovascularization which occurs normally in wound healing. However, neoangiogenesis is unfortunately also associated with various pathological ocular conditions including corneal neovascularization secondary to graft rejection and traumatic, chemical, and infectious insults; diabetic complications in both the anterior and posterior segments; retinoproliferative disease secondary to vaso-occlusive events; as well as choroidal neovascularization associated with trauma, high myopia, genetic disease, and age-related macular degeneration (AMD). Of these, AMD is currently the leading cause of blindness in the developed world. As such, much effort and expense is and has been invested in understanding and seeking cures for this devastating condition. Although there is little direct evidence linking PPAR action to AMD, there is a growing body of literature demonstrating that PPARs may be involved in various chemical pathways associated with AMD. In this issue, three papers authored by respected experts in the field are presented which review what we now know about the relationship between the 3 PPAR isoforms, α, β, and δ, and ocular angiogenesis with emphasis on AMD. Bishop-Bailey has reviewed PPARβ/δ-mediated angiogenesis in the context of ocular disorders. Gehlbach et al. have briefly discussed the PPAR-α ligands as potential therapeutic agents for wet AMD. Chan et al. have comprehensively described PPARs with the development of AMD. There now appears to be ample data in the peer-reviewed literature to encourage further study of the link between PPARs and AMD, and investigate the therapeutic potential of PPARs. In addition, an authoritative fourth paper authored by Pershadsingh is also offered to address PPARγ agonists as potential therapeutics for non-AMD proliferative retinopathies.

Inflammatory signaling participates in the development of different forms of eye diseases. Inflammatory injury happens under the conditions in which pathoangiogenic signaling is activated in acute inflammatory responses; chronic inflammation is triggered by oxidative stress in diabetic retinopathy and atrophic AMD. The majority of reports documented in the literature support an anti-inflammatory role of PPARs, in particular PPARγ, by blocking the release of inflammatory mediators from activated immune cells in vitro and dampening inflammation in animal models. Minghetti et al. have explored the roles of PPARγ in microglial cell functions and therapeutic potentials of PPARγ ligands on ocular diseases such as AMD, diabetic retinopathy, autoimmune uveitis, and optic neuritis. Yanagi has evaluated the role of PPARγ in the breakdown of blood-retinal barrier, providing strong evidence that targeting PPARγ would be beneficial to diabetic retinopathy via maintaining the integrity of blood-retinal barrier. Phipps et al. have extensively reviewed the literature regarding the role of lymphocytes in thyroid eye disease-related inflammation, offering PPARγ ligands as a therapeutic approach via inhibition of inflammatory signaling in activated lymphocytes and fibroblasts.

The potential regulation of redox balance by PPARs in the eye has been recently suggested and may constitute a new,

exciting research field over the next few years. Phagocytosis of tips of rod outer segments' selectively upregulates expression of PPARγ in retinal pigment epithelial (RPE) cells, suggesting that PPARγ activation might deal with oxidative stress during RPE cell phagocytosis. Oxidative stress is a major risk factor causing RPE cell degeneration since RPE cells are exposed to high levels of free radicals due to phagocytosis of oxidized photoreceptor outer segments, intense light irradiation, and high oxygen consumption in the macular area. PPARγ ligands protect a variety of cell types from oxidative stress injury in vitro, including retinal cells, though no in vivo data are available yet. Chang et al. have briefly reviewed the cytoprotective effects of an endogenous PPARγ agonist, 15d-PGJ_2, on oxidative stress-induced RPE cell death.

PPARs are emerging as potential targets for drugs that might be used in the treatment of ocular diseases in which PPAR activities play a key role in disease pathology. It is our hope that this special issue will serve as a seed stimulating broad interests to pursue therapeutic avenues of PPARs in eye diseases. The outcomes of such investigations will undoubtedly shed light on the roles of PPARs in eye diseases and possibly identify new roles of PPARs in the etiology of eye diseases.

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Review Article

A Role for PPARβ/δ in Ocular Angiogenesis

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Recommended by R. Chuck

The uses of highly selective PPARβ/δ ligands and PPARβ/δ knockout mice have shown a direct ability of PPARβ/δ to regulate angiogenesis in vitro and in vivo in animal models. PPARβ/δ ligands induce the proangiogenic growth factor VEGF in many cells and tissues, though its actions in the eye are not known. However, virtually, all tissue components of the eye express PPARβ/δ. Both angiogenesis and in particular VEGF are not only critical for the development of the retina, but they are also a central component in many common pathologies of the eye, including diabetic retinopathy and age-related macular degeneration, the most common causes of blindness in the Western world. This review, therefore, will discuss the recent evidence of PPARβ/δ-mediated angiogenesis and VEGF release in the context of ocular disorders.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPAR's) belong to the steroid receptor superfamily of ligand-activated transcription factors [1]. Three PPAR's, PPARα, PPARβ/δ, and PPARγ, have been identified [2]. PPARα is predominantly expressed in liver, heart, kidney, brown adipose tissue, and stomach mucosa; PPARγ is found primarily in adipose tissue; PPARβ/δ is the most ubiquitously expressed [3, 4], though its roles in physiological and pathophysiological processes are far from clear, particularly, in human tissue. The recent development of PPARβ/δ knockout and transgenic mice has started to implicate roles for PPARβ/δ in adipose tissue formation, metabolism, wound healing, brain development, placental function, atherosclerosis, colorectal carcinogenesis, and skeletal muscle function [5–7]. In this review, the emerging role of PPARβ/δ in regulating endothelial function and angiogenesis will be discussed with a particular emphasis to its relevance in the eye.

2. PPARβ/δ LIGANDS

A number of synthetic PPARβ/δ compounds have been described including GW0742X, GW2433, GW9578, L-783,483, GW501516, L-796,449, L-165,461, and compound F [8, 9]. In addition, putative endogenous PPARβ/δ activators include fatty acids [3, 10], triglycerides [11], the cyclooxygenase (COX) product, prostacyclin [10], the COX/prostacyclin synthase derived endocannabinoid metabolites [12], and *all-trans* retinoic acid (ATRA) [13]. ATRA is derived from vitamin A (retinol) which is found at its highest levels in the eye and is essential for its development and function [14]. Retinol is converted to retinaldehyde, a component of rhodopsin [14] and a functional PPARγ antagonist [15, 16], which in turn is metabolised to ATRA by retinal dehydrogenases [14]. ATRA has its own family of high-affinity nuclear receptors, the retinoic acid receptor (RAR)α, -β, and -γ, which like the PPAR's act as heterodimers with RXRα, -β, and -γ, the receptors for the ATRA isomer 9-cis retinoic acid [17]. Although ATRA can activate PPARβ/δ, it is not known which, if any, of its actions are mediated by PPARβ/δ. However, since ATRA is present in such large quantities in ocular tissue, it is potentially an important site for its actions.

3. PPARβ/δ AND ENDOTHELIAL CELLS

Endothelial cells play critical roles in vascular biology, being both the protective inner lining of vessels and the local site for delivery of oxygen to all tissues. Angiogenesis is the process

of new blood vessel/capillary formation from existing vessels, and hypoxia is a major signal which drives the process [18]. PPARα, PPARβ/δ, and PPARγ are all expressed in endothelial cells [19]. PPARα and PPARγ have well-characterised roles in endothelial cells, both being in general anti-inflammatory, antiproliferative [1], and antiangiogenic in a variety of in vitro and in vivo models, including tumorigenesis [20] and laser-induced retinal injury [21]. In contrast, the role of PPARβ/δ in this important cell type has only recent starting to be elucidated. Initial reports using prostacyclin as a ligand suggested that like PPARα and PPARγ, PPARβ/δ promoted endothelial cell apoptosis [22]. In contrast, the use of highly selective synthetic ligands has revealed a contradictory role for PPARβ/δ regulating endothelial cell survival, proliferation, and angiogenesis.

3.1. PPARβ/δ and endothelial cell proliferation and survival

Long- [23] and short-term [24] culture of endothelial cells with the selective ligand GW501516 induces endothelial cell proliferation, an effect associated with the induction of the VEGF receptor (Flt-1; VEGF R1) and VEGF production [23, 24]. In addition to inducing proliferation, PPARβ/δ activation protects cells from oxidant-induced apoptosis. Synthetic PPARβ/δ ligands or activation of the COX-prostacyclin pathway, which signals through PPARβ/δ, induce the endothelial expression of 14-3-3α protein [25]. 14-3-3 proteins are anti-apoptotic and anti-inflammatory molecules [26]. PPARβ/δ-induced 14-3-3α blocks oxidant- (H_2O_2-) induced apoptosis by sequestering the proapoptotic protein Bad, stopping its translocation to mitochondrial membranes, where it initiates cytochrome c release and the subsequent activation of the proapoptotic caspase cascade [25].

3.2. PPARβ/δ and angiogenesis

In addition to having effects on endothelial cell proliferation, PPARβ/δ activation potently induces angiogenesis of human vascular endothelial cells in tumour extracellular matrix in vitro and in a murine matrigel plug model in vivo [24]. In addition, the putative PPARβ/δ ligand prostacyclin analogues [27] and ATRA [28] also induce angiogenesis, though the latter appears mostly dependent on its RARα receptor rather than PPARβ/δ [29]. In human endothelial cells, a major trigger for morphogensis induced by PPARβ/δ stimulation was the stimulated release of VEGF [24]. In addition to VEGF, mRNA for the matrix metalloproteinase (MMP)-9, a protease important for cell migration was also elevated by PPARβ/δ activation [24]; however, whether this was secondary to VEGF release was not tested. VEGF is expressed as four main splice variants (by amino acid size: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$) [29]. VEGF (VEGF-A; $VEGF_{165}$) is a well-characterised central mediator of endothelial cell growth and angiogenesis [29, 30]. Two endothelial VEGF tyrosine kinase receptors have been identified: VEGFR-1/Flt-1, and VEGFR-2/KDR/Flk1. VEGF R2 appears to be the most important receptor in VEGF-induced mitogenesis and permeability [29, 30]. In addition, in two

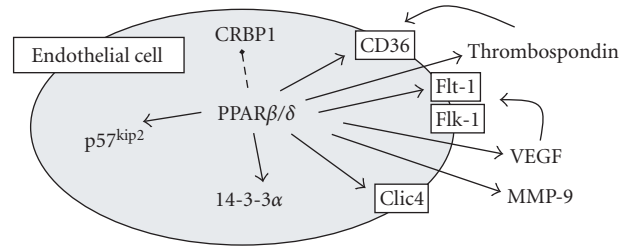

Figure 1: Proangiogenic/prosurvival pathways of PPARβ/δ in endothelial cells. PPARβ/δ is expressed in endothelial cells. PPARβ/δ activation induces (solid line) the expression of VEGF and its receptor Flt-1, matrix metalloproteinase (MMP)-9, thrombospondin and its receptor CD36, the chloride intracellular channel protein (CLIC)-4, the cell cycle inhibitor $p57^{kip2}$, and the antiapoptotic protein 14-3-3α. In contrast, the cellular retinol binding protein-1 is decreased (dashed line) by PPARβ/δ activation. For those interested, a complex transcriptional map of the potential role of PPARβ/δ as a hub node in tumour angiogenesis has recently also been formed as detailed in [32].

recent studies, the growth of PPARβ/δ wild-type tumours or angiogenesis in matrigel plugs in PPARβ/δ knockout mice was tested [31, 32]. The tumours in PPARβ/δ knockout mice compared to wild-type mice were associated with a diminished blood flow and an immature hyperplastic microvascular structures. Moreover, the retroviral introduction of PPARβ/δ into matrigel plugs was able to rescue the knockout phenotype by triggering microvessel maturation [31]. In the latter of these studies, PPARβ/δ was examined in tumours from patients who had undergone "angiogenic switch" a proangiogenic state involved in tumour progression [32]. PPARβ/δ correlated with advanced pathological tumor stage, increased risk for tumor recurrence, and distant metastasis, and was, therefore, suggested as a hub node transcription factor regulating tumour angiogenesis [32].

Genomic and proteomic analyses of the PPARβ/δ knockout endothelial cells isolated from matrigel plugs have also led to the identification of a number of additional candidate genes to mediate the actions of PPARβ/δ in angiogenesis. In particular, the Cdkn1c gene which encodes the cell cycle inhibitor $p57^{Kip2}$ is a direct PPARβ/δ target gene that mediates PPARβ/δ effects on cell morphogenesis [31]. In addition, CD36 and thrombospondin were also decreased in matrigel-invading endothelial cells from PPARβ/δ knockout mice [31]. Thrombospondins by directly interacting with CD36 inhibit angiogenesis in vivo [33, 34]. Similarly, a proteomic analysis by the same group [35] on PPARβ/δ knockout endothelial cells has also revealed a decreased expression of the chloride intracellular channel protein (CLIC)-4 in migrating endothelial cells from PPARβ/δ knockout mice. In contrast, the expression of cellular retinol binding protein CRBP1 is increased in migrating endothelial cells from PPARβ/δ knockout mice [35]. CLIC-4 promotes and plays an essential role during tubular morphogenesis [36], while CRBP1 inhibits cell survival pathways by acting as an inhibitor of the AKT signalling pathway [37], an additional important signalling signal for angiogenesis to occur [38].

The combination of these studies show PPARβ/δ activation induces endothelial cell mitogen and differentiation signals, including VEGF, 14-3-3α, CD36 and thrombospondin, *CLIC4*, CRBP-1, and $p57^{KIP2}$, all of which may act in a coordinate manner to bring about the functional morphogenic changes associated with angiogenesis.

3.3. PPARβ/δ and VEGF

Although the direct evidence for a role of PPARβ/δ in angiogenesis is relatively new, there has been an increasing literature regarding PPARβ/δ regulated tumour cell growth via inducing tumour cells to release VEGF. PPARβ/δ ligands induce VEGF in bladder cancer cells [39], human breast (T47D, MCF7), and prostate (LNCaP, PNT1A) cancer cell lines, along with its receptor flt-1 [22], but not (HT29, colon; HCT116, colon; LS-174T, colon; HepG2, hepatoma; and HuH7, hepatoma) cell lines [40].

In a genetic model of intestinal polyp development APC/min mouse, deletion of PPARβ/δ decreases intestinal adenoma growth and inhibits tumour-promoting effects of the PPARβ/δ agonist GW501516 [41]. Moreover, activation of PPARβ/δ upregulated VEGF in colon carcinoma cells, promoting colon tumour epithelial cell survival through activation of AKT signalling [41]. Angiogenesis was not studied in this model, however, any substantial tumour growth requires a blood supply and angiogenesis to allow it to develop. In contrast, in human colon and liver cancer cell lines [40], PPARβ/δ ligands had no effect on human cancer cell growth, AKT, VEGF or COX-2 expression in vitro or on these makers in the liver, colon, and colon polyps in mice treated in vivo [40]. The roles of PPARβ/δ in VEGF- mediated tumorigenesis are, therefore, still in need of further clarification.

3.4. Expression of PPARβ/δ in the eye

Angiogenesis regulates both the physiological development and many of the most common pathophysiology's of the eye. As yet, there is no direct evidence linking PPARβ/δ and angiogenesis in the eye, however, PPARβ/δ is clearly expressed at least in murine ocular tissue. PPARβ/δ is expressed in the eye ciliary body epithelial cells, cornea epithelial cells, cornea endothelium, cornea fibroblast, retina inner nuclear layer, and retina ganglion cell layer [42]. Although one must be cautious interpreting data from nonocular tissue to the eye [43], as discussed previously and following, pathways that have direct relevance to ocular angiogenesis are clearly regulated by PPARβ/δ and are therefore worthy of discussion.

4. VEGF AND OCULAR ANGIOGENESIS

VEGF is essential in retinal vasculature development [44]. Initially blood vessels grow from the optic nerve outwards. As the retinal tissue develops via a complex interplay between different cellular components such as neurons, glia, endothelial cells, pericytes, and immune cells, the increased oxygen demand induces hypoxia, the main stimulant for new vessel growth via angiogenesis. As the tissue/vasculature develops and gets oxygenated, hypoxia and VEGF decrease limiting new vessel growth [44].

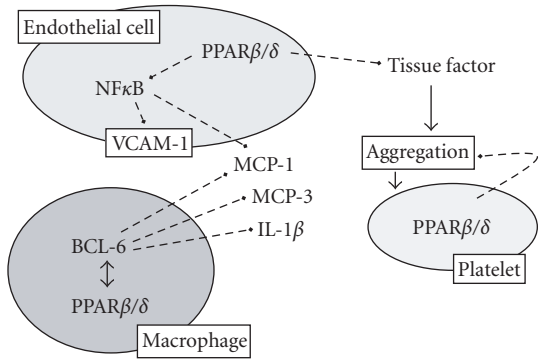

Figure 2: Antiinflammatory/anticoagulation pathways of PPARβ/δ. PPARβ/δ activation in endothelial cells reduces NFκB activation and the induction of vascular cell adhesion molecule (VCAM)-1, and monocyte chemoattractant protein (MCP)-1, along with the release of tissue factor. PPARβ/δ is expressed in platelets and monocytes/macrophages. PPARβ/δ ligands reduce platelet aggregation via a rapid nongenomic mechanism. In macrophages, PPARβ/δ ligands release the transcriptional corepressor BCL-6 from its complex with PPARβ/δ. Free BCL-6 suppresses the release of MCP-1, MCP-3, and IL-1β.

In contrast, neovascularisation of the adult eye via angiogenesis is a critical component of many disorders of the eye including retinopathy of prematurity, diabetic retinopathy, and age-related macular degeneration, the latter two being the leading causes of blindness in the Western world (as reviewed in detail elsewhere [29, 45–48]). Pathological neovascularisation resulting from tissue damage and hypoxia results in a more complex "inflammatory" angiogenesis. These new vessels are often fragile and leaky leading to haemorrhage and vision disturbance and loss. The main trigger for this new vessel growth still appears to be hypoxia induced VEGF expression [29, 45–48]. Angiogenesis is a homeostatic repair mechanism that is required for the reoxygenation of the damaged ischemic tissue [29, 45–48]. The problems that arise with pathologies such as age-related macular degeneration and diabetic retinopathy are that this new vessel growth is leaky and has a critical inflammatory component. VEGF (in particular VEGF A; $VEGF_{165}$) in addition to directly stimulating angiogenesis is also a potent vascular permeability factor and appears to play a role in regulating the local inflammation associated with pathological neovascularisation [49]. VEGF has become a clear therapeutic target for the treatment of angiogenesis in the eye. The clinical importance of VEGF as a target has recently been further demonstrated with the development and use of two new drugs targeting its actions: Macugen (pegaptanib), an aptamer, and Lucentis (ranibizumab), a FAB fragment, from a humanised monoclonal antibody, which both functionally block VEGF. Moreover, Macugen and Lucentis both show clinical efficacy in patients with age-related macular degeneration [50]; especially when treated early and a mature neovasculature has yet to form. These therapies require local delivery by intravitriol

injection, which although having the benefit of overcoming problems such as systemic VEGF blockade, they are clearly still not ideal, and show that new therapies are still required.

5. PPARβ/δ OCULAR ANGIOGENESIS, INFLAMMATION, AND COAGULATION

Angiogenesis associated with pathophysiology is often associated with multiple process such as tissue damage, inflammation, and coagulation. In contrast, developmental angiogenesis may be a simpler hypoxia driven event. Indeed, an inflammatory response is induced by VEGF during pathological but not physiological ischemia-induced retinal angiogenesis [51, 52]. Moreover, specifically blocking inflammatory cytokines monocyte chemotactic protein-1 and macrophage inflammatory protein-1a can reduce retinal neovascularisation [53]. Tissue factor is a critical initiator of blood coagulation, and is associated with tumour aggressiveness and angiogenesis in a variety of cancer cells [54], as well as in choroidal neovascularisation where it promotes fibrin formation and the growth of the choroidal angiogenic complex [55]. One important facet of pathological angiogenesis may therefore be this involvement additional pathways, and a complex interplay between processes of tissue damage, hypoxia, inflammation, and coagulation. A long-term therapeutic aim may therefore be to have revascularisation of hypoxic tissue similar to development without these additional inflammatory/coagulation processes.

PPARβ/δ induces VEGF in a number of cell types and induces angiogenesis. Therefore, one may predict that a PPARβ/δ antagonist would be useful to treat or at least test in models of eye disease that involve neovascularisation. However, PPARβ/δ seems consistent with other PPAR's in that it also has anti-inflammatory and anticoagulation properties, suggesting that its properties in ocular angiogenesis may be more complex than one would originally predict.

PPARβ/δ activation suppresses endothelial cell tissue factor expression [12]. PPARβ/δ is also expressed in platelets where its ligands reduce platelet aggregation to a variety of stimuli [56]. Similar to PPARα and PPARγ, PPARβ/δ ligands are anti-inflammatory in endothelial cells, inhibiting TNFα-induced upregulation of expression of vascular cell adhesion molecule-1, monocyte chemoattractanct protein-1, and nuclear factor (NF)κB translocation [57]. In macrophages, PPARβ/δ controls inflammatory status by its association and disassociation with the transcriptional repressor BCL-6 [58]; in the absence of ligand, PPARβ/δ physically associates with and inhibits this anti-inflammatory BCL-6. When a PPARβ/δ ligand is added, BCL-6 dissociates from PPARβ/δ and represses the inflammation and levels of monocyte chemoattractanct protein-1, -3, and IL-1β [58].

6. CONCLUSION

PPARβ/δ induces angiogenesis and protects endothelial cells from oxidant damage. A common signal for PPARβ/δ activation in endothelial cells or surrounding tissue may be the induction of VEGF. PPARβ/δ is expressed in all tissues in the eye, however its function has yet to be tested in physiological processes, development, or pathophysiological disorders. The development of both the eye and common pathological disorders requires angiogenesis, with VEGF being a primary signalling molecule. Blocking PPARβ/δ may therefore provide a new therapy to treat angiogenic eye disorders. The difference between "physiological" and "pathophysiological" angiogenesis may be additional components of inflammation and coagulation. PPARβ/δ ligands reduce inflammation and components of the coagulation cascade. It will be of great interest to test the roles of PPARβ/δ in the eye as a potential proangiogenic stimulus reliving the hypoxia, while potentially still capable of reducing the damaging inflammatory/coagulation signals.

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Review Article

PPAR-α Ligands as Potential Therapeutic Agents for Wet Age-Related Macular Degeneration

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The peroxisome proliferator-activated receptors (PPAR's) are members of the steroid/thyroid nuclear receptor, superfamily of transcription factors. There are currently three known PPAR subtypes, α, β, and γ. The PPARs are now recognized participants in a number of biological pathways some of which are implicated in the pathogenesis of age-related macular degeneration (AMD). These include immune modulation, lipid regulation, and oxidant/antioxidant pathways important to the onset and progression of "dry" AMD, and vascular endothelial growth factor (VEGF) mediated pathways that stimulate choroidal neovascularization (CNV), characteristic of "wet" AMD. PPAR-α is found in retina and also on vascular cells important to formation of CNV. At this time, however, relatively little is known about potential contributions of PPAR-α to the pathogenesis of dry and wet AMD. This review examines current literature for potential roles of PPAR-α in the pathogenesis and potential treatment of AMD with emphasis on prevention and treatment of wet AMD.

1. INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of new blindness in the Western World and is currently responsible for more than half of all legal blindness in the United States. There are approximately 8 million people in the U.S. with early or intermediate stage AMD. Approximately one million of these people will develop advanced disease within the next five years [1–5]. Currently AMD is estimated to affect about 50 million people worldwide. With aging of the population this number is expected to double by the year 2020. Strategic approaches to management of AMD include delaying onset and progression of nonneovascular "(dry)" disease; preventing conversion from dry to wet disease and treatment of wet disease.

While specific antioxidant vitamin formulations are now known to delay progression of intermediate disease, current treatment of AMD focuses largely on providing therapeutic intervention following the progression of intermediate "(dry)" disease to late stage "(wet)" disease. The neovascular ("wet" or "exudative") form of AMD can lead to rapid visual decline and accounts for nearly 90% of vision lost. It is characterized by development of pathologic choroidal neovascularization (CNV). Early strategies to ablate CNV used thermal laser or photodynamic therapy. These are now less frequently used as treatments that antagonize the effects of vascular endothelial growth factor (VEGF), continue to enhance efficacy, and improve outcomes. Currently pegaptanib, ranibizumab, and bevacizumab are considered relatively safe and achieve therapeutic effects that may include inhibition/regression of CNV, decreased vascular leakage, absorption of subretinal fluid, and improved vision [6–10].

The peroxisome proliferator-activated receptors (PPAR's) are not, at the present time, known as direct treatment targets in the management of AMD. Each represents a separate nuclear receptor of the steroid super-family of ligand activated transcription factors that induce steroid hormones, thyroid hormones, vitamin D, and retinoid acid receptor [11]. PPAR's comprise a family of three ligand-activated transcription factors (α, β, and γ) that are characterized by distinct function, ligand specificity, and tissue distribution. The PPAR transcription factors regulate transcription of many genes involved in differentiation, proliferation, and apoptosis, in a variety of cell types. During gene expression the PPAR forms a heterodimer receptor complex with the 9-cis-retinoic acid receptor (RXR). The PPAR/RXR

heterodimer is associated with a multiprotein corepressor. When a ligand or agonist binds to the receptor, the corepressor complex dissociates. The heterodimer receptor then binds with peroxisome proliferator response elements on the promoter domain of target genes to stimulate transcription [12].

Three distinct PPAR's had been identified in mammals, PPAR-α, PPAR-γ, and PPAR-δ (also referred to as PPAR β). The first PPAR entity identified was PPAR-α agonist, which has multiple functions that result in an improved lipid profile, increasing high density lipoprotein cholesterol (HDL-C), decreasing triglycerides and free fatty acids, and shifting low density lipoprotein cholesterol (LDL-C) to larger less atherogenic particles. Each of these improvements in the lipid profile is potentially beneficial and may in theory lead to delay in AMD onset and progression thereby avoiding late stage or "wet" disease.

PPAR-α is transcribed from chromosome 22q12-13.1, is primarily expressed in tissues with elevated mitochondrial and peroxisomal fatty acids β-oxidation rates, such as liver, heart muscle, kidney, skeletal muscle, retina, and brown fat [13–15] and may have a potential role in oxidant/antioxidant pathways now strongly implicated in the pathogenesis of dry AMD. PPAR-α is also present in cells of the arterial wall associated with smooth muscle cells [16] and endothelial cells [17] and is found in monocytes and macrophages [18] that participate in CNV formation, characteristic of wet AMD [19]. The PPAR's are activated by a number of ligands including eicosanoids and fatty acids. In addition, synthetic antidiabetic and lipid lowering fibrates have been shown to activate PPAR-γ and PPAR-α, respectively. PPAR-α is the main target of fibrate drugs, a class of amphipathic carboxylic acids (gemfibrozil, fenofibrate, clofibrate) used in managing elevated triglycerides and cholesterol. PPAR-γ is highly expressed in adipose tissues and is a key mediator of adipogenesis [20, 21] and glucose homeostasis [22]. Little is known about the PPAR-δ which is expressed ubiquitously and has now been linked to obesity.

2. PPARs IN THE VASCULATURE

In addition to well established roles for the PPAR's in metabolic pathways, recent work suggests that the PPAR's may be involved in vascular regulation. Several groups have identified PPAR-γ and PPAR-α expression in monocytes/macrophages, vascular smooth muscle cells, and endothelial cells [16–18]. In the endothelium, PPAR-γ has been identified by PCR reaction [23], western blot and immunoprecipitation. PPAR-α has been demonstrated in the vascular endothelium by immunohistochemical technique [24]. While PPAR-γ has been widely studied for its antiangiogenic properties [25], recent studies now indicate that PPAR-α may have antiangiogenic properties as well [26, 27], a finding with potential therapeutic implications for wet AMD. PPAR-α agonists have recently been shown to inhibit expression of VEGF receptor 2 (VEGFR2) upregulation in neovascularization [26]. Varet et al. have demonstrated that fenofibrate, a PPAR-α ligand, inhibits angiogenesis in vitro and in vivo. They have also shown that fenofibrate reduces endothelial cell growth rate, endothelial cell mediated wound repair, and capillary tube formation. Interestingly fenofibrate has been shown to inhibit bFGF-induced angiogenesis in vivo [27]. Simultaneous inhibition of VEGFR2, bFGF, and VEGF would in theory have a profound effect on pathological angiogenesis in the eye.

PPAR-α and PPAR-γ are associated with anti-inflammatory and antioxidant activity [28–30] and have antiatherogenic effects [31]. Each of these pathways is considered important to the onset and progression of early AMD and to development of late choroidal neovascularization. PPAR-α activators inhibit expression of vascular cell adhesion molecules on the endothelium that are important for the development of new blood vessels and for atherogenesis [32]. Experimental evidence suggests that the PPAR activators prevent in vitro vascular muscle cell growth [33], limit inflammatory responses [16], and are proapoptotic indicating a potential role in vascular remodeling [34]. Such activity could theoretically inhibit the transition from dry to wet AMD. PPAR-α agonists also inhibit interleukin-1-induced production of interleukin-6 and prostaglandins [16]. Moreover, Delerive et al. have demonstrated prolonged inflammatory responses and increased interleukin-6 production in aortic explants of PPAR-α deficient mice [35] underscoring the anti-inflammatory potential of PPAR-α.

3. PPAR-α IN ANGIOGENESIS

Pathological angiogenesis leading to choroidal neovascularization is pathognomonic of "wet" AMD. Angiogenesis is the formation of new blood vessels from preexisting vessels and involves endothelial cell proliferation, migration, and organization into new capillary tubes. Pathological angiogenesis is integral to a number of prevalent ocular diseases characterized by the development of ocular neovascularization including but not limited to wet AMD, diabetic retinopathy, corneal neovascularization, the occlusive retinal vasculopathies, and retinopathy of prematurity. Inhibitors of ocular angiogenesis therefore have broad therapeutic implications for patients with these diseases.

Varet et al. demonstrated inhibition of angiogenesis by the PPAR-α ligand fenofibrate [27]. The antiangiogenic properties exhibited were characterized by a dose-dependent decrease in endothelial cell proliferation and apoptosis. Fenofibrates also reduced endothelial cell migration in vitro and capillary tube formation in a matrigel assay. Meissner et al. have also reported a reduction in endothelial cell proliferation, migration, and tube formation following treatment with fenofibrates and also with the PPAR-α agonist Wy14643 [26]. In further support of the evident antiangiogenic effect is the observation that several PPAR-α agonists decrease expression of VEGF receptor 2 (VEGFR2) in human umbilical endothelial cells (HUVECs) [26].

VEGFR2 is the most potent of the VEGF receptors. When activated VEGFR2 initiates signaling that leads to endothelial cell proliferation and also to expression of cytoprotective antiapoptotic molecules [36]. VEGFR2 is detectable only at relatively low levels in the adult vasculature; it is markedly up regulated by blood vessels during chronic inflammation,

hypoxia, tumor growth, and wound repair. VEGFR2 and VEGF expression both increase as part of the angiogenic response and this coordinate response is observed in wet AMD as well as other ocular diseases characterized by pathological neovascularization [37, 38]. VEGF has been identified in fibroblastic cells and transdifferentiated RPE cells in surgically excised choroidal neovascular membranes (CNV) [39, 40]. VEGF expression is also increased in macular RPE cells in patients with AMD [41]. Vitreous VEGF levels are significantly higher in AMD patients with CNV as compared to healthy controls [42]. VEGF production is also increased in RPE cells, retinal vascular endothelial cells, retinal pericytes [43–45], and Muller cells [46]. The endothelial cells of the retinal vasculature possess numerous high-affinity VEGF receptors.

PPAR-α agonists have been associated with a reduction in VEGF levels in OVCAR-3 tumor as well as in DISS-derived ascites [47]. They also reduce microvessel density in these tumors. Other studies have similarly demonstrated that a reduction in PPAR-α message and activity is associated with hypoxia [48]. Hypoxia-induced VEGF expression contributes to choroidal and retinal neovascularization. The relative significance of the effect of PPAR-α on VEGFR2 and VEGF expression in the setting of AMD is not yet known.

4. PPAR-α AND WET AMD

Fenofibrates and other PPAR-α agonists are reported to decrease expression of VEGF and VEGFR2 that are central to the VEGF/VEGFR signaling cascade and important to the development of pathological CNV in AMD. Growth of experimentally induced CNV, via laser rupture of Bruch's membrane in a rat model, is inhibited by intravitreous treatment with a PPAR-γ agonist [49]. At the time of this writing, similar data has not been reported for PPAR-α. Evaluation of this question is however supported by evidence of PPAR-α reduction of VEGFR2 expression in endothelial cells [26] and reported decreases in tissue VEGF levels [47]. PPAR-α activators have also been shown to limit the expression of vascular cell adhesion molecules in the endothelium, an early step in atherogenesis and an important step in the development of new blood vessels [32]. Inhibition of CNV initiation and early progression of CNV are therefore theoretical benefits of PPAR-α agonist treatment. Described proapoptotic effects also suggest therapeutic roles in early CNV development or late regression of CNV [34].

With reports that the PPAR's limit inflammatory as well as oxidative responses and improve lipid profiles [16, 28, 29, 35, 37, 50], it is tempting to speculate on a potential role in delaying onset and progression of nonneovascular "(dry)" disease, thereby potentially preventing latter "wet" stages of disease. There is a substantial literature linking oxidative damage to dry AMD pathogenesis [51]. PPAR-α could theoretically inhibit AMD progression via effects on oxidative pathways. It has been previously reported that PPAR-α activation induces the expression and activation of antioxidant enzymes, such as super oxide dismutase and glutathione peroxidase [29]. It has also been reported that PPAR-α agonists are neuroprotective in the CNS, and that this neuroprotection has been associated with a decrease in cerebral oxidative stress. Consumption of direct acting antioxidants to provide protection to the retina and the RPE is supported by the AREDS clinical trial that has added antioxidant formulation to the routine care of dry AMD. Whether the antioxidant effects of PPAR-α activation are comparable to those of AREDS formulation is not known.

Because fenofibrates are orally administered and have an established safety profile in the treatment of atherosclerosis, investigations pertaining to the impact of oral therapy on oxidative stress, VEFGR2, VEGF, and CNV growth are important. It is also important to consider examining for potential beneficial effects on onset and progression of nonneovascular "(dry)" disease and conversion from dry to wet disease. These and other factors support a hypothesis that asks whether PPAR-α may play a therapeutic role in either prevention or treatment of wet AMD.

5. SUMMARY

AMD remains the leading cause of new blindness in people over 65 years of age and is the leading cause of new blindness in the Western World. The conversion of dry AMD to wet AMD is associated with most of the attendant visual decline. Currently a variety of antiangiogenic treatments directed at halting CNV growth and leakage are the mainstay of therapy. The most frequently injected agent ranibizumab (Lucentis) results in stabilization of visual acuity at the pretreatment level for a majority of patients and results in improvement of visual acuity by 3 or more lines in about 1/3 of those treated. The therapy does not however restore visual acuity to normal levels in the majority of those treated. Moreover, therapy with ranibizumab and other currently available VEGF antagonists requires frequent intravitreous injections and is associated with significant expense, some risk, and for most, incomplete recovery of vision.

An oral therapy with an established safety profile that favorably modified VEGF/VEGFR signaling and increased the antioxidant capacity could significantly impact the therapy of wet AMD. Taken collectively, the PPAR's demonstrate favorable biological activity in pathophysiological pathways relevant to the onset and progression of nonneovascular and neovascular age-related macular degeneration. The relative importance of the PPAR-α pathway in AMD is not yet known. There is, however, sufficient preliminary evidence to support further study of a potential role for PPAR-α pathway modulation as an adjuvant or primary treatment in AMD.

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Review Article

Peroxisome Proliferator-Activated Receptor and Age-Related Macular Degeneration

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Age-related macular degeneration (AMD) is the leading cause of new blindness in the western world and is becoming more of a socio-medical problem as the proportion of the aged population increases. There are multiple efforts underway to better understand this disease process. AMD involves the abnormal retinal pigment epithelium (RPE), drusen formation, photoreceptor atrophy, and choroidal neovascularization. Peroxisome proliferator-activated receptors (PPARs) play an important role in lipid degeneration, immune regulation, regulation of reactive oxygen species (ROSs), as well as regulation of vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and docosahexaenoic acid (DHA). These molecules have all been implicated in the pathogenesis of AMD. In addition, PPAR gamma is expressed in RPE, an essential cell in photoreceptor regeneration and vision maintenance. This review summarizes the interactions between PPAR, AMD-related molecules, and AMD-related disease processes.

1. INTRODUCTION

Improvements in public health and medical advancements have led to increasing lifespan among the population today and consequently, a mounting burden of many disorders of deteriorating body systems such as age-related macular degeneration (AMD). Currently AMD is the leading cause of blindness in developed countries [1]. With the general aging of the population, this debilitating disease promises to become an even bigger health care problem. As the demand for therapy increases, much effort is being directed toward the elucidation of the mechanisms underlying AMD pathogenesis.

Peroxisome proliferator-activated receptors (PPARs) are members of the steroid/thyroid nuclear receptor superfamily of ligand-activated transcription factors. PPARs are involved in lipid and glucidic metabolism, immune regulation, and cell differentiation. Because of these functions, PPARs and their synthetic agonists have been marketed as fibrates and thiazolidinediones for hypercholesterolemia and type 2 diabetes mellitus, respectively [2]. There is much speculation regarding the potential role of PPARs in other disease mechanisms. Recently, PPARs have been associated with age-related changes in Alzheimer's disease [3] and Parkinson's disease [4], suggesting that PPARs might also play a role in the pathogenesis of AMD.

2. AGE-RELATED MACULAR DEGENERATION

The normal aging process of the eye can include a spectrum of changes in the eyes [5] as follows. Photoreceptors decrease in density, retinal pigment epithelium (RPE) undergoes loss of melanin; formation of lipofuscin granules, and accumulation of residual bodies; and basal laminar deposits accumulate in Bruch's membrane. AMD is a degenerative disease of the central portion of the retina (the macula) which results primarily in loss of central vision [6]. The disease can progress in two different ways and, therefore, can be classified into a dry form (geographic atrophy) and a wet form (neovascular AMD).

In both subtypes of AMD, the RPE is a crucial cell in the pathogenesis of AMD [6]. A pivotal function of the RPE is the phagocytosis of the outer segments of the photoreceptors and subsequent regeneration of the rods and cones. As one

ages, metabolic waste builds up and imposes an increasing burden on the RPE. The waste, now partially degraded in a phagolysosome, is visualized histologically as residual bodies and serves as a substrate for lipofuscin formation. These residual bodies increase in number until they are extruded and accrue in Bruch's membrane, thickening the membrane itself and forming dome shaped basal linear deposits in Bruch's membrane referred to as drusen. When the deposits become large (>125 μm in diameter), soft (amorphous and poorly demarcated), and confluent, they cause interruptions in the choroidal capillaries, compromising blood flow within the RPE layer. The extracellular deposits in Bruch's membrane also instigate chronic inflammation, promoting invasion by phagocytes and other immune cells, cytokine release, and production of reactive oxygen species (ROSs) [7].

The retina, because of its high oxygen consumption, its high levels of cumulative irradiation, and its composition of polyunsaturated fatty acids, which are readily oxidized and can initiate a cytotoxic chain reaction, is an ideal environment for the generation of ROS [8]. Moreover, the process by which RPE phagocytizes is itself an oxidative stress that results in ROS generation. The combined effects from chronic sustained inflammation and ROS generation promote the development of RPE damage seen in AMD [6, 9, 10]. Thinning or destruction of theRPE leads to its degeneration and to the subsequent death of rods and cones that depend on the RPE for their nutrition. This translates into visual loss. As the RPE degenerates, choriocapillaris beneath the RPE becomes less fenestrated, reducing the transport of macromolecules between the retina and choroidal blood supply and then disappearing altogether, creating a hypoxic environment. Hypoxia then increases the secretion of growth factors such as vascular endothelial growth factor (VEGF) that promotes choroidal neovascularization (CNV). The friable, small vessels comprising CNV are easily damaged and leak, creating the wet or exudative form of macular degeneration. The other more-common and less-severe form, termed dry AMD, occurs in the absence of neovascularization and with a region of atrophy in a geographic distribution [6].

2.1. Risk factors for AMD

The etiology of AMD remains elusive. A major feature of AMD is its association with age, with the highest prevalence among those 85 years of age or older [1]. Other certain risk factors include smoking and family history or genetics [6, 11–17]. There have been recent studies showing certain association between AMD and *CFH* [18–23], *LOC38775/ARMS2* (age-related maculopathy susceptibility 2) [24–27], *HrtA-1* [28, 29], and *APOE* [30–34]genes. Recently, VEGF single nucleotide polymorphism and matrix metalloproteinases (MMP)-9 microsatellite polymorphism are reported to be associated with wet AMD [35–37]. Studies have also considered an association between exposure to sunlight and AMD [6].

The Age-Related Eye Disease Study (AREDS), a controlled randomized clinical trial reports the use of high doses of antioxidants (vitamin C, vitamin E, and beta carotene) and zinc reduce the risk of advanced AMD by about 25% in patients with moderate risk of developing AMD [38]. Supplementation of various nutrients in the literature have demonstrated risk reduction for AMD, and these findings support the potential role of PPARs in AMD, especially since diet is an important modifiable risk factor when discussing PPARs, which regulate lipid metabolism and homeostasis [39, 40]. PPAR is one of the two characterized types of polyunsaturated fatty acid-responsive transcriptional factors. Because humans do not have the capability for de novo synthesis of essential fatty acids, which are particularly rich in long-chain polyunsaturated fatty acid (LCPUFA), we are dependant on dietary sources of these compounds [9]. Importantly, a recent AREDS study has demonstrated that participants reporting high-dietary intake of lutein/zeaxanthin, an LCPUFA which counteracts photochemical damage and generation of reactive oxygen species that attack cellular lipids, proteins, and other nuclear material, are statistically less likely to have advanced AMD (both neovascularizationand geographic atrophy) or large or extensive intermediate drusen than thosereporting lowest dietary intake of lutein/zeaxanthin [41]. Thus, it is possible that the beneficial effects of lutein/zeaxanthin LCPUFAs are related to their ability to activate fatty acid-responsive PPARs, suggesting a protective role of PPARs in AMD pathogenesis.

2.2. Clinical presentation

Though the etiology of AMD remains unclear, the clinical progression of this disease is well characterized. With dry AMD, patients may complain of a gradual loss of vision, from several months to years, in one or both eyes due to progressive loss of photoreceptors [42]. This gradual loss of vision is often first noticed as difficulty in reading or driving, scotomas, or increased reliance on brighter light or a magnifying lens for tasks that require fine visual acuity [43]. Vision loss that has occurred acutely over a period of days or weeks may represent wet AMD due to subretinal/retinal hemorrhage resulting from leakage or breaks of choroidal neovascular vessels. These patients may report an acute distortion in vision due to retinal hemorrhage, especially distortion of straight lines, or loss of central vision. Symptoms of wet AMD usually appear in one eye although AMD pathology is generally present in both eyes [44].

2.3. Pathological findings

The nonneovascular abnormalities in AMD include drusen as well as abnormalities of the RPE highlighted by accumulation of lipofuscin granules. The main component of lipofuscin is A2E, which is cytotoxic to RPE and induces RPE apoptosis. Clinically, drusen are round, dull yellow lesions, located under the sensory neuroretina and RPE, which upon fluorescein angiography, light up and stain late with no leakage. Histologically this material corresponds to the abnormal thickening of the inner aspect of Bruch's membrane. The thickening involves basal laminar deposits, collagen accumulation between the plasma membrane of the RPE cells and the inner aspect of the basement membrane of the RPE, as well

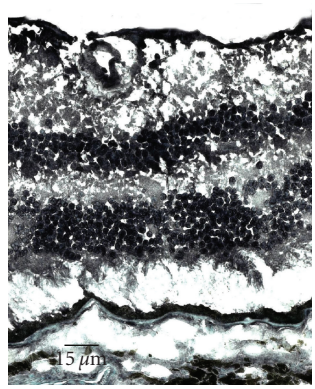

Figure 1: Microphotograph showing normal human retina stained for PPARγ in the ganglion cell, inner nuclear layer, outer nuclear layer, and RPE (avidin-biotin-complex immunoperoxidase).

as basal linear deposits outside the RPE basement membrane referred to as drusen [6].

How and why drusen develop is unknown, however much is deduced from its contents. Drusen often have a core of glycoproteins and their outer domes contain crystallins, chaperone proteins, apolipoprotein E, vitronectin, proteins related to inflammation (amyloid P, C5, and C5b-9), and sometimes fragments of RPE cells [45]. Drusen appear as electron-dense granules within the inner aspect of Bruch's membrane. The thickening of the membrane causes a sharp reduction in fluid and nutrient transport across the membrane. Its diminished function also results in decreased cell adhesion and anoikis of the photoreceptors, RPE cells, and possibly choriocapillaris endothelial cells [6]. These deposits around Bruch's membrane are also the cause of chronic local inflammation further promoting AMD development and progression.

The presence of drusen may lead to RPE degeneration and subsequently, deterioration of photoreceptors, which are dependent upon maintenance by RPE [46]. When the atrophy of the RPE and photoreceptors covers a distinct and contiguous area, it is termed geographic atrophy. Histologically, geographic atrophy is characterized by roughly oval patches of hypopigmentation as a consequence of RPE atrophy. The underlying choroidal vessels are more readily visible and the outer retina may appear thin secondary to loss of the photoreceptor and RPE cells. At the periphery of the hypopigmented regions there may be hyperpigmented changes from RPE cell proliferation. If the atrophy is less defined, with a mottled appearance, then it is called nongeographic atrophy. If the disease continues to progress, there comes a point when the components of the drusen begin to disappear; this is termed regressed drusen [46]. Additionally there may be small pinpoint glistening of the drusen where calcium has been deposited.

The third key component of AMD is choroidal neovascularization [47]. With the thinning and destruction of the RPE the underlying choriocapillaries become less fenestrated, impairing transport of macromolecules, such as oxygen, between the retina and choroidal blood supply. The resulting hypoxia stimulates neovascularization through vascular endothelial growth factor (VEGF). VEGF, which will be discussed in more detail below, acts as a stimulus for neovascularization [48]. There can be both new vascular growths from the choroidal vessels, growing through Bruch's membrane into the subretinal space. Clinically CNV appears as a purple-grey discoloration beneath the retina. With the increase in blood flow within the retina due to CNV, there may even be a focal sensory retinal detachment and cystoid edema. New vessels also promote fibroblast proliferation and disruption of normal retinal architecture. Moreover, these neovascular blood vessels are extremely leaky, and hemorrhage from these friable vessels leads to sudden vision loss secondary to accumulation of fluid or blood in the subretinal space and/or within the retina itself [49].

3. PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

Peroxisome proliferator-activated receptors (PPARs) seem to be associated with chronic diseases such as diabetes mellitus, obesity, atherosclerosis, cancer, and neurodegenerative diseases [2, 4, 50]. Like androgens, steroids, retinoid, and thyroid hormone receptors groups, PPARs are members of nuclear receptor superfamily of ligand-activated transcription factors [2]. Though they are among the best-categorized nuclear receptor families, the evolution of these molecules remains unclear. PPARs have three known subtypes: α, β, and γ. The α subtype is present in adipose tissue, liver, brain, heart, and skeletal muscle. A synthetic agonist to this subtype has been created as a cholesterol-lowering therapy. The PPAR β subtype, also known as δ or NUC1, is present in the gut, kidney, brain, and heart. PPARγ, the subtype most widely studied, is expressed on adipocytes, colon, brain, renal epithelium, monocytes, and macrophages. The γ subtype is the model for therapy such as thiazolidinediones (troglitazone, rosiglitazone, pioglitazone) for increased insulin sensitivity in noninsulin-dependent diabetes (type 2) [51, 52]. This receptor is also expressed in the retina, specifically in the RPE and choroidal vascular endothelial cells [53]. Figure 1 shows positive immunoreactivity against PPARγ in the normal human retina. The association of PPAR with RPE cells, as well as neuronal cells, supports the hypothesis that PPAR may play a role in the pathogenesis of AMD; therefore, PPAR may present a possible target for AMD treatment.

In response to binding by fatty acids, PPARs form heterodimers with retinoid X receptor (RXR), and the PPAR-RXR heterodimer binds to specific response elements (PPREs) consisting of a direct repeat of the nuclear receptor hexameric DNA core recognition motif spaced by one nucleotide to influence the transcription of numerous target genes [54]. Because PPAR is widely expressed as a transcription factor, it also plays a role in many processes including lipid homeostasis, glucose regulation, inflammation, atherosclerosis, ischemia, cancer, and neurodegenerative diseases [2, 36, 37, 54–62] with the subtypes overlapping in activity, function, and location.

4. PROPOSED MECHANISMS OF AMD AND THE LINKS TO PPAR

The etiology of AMD is not well understood, an explanation in itself for the various proposed mechanisms for how and why AMD progresses. Theories include aging, oxidative stress, endoplasmic reticulum stress, and inflammation. Interestingly, these processes are shared among diseases with similar pathophysiological changes to those seen in AMD and also involve PPAR.

Oxidative stress arises from a significant increase in reactive oxygen species (ROS) concentration and/or a decrease in detoxification mechanisms. ROS include free radicals, hydrogen peroxide, and singlet oxygen. There are many natural sources of oxidative stress such as exposure to environmental oxidants, ionizing and UV radiation, heat shock, and inflammation. The ROSs usually have one or more unpaired electrons in their outer orbits, and in order to achieve a stable state, extract electrons from other molecules, which themselves become unstable, causing a chain reaction [8]. High levels of oxidative stress exert a toxic effect on biomolecules, such as DNA, proteins, and lipids. As we know ROS may start an oxidative cascade, mediated in part by ROS-induced activation of NF-κB, STAT, and AP-1transcription factors, altering the composition of the cellular membrane, changing protein conformations, and lead to an upregulation of proinflammatory genes and cytokines, further potentiating damage [62, 63].

Oxidative stress plays a role in ischemic-reperfusion injuries, atherosclerosis, hypertension, inflammation, cystic fibrosis, type 2 diabetes, Alzheimer's, and Parkinson's disease [62]. Oxidative stress has also been linked to aging [64]. The retina has a very high concentration of lipids [9] and therefore easily falls pray to such mechanisms of destruction [8].

Oxidative stress such as aging and light exposure is considered to be associated with AMD. RPE and photoreceptors are particularly susceptible to oxidative stress because of high oxygen consumption by photoreceptors [8], high concentration of LCPUFA in the outer segments [65], exposure to visible light, and presence of lipofuscin, a photo-inducible generator of ROS in RPE [66, 67]. Clinical data supporting a beneficial effect of antioxidants in AMD provide direct validation of the role of oxidative injury in AMD treatment. Subgroup analysis of a multicenter, randomized, placebo-controlled AREDS trial revealed that an antioxidant cocktail of vitamins C and E, β-carotene, and zinc can reduce the progression of moderate atrophic AMD to late-stage disease [38]. Epidemiologic data showing that smoking leads to a significantly increased risk of the disease is consistent with the antioxidant approach as smoking is known to depress antioxidants such as vitamin C and carotenoids, and to induce hypoxia and ROS generation [68, 69].

PPARs are known to stimulate peroxisome enlargement and proliferation, as well as upregulation of β-oxidation enzymes. Since the peroxisome houses a variety of oxidative metabolic processes, they are an obvious cause of oxidative stress [64]. Oxidative damage and proinflammatory cytokines, TNF-α, INF-γ, and MMPs have been cited to play roles in each of the disease processes mentioned above [3, 50, 70–74], establishing PPAR as a common link between them.

Another theory regarding drusen formation involves a phenomenon known as endoplasmic reticulum (ER) stress. The ER is central to protein and lipid synthesis and maturation, as most newly formed proteins are assembled in the ER. Incorrectly folded proteins tend to form aggregates that are harmful to the cells and thus, ER-resident and/or visiting chaperone molecules facilitate protein folding and clearance of terminally misfolded proteins [75]. Any condition which impairs protein folding, for example, mutations in proteins that affect folding or ER malfunction, is termed ER-stress. Increased ER-stress, therefore, leads to protein and lipid buildup within cells, and this buildup in the eye might translate into RPE damage and drusen deposition.

The argument for a role for ER stress in AMD pathogenesis is supported by the well-characterized role of ER stress in several AMD-related neurodegenerative diseases. Alzheimer's disease and Lewy Body diseases, such as Parkinson's disease, are characterized by deposition of abnormal substances, which may parallel the abnormal deposition of drusen in the eye. The classical histopathological hallmarks of Alzheimer's disease [3, 4] include deposition of fibrillar amyloid in neuritic plaques as well as intracellular deposits of hyperphosphorylated tau protein. This results in the formationof neurofibrillary tangles and finally neuronal death, causing progressive memory loss and decline in cognitive functions [4]. In Parkinson's disease, suffering dopaminergic neurons are found to contain Lewy bodies and neuromelanin, an end product in catabolism by autoxidation [3]. In atherosclerosis there are abnormal lipid depositions in blood vessels leading to plaque formation and partial occlusion of these vessels [76]. In an AMD model of $Ccl2^{-/-}/Cx3cr1^{-/-}$ deficient mice abnormal ER protein is detected and associated with disease pathogenesis [75].

Recent articles have discovered a role for PPAR in ER stress. Dirkx et al. found that absence of peroxisomes in hepatocytes had repercussions on different subcellular compartments, including mitochondria, ER, and lysosomes [77]. Another study found that intracellular calcium mobilization by PPARγ ligands in rat liver epithelial cells interferes with proper protein foldingin the ER, thus promoting ER stress [73]. A third article discovered that under conditions of impaired translation, PPARγ ligands stimulate the expression of a number of ER stress-responsive genes, such as GADD 153, BiP, and HSP70 in rat pancreatic β cells. They concluded that PPARγ ligands induce ER stress [78].

In addition to the obvious parallels, between amyloid, Lewy bodies, cholesterol, and drusen, there are also similar processes such as inflammation that may play a role in inciting the damage associated with each disease.

Various immunological molecules and inflammatory mediators, cytokines, and chemokines have been identified in AMD lesions [79, 80]. Many of them are produced locally by RPE, choroid, and retina [81]. It has been hypothesized that RPE dysfunction is the critical event in drusen formation, making drusen a product of a localized inflammatory response, possibly involving HLA antigens and the complement system [82]. The hypothesis is based on many different

findings scattered among the literature and within different fields of medicine. Drusen, the hallmark of AMD, are found higher in membranoproliferative glomerulonephritis II (MPGNII), a complement-medicated immune deficiency. These cuticular drusen are identical, clinically, histologically, and immunohistochemically to the drusen in AMD [83–85]. Drusen has also been cited as having similar features to lipid-laden plaques of atherosclerosis [82, 86]. The relationship here is inferred from the histological as well as local inflammatory similarities between dysfunctional endothelial cells and the subendothelial deposition of modified LDL-cholesterol in atherosclerotic deposits within arterial vessels to those of drusen in the eye [86–88]. In addition, molecules such as MMP-9 seem to be involved in both processes. Inhibition of MMP-9 in atherosclerotic lesions has been cited to oppose remodeling, as suggested by the inhibition of intimal thickening and outward arterial remodeling [89]; while in AMD it is thought to be involved in microvessel formation duringearly phases of angiogenesis, in the reabsorption ofneovascularization, and in involution and regression of vessels inlater stages [90]. Similarities to the local inflammatory components seen in Alzheimer's also support this theory where accumulations of neurofibrillary tangles or insoluble deposits of beta amyloid peptide are the inciting agents of local inflammation [86].

The association between *complement factor H (CHF)* single nucleotide polymorphisms and increased risk of AMD also uncovers an important link between the complement system (inflammation) and the development of maculopathy (AMD) [18–20, 91]. The gene for *CHF* is located within the chromosomal region (1q32) linked to AMD [82, 92]. The *CHF* gene encodes a protein, complement response factor (CRF), that functions as part of the complement system and has been found in drusen from AMD patients [82, 93]. Furthermore, the same environmental risk factors, smoking, that influence levels of complement in serum are also associated with increased risk of developing AMD [86, 94].

In Alzheimer's, atherosclerosis, and AMD similar local proinflammatory pathways are stimulated, thereby leading to the deposition of activated complement components, acute-phase proteins, and other inflammatory mediators in tissues affected by each disease process. The cumulative impact is chronic tissue-specific low-grade inflammation exacerbating the effects of the primary pathogenic lesion [86]. PPARs act to inhibit many proinflammatory genes, which may result in protection of these diseases.

5. MOLECULES THAT INTERACT WITH PPAR AND THEIR RELATIONSHIP WITH AMD: AN INTRODUCTION TO VEGF, MMP, AND DHA

5.1. Vascular endothelial growth factor A, VEGF

VEGF was first identified in the early 1970s as a tumor-angiogenesis factor that is mitogenic to capillary endothelial cells in human tumors [95]. VEGF is now recognized as an essential regulator of normal and abnormal vessel growth. It regulates both vascular proliferation, as well as permeability, and functions as an antiapoptotic factor for newly formed blood vessels [95]. VEGF is expressed in response to hypoxia, oncogenes, or cytokines [96]. In this process, VEGF binds to and stimulates autophosphorylation of two distinct receptor tyrosine kinases, VEGFR1 or Flt-1 (fms-related tyrosine kinase 1) and VEGFR2 or KDR/FlK-1 (kinase insert domain containing receptor/fetal liver kinase 1) [97]. This activates an MAPK pathway causing neovascular channel growth from the choroidal vasculature and extension into the space between the RPE and Bruch's membrane thus activating the RPE to migrate into stroma of the CNV lesion [98, 99]. VEGF blockade has been shown to have a direct and rapid anti-vascular effect in tumors by deprivation of tumor vascular supply and inhibition of endothelial proliferation. Recently, VEGF has also been shown to target CNV in AMD [100]. The first anti-VEGF compound, pegaptanib, was approved by the FDA in 2004 and followed closely by approval of two other treatments, bevacizumab (Avastin) and ranibizumab (Lucentis). With monthly intravitreal injections of ranibizumab, growth of neovascular membranes is halted and there is prevention of severe vision loss in 90% of patients and improvement of visual acuity in 30–40% of patients [101–104].

5.2. Matrix metalloproteases, MMPs

The regulated turnover of extracellular matrix macromolecules is crucial to a variety of important biological processes. MMPs, a member of the class of proteases, degrade components of extracellular membranes [105]. MMPs, zinc-dependent endopeptidases, are expressed by activated macrophage foam cells and smooth muscle cells, and are important in the resorption of extracellular matrixes in both physiological and pathological processes. MMPs are secreted by macrophages as a proenzyme and once activated can completely degrade extracellular matrix components, such as elastin and collagen, including the structural backbone of the basement membrane, type IV collagen. Mostly this group of enzymes acts locally where they are expressed to aid in cell migration by clearing a path through the matrix, exposing cryptic sites on the cleaved proteins that promote cell binding and/or cell migration, promoting cell detachment so that a cell can move onward, or by releasing extracellular signal proteins that stimulate cell migration [105].

MMP-9, a specific MMP, is thought to degrade the fibrinous cap found on atherosclerotic plaques, destabilizing the plaque, and priming it for rupture [106]. Since AMD is associated with sustained chronic inflammation and loss of integrity of Bruch's membrane, it has been hypothesized that MMPs may play a role in the pathogenesis of the disease [107]. MMP-9 and MMP-2, two subtypes of MMPs, have been identified in Bruch's membrane in AMD eyes, and cell-culture studies have documented its role in the development of CNV [108–110]. A recent study found the first association between AMD and MMP-9 [108]. Significantly elevated plasma MMP-9 levels were reported in both wet and dry AMD patients as compared to age-matched controls. In addition, circulating plasma levels of MMP-9 were approximately three times higher in AMD patients than in control patients with no confounding illnesses. MMP transcriptional activity is regulated by genetic polymorphisms of the promotor

region and carriers repeats of the MMP-9 promotor, numbering greater than or equal to 22, have a more than doubled risk of developing AMD [37]. Facilitating this MMP-9 expression may act as a factor in increasing vascular permeability of the vessels or in the neovascularization seen in exudative AMD.

5.3. Docosahexaenoic acid, DHA

Docosahexaenoic acid (DHA) is a major dietary omega-3 LCPUFA. It is also a major structural lipid of retinal photoreceptor outer segment membranes with the highest concentrations per unit weight found here. Omega-3 LCPUFA have the capacity to play roles in many processes of AMD, such as retinal neovascularization, inflammation of the retinal vasculature, and alterations in the retinal capillary structure and integrity [9]. DHA has been shown to promote survival, inhibit apoptosis of photoreceptors, possibly via signaling cascades, play a role in rhodopsin regeneration, and exert neural protection through an RPE-secreted neuroprotective mediator, NPD-1. Tissue DHA insufficiency can affect retinal signaling and is associated with alterations in retinal function [9]. It has also been documented that there exists an inverse relationship between dietary intake of the omega-3 LCPUFA and risk of developing AMD [111].

Despite the benefits of polyunsaturated fatty acids, humans lack the Δ15 and 12 desaturase enzymes to synthesize these compound de novo and are dependent on dietary sources. In addition, the biochemical nature of DHA and the proximity of these compounds to metabolically active ocular tissue and high oxygen tension of the choriocapillaries facilitate the formation of ROSs. ROSs may start an oxidative cascade altering the DHA and changing the composition of the cellular membrane and increasing the expression ofproinflammatory genes and cytokines, thereby damaging the retina [62, 63]. ROS are therefore extremely dangerous because they damage DHA, a necessary yet limited resource needed to keep retina healthy.

6. IMPORTANT MOLECULES INVOLVED IN PPAR's POTENTIAL ROLE IN AMD

6.1. VEGF, PPARγ, and their role in AMD

As previously discussed, VEGF has been shown to play a critical role in neovascularization via the MAPK kinase pathway, associated with the wet form of AMD [103, 104]. PPARγ with expression localized to the RPE and choroidal endothelial cells of ocular tissue [53] may have an effect on endothelial cells and may have a direct antagonistic relationship with VEGF.

It has been demonstrated that vascular endothelial cells express PPAR-γ mRNA and protein [61, 112]. PPAR-γ ligands inhibit growth factor-induced proliferation of endothelial cells, increase plasminogen activator inhibitor-1 expression and suppress endothelin-1 secretion [113, 114], overall providing support to the theory that PPAR-γ plays an antagonistic role to that of VEGF [115]. More directly Murata and colleagues demonstrated that PPARγ inhibits MAPK-dependent migration of smooth muscle and may act as a downstream inhibitor to VEGF. This group also showed that troglitazone and rosiglitazone, synthetic agonists of PPARγ, inhibited the endothelial effects of VEGF in a dose-dependent manner. In vivo studies with the troglitazone demonstrated that intravitreal injections dramatically inhibited the percentage of lesions as well as leakage per lesion, making a strong case for therapeutic value of this drug [53].

6.2. Matrix metalloproteinase (MMP), PPARγ, and their role in AMD

Ricote showed that PPARγ inhibits the expression of MMP-9 in response to a naturally occurring ligand, prostaglandin D2 metabolite 15-deoxy-$\Delta^{12,14}$ prostaglandin J2 (15d-PGJ2), and synthetic PPARγ ligands activated macrophages by antagonizing the activities of the transcription factors AP-1, STAT, and NF-κB [52]. PPARγ activators decrease MMP-9 expression in vascular smooth muscle [116] and treatment with PPAR agonist troglitazone has shown decreased atherosclerotic lesions in various animal models [107]. In addition PPARγ-mediated suppression of NF-κB activity may decrease proinflammatory cytokines in macrophages, including MMP-9 [117].

This intricate relationship demonstrates that PPARγ downregulates MMP expression and inhibits MMP-9's subsequent accumulation in Bruch's membrane where it may play an integral role in the degradation of the extracellular matrix and be a stimulus for migration of the RPE into Bruch's membrane, in this way contributing to the pathophysiology of AMD.

6.3. DHA, PPARγ, and their role in AMD

DHA is a naturally occurring ligand to all subtypes of the PPAR family. It binds specific DNA motifs to modulate the activity of PPAR and RXR as transcription factors [9]. As being well known, PPARs play an important regulatory role in oxidative stress by inducing the transcription of antioxidant genes, such as glutamate cysteine ligase (GCL) and heme oxidase-1 (HO-1) [118]. These antioxidants then work through MAPK kinase pathways to curb ROS. A functional PPRE is located at the catalase gene promoter, a gene known to protect cells from the toxic effects of hydrogen peroxide (H_2O_2) by catalyzing its decomposition, indicating that catalase expression is directly regulated by PPARγ [62]. To further test this relationship, catalase expression was analyzed in the striatum of rats subjected to intracranial bleeds with and without 15-dPGJ2 treatment. Treated rats showed 1.6-, 2.1-, and 1.7 fold higher levels of catalase mRNA expression compared to the saline controls at 1, 3, and 24 hours [63]. Girnun et al. found similar increases in catalase mRNA when using known PPAR agonists rosiglitazone and ciglitazone in rat brain microvascular endothelium cells, one of the cell types damaged during inflammatory responses induced by ROS generation [62].

In short, PPARγ has a special role in counteracting the damaging effects of ROS generation by upregulating antioxidant genes and downregulating proinflammatory

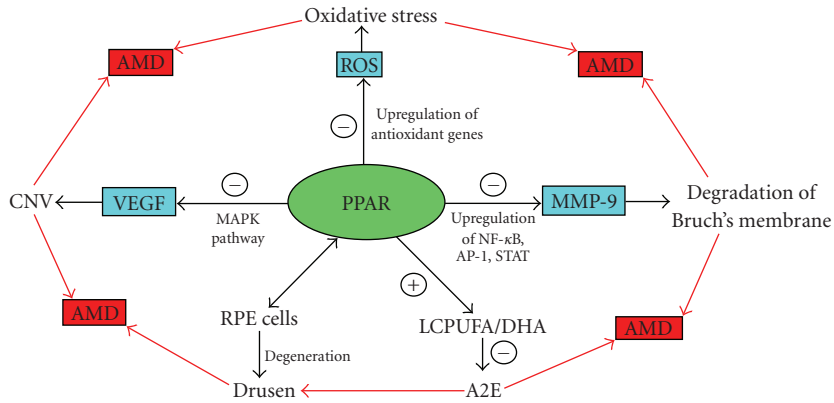

Figure 2: Schematic graph showing PPAR interactions with VEGF, ROS, MMP-9, LCPUFA, DHA, and RPE cells and their role in the development of AMD.

genes. By decreasing damage to LCPUFAs, such as DHA, there is preservation of the protective effects these essential molecules confer to the retina.Enhancing this ability of the RPE to protect itself from oxidative injury may provide a therapeutic opportunity to delay or hinder the development of AMD.

7. SUMMARY

Though there is limited literature directly linking PPAR dysfunction with AMD pathology, there is evidence that PPARs may be involved in various mechanisms and pathways associated with this disease process. PPARγ is localized to the neuroretina and RPE, the essential component to photoreceptor degeneration and vision loss. PPAR acts to inhibit inflammatory processes, which are linked to AMD. VEGF is a known driving factor for neovascularization, a main causal element of wet macular degeneration and PPARs directly inhibit VEGF function. High levels of MMP-9 have been detected in retinas afflicted with AMD. In turn, PPARs are known to decrease expression of MMP. PPARs play a direct role in upregulation of antioxidative enzymes, one of the many possible causes of macular pathology. PPARs bind various ligands including LCPUFAs and their metabolites, possibly shedding light on how PPARs interfere with NFκB as one way in which omega-3 LCPUFAs are protective against AMD. It is evident that PPARs must play a certain role in the development of AMD. Figure 2 demonstrates the many ways that PPARs interact with processes closely related to progression of AMD. Future studies are warranted to better elucidate the pathogenic and therapeutic potentials of PPARs in AMD.

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Review Article

PPARγ Agonists: Potential as Therapeutics for Neovascular Retinopathies

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The angiogenic, neovascular proliferative retinopathies, proliferative diabetic retinopathy (PDR), and age-dependent macular degeneration (AMD) complicated by choroidal neovascularization (CNV), also termed exudative or "wet" AMD, are common causes of blindness. The antidiabetic thiazolidinediones (TZDs), rosiglitazone, and troglitazone are PPARγ agonists with demonstrable antiproliferative, and anti-inflammatory effects, in vivo, were shown to ameliorate PDR and CNV in rodent models, implying the potential efficacy of TZDs for treating proliferative retinopathies in humans. Activation of the angiotensin II type 1 receptor (AT1-R) propagates proinflammatory and proliferative pathogenic determinants underlying PDR and CNV. The antihypertensive dual AT1-R blocker (ARB), telmisartan, recently was shown to activate PPARγ and improve glucose and lipid metabolism, and to clinically improve PDR and CNV in rodent models. Therefore, the TZDs and telmisartan, clinically approved antidiabetic and antihypertensive drugs, respectively, may be efficacious for treating and attenuating PDR and CNV humans. Clinical trials are needed to test these possibilities.

1. INTRODUCTION

Angiogenesis and neovascularization involve formation and proliferation of new blood vessels and have a vital role normal growth and development, such as embryogenesis, wound healing, tissue repair [1, 2]. However, in pathological neovascularization, angiogenesis is aberrant and unregulated resulting in the formation of dysfunctional blood vessels [3]. The latter occurs in proliferative diabetic retinopathy (PDR) and choroidal neovascularization (CNV), "wet" or exudative age-dependent macular degeneration (AMD), wherein pathological neovascular vessels proliferate and leak fluid leading to retinal edema, subretinal and retinal/vitreous hemorrhage, retinal detachment, and blindness. In the United States, PDR is the most common preventable cause of blindness in adults <50 years [4], whereas CNV/AMD is the leading cause of blindness among people of European origin >65 years [5]. Both retinopathies are progressively destructive, leading to eventual and irreversible blindness. PDR is a serious microvascular complication of both type 1 and type 2 diabetes [6]. Type 2 diabetes is rapidly expanding worldwide and is estimated to reach 380 million by 2025 [7, 8]. PDR is progressive and compounded by persistent and substandard control of hyperglycemia, and concomitant cardiovascular risk factors, especially hypertension [9–11]. Nearly, all type 1 diabetics and >60% of type 2 diabetics have significant retinopathy after 20 years, emphasizing the need for more cost-effective therapy [6, 10, 11]. Hyperglycemia, advanced glycation end-products (AGEs), and hypoxia are believed to induce pathological angiogenesis and neovascularization within the retina [12]. Prevention of end-organ damage by early and aggressive diabetes management is the best approach to treating diabetic retinopathy (DR) [6, 12].

Visual acuity depends on a functional macula, located at the center of the retina where cone photoreceptors are most abundant. Exudative (wet) AMD is complicated by CNV, involving activation and migration of macrophages, and normally quiescent retinal pigment epithelial cells from the choroid and invasion of defective neovascular blood vessels into the subretinal space [13, 14]. Bleeding and

Table 1: Growth factors, cytokines, chemokines, and other proinflammatory mediators downregulated by PPARγ activation. PDGF-BB, platelet-derived growth factor-BB homodimer; AP-1, activated protein-1; NF-κB = nuclear factor-κB; NFAT = nuclear factor of activated T lymphocytes; STAT = signal transducer and activator of transcription; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; iNOS, inducible nitric oxide synthase. (Adapted with permission from: B. Staels, "PPARγ and atherosclerosis." Current Medical Research and Opinion, vol. 21, Suppl. 1, pp. S13-S20, 2005; H. A. Pershadsingh, "Dual peroxisome proliferator-activated receptor-alpha/gamma agonists : in the treatment of type 2 diabetes mellitus and the metabolic syndrome." Treatments in Endocrinology, vol. 5, no. 2, pp. 89-99, 2006.)

Growth factors	Cytokines	Chemokines	Nuclear transcription factors	Other molecules
ATII	IL-1β	IL-8	AP-1	IFN-γ
TGF-β	IL-2	MCP-1	NF-κB	iNOS
ET-1	IL-6	RANTES	STAT	PAI-1
bFGF	TNF-α		NFAT	MMP-2
PDGF-BB				MMP-9
EGF				VCAM-1
VEGF				ICAM-1
				E-selectin

Table 2: Growth factors, cytokines, chemokines, and other proinflammatory mediators upregulated by angiotensin II stimulation. ET-1, endothelin-1; TGF-β, transforming growth factor-β; CTGF, connective tissue growth factor; bFGF, basic fibroblast growth factor; PDGF-AA, platelet-derived growth factor-AA homodimer; EGF, epidermal growth factor; VEGF, vascular endothelial cell growth factor; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; NF-κB, nuclear factor-κB; NFAT, nuclear factor of activated T lymphocytes; STAT, signal transducer and activator of transcription; RANTES, regulated on activation, normal T-cell expressed and secreted; IFN-γ, interferon-γ; PAI-1, plasminogen activator inhibitor type 1; AP-1, activated protein-1. (Adapted with permission from: R. E. Schmieder, K. F. Hilgers, M. P. Schlaich, B. M. Schmidt, "Renin-angiotensin system and cardiovascular risk." Lancet, vol. 369, no. 9568, pp. 1208-1219, 2007.)

Growth factors	Cytokines	Chemokines	Other proinflammatory molecules
ET-1	IL-1β	IL-8	IFN-γ
TGF-β	IL-6	MCP-1	Tissue factor
CTGF	IL-18	MIP-1	PAI-1
bFGF	GM-CSF	RANTES	
PDGF-AA	TNF-α		
EGF			
VEGF			

lipid leakage from these immature vessels damage the retina and lead to severe vision loss and blindness [14, 15]. Current therapies of AMD are limited to treating the early stages of the disease, and include laser photocoagulation, photodynamic therapy, surgical macular translocation, and antiangiogenesis agents [13–16]. These invasive procedures are expensive, require repetition, whereas pharmacologic approaches could simplify therapy and reduce cost.

The peroxisome proliferator-activated receptor (PPAR) class of nuclear receptors (PPARα, PPARβ/δ, and PPARγ) belongs to the nuclear receptor superfamily that include the steroid, thyroid hormone, vitamin D, and retinoid receptors [17, 18]. In 1995, Lehmann et al. [19] discovered that PPARγ was the intracellular high affinity receptor for the insulin-sensitizing, antidiabetic thiazolidinediones (TZDs), the activation of which also promotes growth arrest of preadipocytes, differentiation, adipogenesis, and differentiation into mature adipocytes [20]. Ligand activation of PPARγ also downregulates the transcription of genes encoding inflammatory molecules, inflammatory cytokines, growth factors, proteolytic enzymes, adhesion molecules, chemotactic, and atherogenic factors [21–25] (Table 1).

Angiotensin II (AII) and components of the renin-angiotensin system (RAS) are expressed in the retina [26, 27]. AII promotes retinal leukostasis by activating the angiotensin type 1 receptor (AT1-R) pathway that propagates proinflammatory, proliferative mediators (Table 2) leading to the development and progression of PDR [28–30] and CNV [31]. By selectively blocking the AT1-R, angiotensin receptor blockers (ARBs) or "sartans," for example, valsartan and telmisartan have been shown to confer neuroprotective and anti-inflammatory effects in animal models of retinal angiogenesis and neovascularization [32–36]. Among the seven approved ARBs, telmisartan and irbesartan were recently shown to constitute a unique subset of ARBs also capable of activating PPARγ [37–39]. Valsartan and the remaining ARBs were inactive in the PPARγ transactivation assay. In fact, telmisartan was shown to downregulate AT1 receptors through activation of PPARγ [40]. Telmisartan was shown to provide therapeutic benefits in rodent models of PDR [33, 41–44] and CNV [45] but data with irbesartan is unavailable. Therefore, telmisartan and possibly irbesartan (data unavailable) may have enhanced efficacy in treating proliferative retinopathies. ARBs are safe and have beneficial

Table 3: Comparison of pharmacological and other relevant properties of thiazolidinedione (TZD) full PPARγ agonists and dual angiotensin II type 1 receptor blocker/selective PPARγ modulator (ARB/SPPARγM).

Parameter	TZDs†			ARBs*	
	Troglitazone	Pioglitazone	Rosiglitazone	Telmisartan	Irbesartan
Primary pharmacological target	PPARγ	PPARγ	PPARγ	AT1-R	AT1-R
Type of PPARγ agonists	Full PPARγ agonists			Selective PPARγ modulator (SPPARγM)	
Drug class (common names)	Thiazolidinedione (TZDs)			Angiotensin receptor blockers (ARBs)	
PPARγ activation (EC_{50} in μM)	0.55	0.58	0.043	4.5	27
Therapeutic indication	Treatment of type 2 diabetes mellitus			Treatment of hypertension	
Primary therapeutic mechanism	Increase insulin sensitivity			Lower blood pressure	
Serious adverse effect (Black box warning)	Fluid retention/weight gain/heart failure			None	None
Supplier/Pharmaceutical Co.	Sigma-Aldrich, St. Louis, Mo, USA	Takeda Pharmaceuticals Deerfield, Ill, USA	GlaxoSmithKline, NC, USA	Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield, Conn, USA	Sanofi-Aventis, Bridgewater, NJ, USA

† Thiazolidinedione full PPARγ agonists; troglitazone was withdrawn from the market (1998) because of association with rare cases of fatal hepatic failure. Rosiglitazone and pioglitazone have no such known association.
*Other FDA-approved ARBs had EC_{50} values > 100μM (see [37, 38]). EC_{50} values shown were determined using the standard PPARγ-GAL4 transactivation assays.

cardiometabolic, anti-inflammatory, and antiproliferative effects. Among these telmisartan and irbesartan may have improved efficacy for targeting proliferative retinopathies. Table 3 provides relevant information on the various drugs described herein.

2. TISSUE DISTRIBUTION PPARγ

Four PPARγ mRNA isoforms have been identified [46] that encode two proteins, PPARγ1 and PPARγ2 [47, 48]. PPARγ1 is the principal subtype expressed in diverse tissues, whereas PPARγ2 predominates in adipose tissue [49, 50]. The PPARγ2 protein differs from PPARγ1 by the presence of 30 additional amino acids [49]. Tissue-specific distribution of isoforms and the variability of isoform ratios raise the possibility that isoform expression might be modulated by or reflect disease states in which PPARγ activation or inactivation has a role. In humans, PPARγ is most abundantly expressed mainly in white adipose tissue and large intestine, and to a significant degree in kidney, heart, small intestine, spleen, ovary, testis, liver, bone marrow, bladder, epithelial keratinocytes, and to a lesser extent in skeletal muscle, pancreas, and brain [51].

2.1. PPARγ expression in the eye

PPARγ is heterogeneously expressed in the mammalian eye [51–53]. PPARγ was found to be most prominent in the retinal pigmented epithelium, photoreceptor outer segments, choriocapillaris, choroidal endothelial cells, corneal epithelium, and endothelium, and to a lesser extent, in the intraocular muscles, retinal photoreceptor inner segments and outer plexiform layer, and the iris [52]. Ligand-dependent activation of PPARγ evokes potent inhibition of corneal angiogenesis and neovascularization [53–55]. The prominent expression of PPARγ in selected tissues of the retina [52–54] provides the rationale for pharmacotherapeutic targeting of PPARγ for treating ocular inflammation and proliferative retinopathies [53–56].

2.2. Importance of PPARγ in proliferative retinopathy

To determine whether endogenous PPARγ played a role in experimental DR, Muranaka et al. [54] evaluated retinal leukostasis and retinal (vascular) leakage in streptozotocin-induced diabetic C57BL/6 mice deficient in PPARγ expression (heterozygous genotype, PPARγ+/−) after 120 days. Retinal leukostasis and leakage were greater (205% and 191%, resp.) in the diabetic PPARγ+/− mice, compared to diabetic wild-type (PPARγ+/+) mice. In streptozotocin-induced diabetic Brown Norway rats, oral administration of the TZD PPARγ ligand, rosiglitazone for 21 days (3 mg/kg body weight/day, initiated post-streptozotocin injection) resulted in suppression of retinal leukostasis by 60.9% ($P < .05$), and retinal leakage by 60.8% ($P < .05$) [54]. Expression of the inflammatory molecule. ICAM-1 protein was upregulated in the retina of the rosiglitazone-treated group, though the levels of VEGF and TNF-α were unaffected [54]. These findings provide strong evidence for a role of PPARγ activity in the pathogenesis of DR and provide novel genomic information that therapeutic targeting of PPARγ with a known PPARγ ligand, the TZD rosiglitazone, can attenuate the progression of PDR. Whether a similar effect may apply to the prevention or attenuation of CNV is currently unknown and should be explored.

3. ANTIDIABETIC THIAZOLIDINEDIONES (TZDs) AND PROLIFERATIVE RETINOPATHIES

The insulin-sensitizing TZDs, rosiglitazone, and pioglitazone are approved for the treatment of type 2 diabetes. Because they increase target tissue sensitivity to insulin without

increasing insulin secretion [57], there is no risk of hypoglycemia, though there is a risk fluid retention in diabetic patients, especially those with coexisting heart failure, or at risk for developing CHF [58].

By activating PPARγ, TZDs modulate groups of genes involved in energy metabolism [59], inflammation, and cellular differentiation [60–64] by down-regulating the activity of the proinflammatory nuclear receptors (NF-κB, AP-1, STAT, NFAT), and inhibiting the activity and expression of inflammatory cytokines (TNF-α, IL-1β, IL-2, IL-6), iNOS, proteolytic enzymes (MMP-3 and MMP-9), and growth factors (VEGF, PDGF-BB, bFGF, EGF, TGF-β) (Table 1). Because of these broadly beneficial and protective actions of PPARγ agonists, TZDs have been under development for the treatment of conditions beyond type 2 diabetes, including atherosclerosis [64, 65], psoriasis [66], inflammatory colitis [67], nonalcoholic steatohepatitis [68], and Alzheimer's disease [69]. More recently, TZDs have been found to protect against glutamate cytotoxicity in retinal ganglia and have antioxidant properties [70] suggesting that PPARγ agonists could prove valuable in targeting retinal complications [71].

3.1. Therapeutic effects on proliferative diabetic retinopathy (PDR)

Retinal capillaries consist of endothelial cells, basement membrane neovascularization, and intramural pericytes within the basement membrane which are important in vascular development and maturation [44]. Selective loss of pericytes from the retinal capillaries characteristically occurs early in diabetic retinopathy (DR) [72]. Diabetic macular edema (DME), often associated with PDR, involves breakdown of the blood-retinal barrier and leakage of plasma from blood vessels in the macula causing macular edema and impaired vision [73, 74]. Resorption of the fluid from plasma leads to lipid and lipoprotein deposition forming hard exudates [75]. In PDR, inflammation leads to endothelial dysfunction, retinal vascular permeability, vascular leakage, and adhesion of leukocytes to the retinal vasculature (leukostasis), progressive capillary nonperfusion, and DME [12]. Intraretinal microvascular abnormalities and progressive retinal ischemia lead to neovascular proliferation within the retina, bleeding, vitreous hemorrhage, fibrosis, and retinal detachment [74–76]. Despite advancements in ophthalmologic care and the management of both type 1 and type 2 diabetes, PDR remains a leading cause of preventable blindness [5–7]. Primary interventions, especially intensive glycemic and blood pressure control, and management of other cardiovascular risk factors are essential [6, 73–75]. Focal laser photocoagulation remains the only surgical option for reducing significant visual loss in eyes with macular edema [6, 9–12]. The risk of blindness with untreated PDR is currently greater than 50% at 5 years, but can be reduced to less than 5% with appropriate therapy [5–7]. At present, there is insufficient evidence for the efficacy or safety of pharmacological interventions, including therapy targeting vascular endothelial growth factor (i.e., anti-VEGF antibody therapy), though intravitreal glucocorticoids may be considered when conventional treatments have failed [6, 12].

Troglitazone and rosiglitazone were shown to attenuate VEGF-induced retinal endothelial cell proliferation, migration, tube formation, and signaling, in vitro [55] by arresting the growth cycle of endothelial cells [62]. Local intrastromal implantation of micropellets containing pioglitazone into rat corneas significantly decreased the density of VEGF-induced angiogenesis, an accepted animal model of retinal neovascularization [53].

Adverse conditions that contribute to macular edema and retinal degeneration in PDR include generation of advanced glycation end products (AGEs), local ischemia, oxidative reactions, and hyperglycemia-induced toxicity [72, 75, 76]. In PPARγ-expressing retinal endothelial cells, troglitazone, and rosiglitazone inhibited VEGF-stimulated proliferation, migration, and tube formation [55, 77]. The effects of troglitazone and rosiglitazone were also evaluated in the oxygen-induced ischemia murine model of retinal neovascularization, an experimental model of PDR [77]. Although the model lacks specific metabolic abnormalities found in diabetes, it isolates the VEGF-driven process in which neovascularization is stimulated by increased VEGF expression in the inner retina [77]. Both troglitazone and rosiglitazone decreased the number of microvascular tufts induced on the retinal surface, suggesting inhibition of an early aspect of neovascularization. The inhibitory effects were dose-dependent ($IC_{50} \simeq 5\,\mu$mo1/L) [77]. These findings support the proposal that TZDs may have beneficial effects by reducing or delaying the onset of PDR in diabetic patients. Prospective clinical trials are required to demonstrate clinical efficacy.

3.2. Therapeutic effects on choroidal neovascularization (CNV)

AMD complicated with CNV involves angiogenesis and neovascularization in the choroid with hemorrhage in the subretinal space, fluid accumulation beneath the photoreceptors within the fovea, and neural cell death in the outer retina [13–16]. CNV is present with vascular inflammation, unbridled vascular proliferation, aberrant epithelial and endothelial cell migration, and inappropriate production of proinflammatory cytokines, inducible nitric oxide synthase, growth factors, proteolytic enzymes, adhesion molecules, chemotactic factors, atherogenic, and other mediators that propagate defective blood vessel proliferation [5, 13–16, 78]. Elevated blood pressure, serum lipids, smoking, and insulin resistance also have an etiological role in CNV development [78]. Therefore, control of cardiometabolic risk factors is important in palliative management of CNV [79, 80]. Recently, therapy for early exudative AMD has been directed toward intravitreal injection of VEGF-directed antibodies or fragments thereof [14–16]. However, excessive cost ($1,950/dose) is a major issue [http://www.globalinsight.com/SDA/SDADetail6273.htm]. Monthly treatments are difficult for patients to tolerate, and the risk of serious adverse effects increases over time [16]. On the other hand, synthetic, nonpeptide PPARγ agonists [81, 82] are straightforward to synthesize, inexpensive to formulate.

CNV comprises the underlying pathology of exudative AMD, principally involving the subretinal vasculature and choriocapillaris, leading to capillary closure and retinal ischemia, angiogenesis, retinal neovascularization, bleeding into the vitreous, retinal detachment and degeneration, and eventually vision loss [13–16]. PPARγ is expressed in the choriocapillaris, choroidal endothelial cells, retinal endothelial cells, and retinal pigmented epithelium [52, 83]. VEGF is a potent inducer of retinal [13–16] angiogenesis and neovascularization. In their landmark study, Murata et al. [83] demonstrated the expression of PPARγ1 in human retinal pigment epithelial (RPE) cells and bovine choroidal endothelial cells (CECs), and that application of the TZDs troglitazone or rosiglitazone (0.1–20 μmol/L) inhibited VEGF-induced proliferation and migration of RPE and CEC cells, and neovascularization [83]. Moreover, in the eyes of rat and cynomolgus monkeys in which CNV was induced by laser photocoagulation, intravitreal injection of troglitazone markedly inhibited CNV compared to control eyes ($P < .001$). The treated lesions showed significantly less fluorescein leakage and were histologically thinner in troglitazone-treated animals, without adverse effects in the adjacent retina or in control eyes [83]. These findings suggest that pharmacological activation of PPARγ by TZDs appear to have a palliative or therapeutic effect on experimental CNV. Again, clinical trials are required to demonstrate efficacy in the clinical setting.

3.3. Adverse effects of TZDs: fluid retention and macular edema

Pioglitazone and rosiglitazone are generally safe though, in type 2 diabetic patients, there is a risk of weight gain (1–3 kg) and fluid retention [58]. The incidence of peripheral edema is greater in those concurrently taking exogenous insulin, increasing from 3.0–7.5% to 14.7–15.3% [58]. The edema may be related to TZD-induced vasodilation, increased plasma volume secondary to renal sodium reabsorption, and reflex sympathetic activation [58]. The association of rosiglitazone treatment with development of macular edema has been reported [84]. In a case review of 11 patients who developed peripheral and macular edema, while on the TZD therapy [85] 8 patients experienced resolution of macular edema with improved vision, without laser treatment, 3 months to 2 years after TZD cessation. Therefore, DME should be considered in type 2 diabetic patients treated with a TZD, especially those with peripheral edema, or other symptoms or risk factors of CHF, or concurrently taking exogenous insulin or nitrates. Drug cessation usually results in rapid resolution of both peripheral and macular edema [85].

4. ANTIHYPERTENSIVE ANGIOTENSIN RECEPTOR BLOCKERS (ARBs) THAT ACTIVATE PPARγ

In their search for PPARγ agonists that lack the adverse effects of TZDs, Benson et al. [37] screened the active forms of all currently available antihypertensive "sartans" (ARBs): losartan, valsartan eprosartan, irbesartan, candesartan, telmisartan, and olmesartan, using the standard GAL-4 cell-based PPARγ transactivation assay. Only telmisartan and irbesartan [37, 38] activated PPARγ and promoted adipogenesis, intracellular lipid accumulation and differentiation of preadipocyte fibroblasts into mature adipocytes, in vitro, hallmark properties of PPARγ agonists [19]. The EC_{50} values for transactivation of PPARγ by telmisartan and irbesartan were 4.5 μmol/L and 27 μmol/L, respectively [37–39] (Table 3). Although the PPARγ transactivation assay may not recapitulate conditions in vivo, based on pharmacokinetic considerations, concentrations of these ARBs required to activate PPARγ in vivo are achievable by standard dosing [86, 87]. By functioning as partial PPARγ agonists this unique subset of ARBs may provide added end-organ benefits in certain patient populations such patients with the metabolic syndrome [87] and other cardiometabolic risk factors, including atherosclerosis, atherogenesis, and may have palliative effects on proliferative retinopathies.

ARBs bear an acidic group (tetrazole or carboxyl group) at the *ortho* position on the terminal benzene ring of the biphenyl moiety, which is essential for AT1 receptor binding. Telmisartan bears a carboxyl and irbesartan, a tetrazole [87, 88]. The active forms of all other ARBs have two acidic groups at opposite molecular poles. This second acidic group limits accessibility, and hinders binding to the hydrophobic region of the PPARγ receptor [87, 88]. Therefore, among currently available ARBs, the molecular dipole appears to be an important structure-functional determinant of ligand binding to the PPARγ receptor [87]. Compared to all other ARBs, telmisartan has a uniquely long elimination half-life (24 hours), and the largest volume of distribution (500 L, and >10-fold in excess of other ARBs) which greatly increases central bioavailability upon oral dosing [86]. Furthermore, telmisartan has been shown to have significant anti-inflammatory and antioxidant activity, which may enhance its effectiveness in attenuating the progression of proliferative retinopathies [89–91].

4.1. Full versus partial PPARγ agonists

The PPARγ receptor is composed of five different domains, an N-terminal region or domain A/B, a DNA binding domain C (DBD), a hinge region (domain D), a ligand binding domain E (LBD), and a domain F [81, 92, 93]. The A/B domain contains an activation function-1 (AF-1) that operates in absence of ligand. The DBD confers DNA binding specificity. PPARγ controls gene expression by binding to specific DNA sequences or peroxisome proliferation-responsive elements (PPREs) in the regulatory region of PPAR-responsive genes. The large LBD ($\sim$1300 Å^3) allows the receptor to interact with a broad range of structurally distinct natural and synthetic ligands [81, 92, 93]. The receptor protein contains 13 helices, and the activation function, AF-2 helix located in the C terminus of the LBD is intimately integrated with the receptor's coactivator binding domain [81]. Ligand-dependent stabilization is required for activation of the downstream transcriptional machinery [81, 92, 93].

Thiazolidinedione full agonists (TZDfa), for example, rosiglitazone and pioglitazone permit certain coactivators to

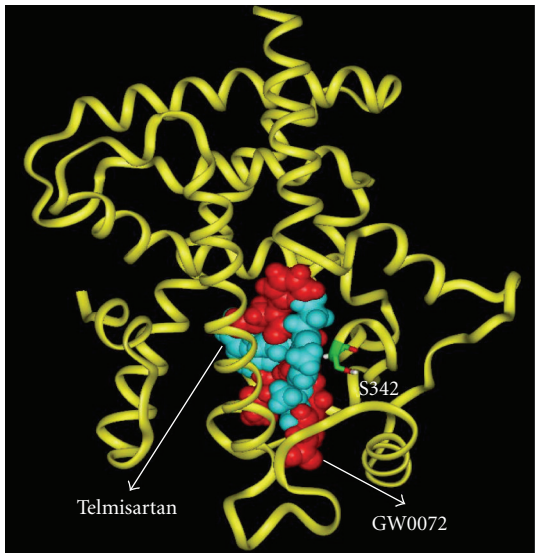

Figure 1: Telmisartan (blue) superimposed on the co-crystal structure of GW0072 (red) bound within the PPARγ-LBD. Telmisartan and GW0072 are Van der Waals space-filling representations, and the protein backbone by the yellow ribbon. Formation of hydrogen bonds and interactions between both ligands and the amide proton of Ser342 contribute toward stabilization of the partial agonists within the PPARγ-LBD. (*Kindly provided by Dr. P.V. Desai & Professor M.A. Avery, Department of Medicinal Chemistry, University of Mississippi, USA.*)

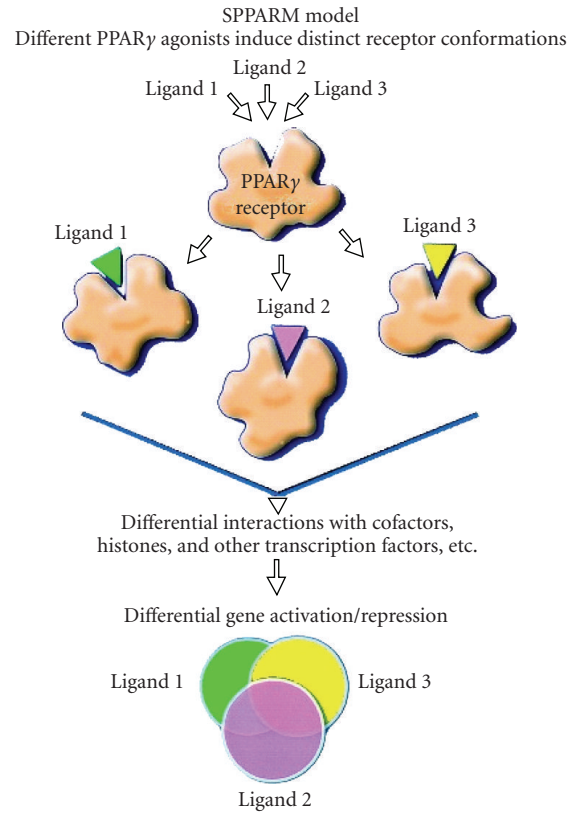

Figure 2: Selective PPARγ modulator (SPPARγM) model of PPARγ ligand action. PPARγ is a multivalent receptor whose ligand binding domain can accommodate different PPARγ ligands. Ligands 1, 2, or 3 (e.g., full agonist, partial agonist, or SPPARγM) are capable of inducing distinct receptor combinations leading to selective gene expression. Each ligand-receptor complex assumes a somewhat different three-dimensional conformation, leading to unique and differential interactions with cofactors, histones (acetylases/deacetylases), and other transcription factors. Consequently, each PPARγ ligand-receptor complex leads to a differential, but overlapping, pattern of gene expression. Thus, each ligand will activate, or repress multiple genes leading to differential overlapping expression of different sets of genes. (Adapted with permission from: J. M. Olefsky, "Treatment of insulin resistance with peroxisome proliferator-activated receptor gamma agonists." Journal of Clinical Investigation, vol. 106, no. 4, pp. 467-472, 2000); H. A. Pershadsingh, "Treating the metabolic syndrome using angiotensin receptor antagonists that selectively modulate peroxisome proliferator-activated receptor-gamma." International Journal of Biochemistry and Cellular Biology, vol. 38, nos 5-6, pp. 766-781, 2006.)

interact with the PPAR-LBD in an agonist-dependent manner and are oriented by a "charge clamp" formed by residues within helix 3 and the AF-2 arm of helix 12 in the LBD [45, 93]. Based on protease digest patterns and crystallographic findings, the PPARγ non-TZD partial agonist (nTZDpa) [94] and PPARγ partial agonist/antagonist, GW0072 [95] are mainly stabilized by hydrophobic interactions with helixes H3 and H7.

The antihypertensive ARBs telmisartan and irbesartan have been shown to function as partial PPARγ agonists, similar to the previously identified nTZDpa [94]. Based on molecular motifs, telmisartan appears to occupy a region in proximity with helix 3, with key interactions between the carboxylic acid group of the ligand and Ser342 near the entrance of the PPARγ pocket [37] (Figure 1). Telmisartan and irbesartan appear to cause an alteration in the conformation of these helixes similar to that induced by nTZDpa [37, 39], promoting differences in receptor activation and target gene expression that confer a low adipogenic potential compared with full agonists (TZDfa) like rosiglitazone and pioglitazone, which are known to have a high adipogenic potential and promote weight gain [58, 81, 94]. Differential binding motifs reflecting full versus partial PPARγ agonism are illustrated in Figure 2.

Several coactivators, including CREB-binding protein complex, CBP/p300, steroid receptor coactivator (SRC)-1, nuclear receptor corepressor (NcoR), DRIP204, PPAR binding protein (PBP)/TRAP220, and PPARγ coactivator-1 (PGC-1), among others, interface functionally between the nuclear receptor and the transcription initiation machinery in ways not well understood [94]. Differential ligand-induced initiation of transcription is the consequence of differential recruitment and release of selective coactivators and corepressors [96] (Figure 3). For example, NcoR a silencing mediator when bound to PPARγ suppresses adipogenesis in the absence of ligand. Activation by TZDfa ligands causes release of NcoR and recruitment of the nuclear receptor coactivator complex, NcoA/SRC-1 which promotes adipogenesis and lipid storage [94].

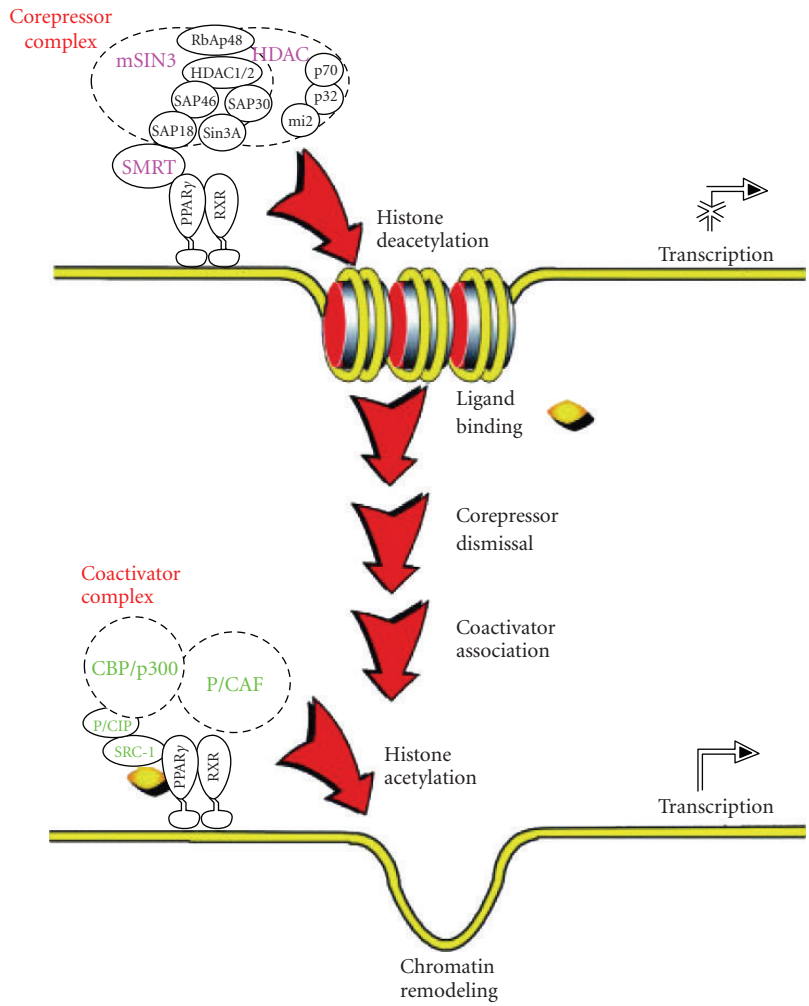

Figure 3: Schematic diagram of the mechanisms of PPARγ action. In the unliganded state (top), the PPARγ receptor exists as a heterodimer with the RXR nuclear receptor and the heterodimer is located on a PPAR response element (PPRE) of a target gene. The unliganded receptor heterodimer complex is associated with a multicomponent corepressor complex, which physically interacts with the PPARγ receptor through silencing mediator for retinoid and thyroid hormone receptors (SMRT). The corepressor complex contains histone deacetylase (HDAC) activity, and the deacetylated state of histone inhibits transcription. After PPARγ ligand binding, the corepressor complex is dismissed, and a coactivator complex is recruited to the heterodimer PPARγ receptor (bottom). The coactivator complex contains histone acetylase activity, leading to chromatin remodeling, facilitating active transcription. (Adapted with permission from: J. M. Olefsky, "Treatment of insulin resistance with peroxisome proliferator-activated receptor gamma agonists." Journal of Clinical Investigation, vol. 106, no. 4, pp. 467-472, 2000); C. K. Glass, M. G. Rosenfeld, "The coregulator exchange in transcriptional functions of nuclear receptors". Genes & Development,vol. 14, no. 2, pp. 121-141, 2000.)

Demonstration of direct interaction between telmisartan or irbesartan with PPARγ protein, by analyzing migration patterns of ligand-PPARγ protein fragments in trypsin digestion experiments, indicated that both ARBs downregulated PPARγ mRNA and protein expression in 3T3-L1 human adipocytes, a known property of PPARγ ligands in adipocytes [39]. In fact, both telmisartan and irbesartan caused release of NCoR and recruitment of NCoA/DRIP205 to PPARγ in a concentration-dependent manner [39]. The transcription intermediary factor 2 (TIF-2), an adipogenic coactivator implicated in PPARγ-mediated lipid uptake and storage, which increased the transcriptional activity of PPARγ, was potentiated by pioglitazone but not by the ARBs [39]. Moreover, irbesartan and telmisartan also induced PPARγ activity in an AT1R-deficient cell model (PC12W), demonstrating that their effects on PPARγ activity were independent of their AT1-R blocking actions [38]. These data demonstrate the functional relevance of selective cofactor docking by the ARBs, and compared to pioglitazone, identify telmisartan and irbesartan as unique selective PPARγ modulators (SPPARγMs) that can retain the metabolic efficacy of PPARγ activation, while reducing adverse effects, in parallel AT1-R blockade [37–39, 88]. Therefore, as dual ARB/SPPARγM ligands, telmisartan and irbesartan have important differential effects on PPARγ-dependent regulation of gene transcription, without the limitations of fluid retention and weight gain, providing improved therapeutic efficacy by combining potent antihypertensive, antidysmetabolic, anti-

inflammatory, and antiproliferative actions in the treatment of the proliferative retinopathies.

4.2. Expression of the renin-angiotensin system in the eye

The RAS evolved to maintain volume homeostasis and blood pressure through vasoconstriction, sympathetic activation, and salt and water retention [97]. AII binds and activates two primary receptors, AT1-R, and AT2-R. In adult humans, activation of the AT1-R dominates in pathological states, leading to hypertension, atherosclerosis, cardiac failure, end-organ demise (e.g., nephropathy), and proliferative retinopathies. AT2-R activation generally has beneficial effects, counterbalancing the actions propagated through AT1-R. ARBs selectively block AT1-R, leaving AII to interact with the relatively beneficial AT2-R. AII is generated in cardiovascular, adipose, kidney, adrenal tissue, and the retina; and through AT1-R activation promotes cell proliferation, migration, inflammation, atherogenesis, and extracellular matrix formation [97].

AII and genes enconding angiotensinogen, renin, and angiotensin converting enzyme (ACE) have been identified in the human neural retina [98]. Prorenin and renin have been identified in diabetic and nondiabetic vitreous, and intravitreal prorenin is increased in PDR [99]. Angiotensin I and AII were found to be present in ocular fluids of diabetic and nondiabetic patients [100]. AII and VEGF have been identified in the vitreous fluid of patients with PDR [101], and AT1 and AT2 were identified in the neural retina [102]. Furthermore, AT1 and AT2, AII, and its bioactive metabolite Ang-(1–7) were identified in blood vessels, pericytes, and neural (Müller) cells suggesting that these glial cells are able to produce and process AII [102]. Thus, AII signaling via the AT1 pathway within the retina may mediate autoregulation of neurovascular activity, and the onset and severity of retino-vascular disease [103].

4.3. Pathophysiological role of AT1 activation in proliferative retinopathies

AT1 activation participates in the pathogenesis of PDR, involving inflammation, oxidative stress, cell hypertrophy and proliferation, angiogenesis, and fibrosis [101, 103]. The RAS is upregulated concomitant with hypoxia-induced retinal angiogenesis [102–104] and is linked to AII-mediated induction of inflammatory mediators and growth factors, including VEGF and PDGF [103–106]. AT1 blockade with candesartan inhibited pathological retinopathy in spontaneously diabetic Torii rats by reducing the accumulation of the advanced glycation end-product (AGE) pentosidine [34]. AGEs contribute to vascular dysfunction by increasing the activity of VEGF and reactive oxygen species [34]. Treatment with candesartan reduced the accumulation pentosidine and VEGF gene expression in the diabetic rat retina [34]. AT1-R, AT2-R, and AII were shown to be expressed in the vascular endothelium of surgical samples from human CNV tissues and chorioretinal tissues from mice in which CNV was laser-induced [40]. Therefore, the retinal RAS appears to have an important pathophysiological role in proliferative retinopathies.

4.4. Therapeutic effects of telmisartan on PDR and CNV

AII is among the most potent vasopressive hormones known and contributes to the development of leukostasis in early diabetes [29]. Hypertension increases retinal inflammation and exacerbates oxidative stress in experimental DR [34, 107], and in diabetic hypertensive rats, prevention of hypertension abrogaIte retinal inflammation and leukostasis in early DR [108]. Therefore, RAS blockade by the dual ARB/PPARγ agonists, telmisartan or irbesartan, may have enhanced effects for abrogating inflammatory and other pathological events that contribute to or exacerbate PDR and CNV/AMD. In clinical studies, reduction of hypertension by any means reduces the risk of development and the progression of DR [109]. ARBs are widely used antihypertensive agents clinically.

Induction of diabetes by streptozotocin injection in C57BL/6 mice caused significant leukostasis and increased retinal expression and production of AII, AT1-R, and AT2-R [30]. Intraperitoneal administration of telmisartan inhibited diabetes and glucose-induced retinal expression of ICAM-1 and VEGF, and upregulation of ICAM-1 and MCP-1, via inhibition of nuclear translocation of NF-κB [33]. There have been no reports on the effects of irbesartan on PDR or CNV/AMD.

In the laser-induced mouse model of CNV, new vessels from the choroid invade the subretinal space after photocoagulation, reflecting the choroidal inflammation and neovascularization seen in human exudative AMD. Based a recent suggestion [110], Nagai et al. [45] evaluated and compared the effects of telmisartan with valsartan, an ARB lacking significant PPARγ activity [38, 39], and suitable control to evaluate the role of telmisartan PPARγ activity. Both ARBs have identical affinities for the AT1-R ($\sim$10 nmo1/L) [97]. Telmisartan (5 mg/kg, i.p.) or valsartan (10 mg/kg, i.p.) significantly suppressed CNV in mice [45]. Simultaneous administration of the selective PPARγ antagonist GW9662, partially (22%) but significantly reversed the suppression of CNV in the group receiving telmisartan but not the group receiving valsartan [45], indicating separate beneficial contributions via AT1 blockade and PPARγ activation, respectively [45]. Using GW9662, similar findings were obtained identifying participation of PPARγ in the suppressive effect of telmisartan on the inflammatory mediators, ICAM-1, MCP-1, VEGFR-1 in b-End3 vascular endothelial cells, and VEGF and in RAW264.7 macrophages, unrelated to AT1 blockade [45]. These findings confirm that the beneficial effects of telmisartan are derived from a combination of AT1 blockade and PPARγ activation. The inhibitory effects of valsartan were insensitive to the presence of GW9662. This is the first known demonstration of PPARγ-dependent inhibitory actions of a non-TZD PPARγ agonist on CNV. There have been no reports on the effects of irbesartan on PDR or CNV.

4.5. Therapeutic potential of dual ARB/SPPARγMs

Reduction in the cardiometabolic risk profile by lowering high blood pressure, improving insulin sensitivity, normalizing the lipid profile, and inhibiting inflammatory pathways are known to impede the pathological evolution of proliferative retinopathies. The dual ARB/SPPARγM ligands, telmisartan has been shown to be effective in this regard in the rodent model, though irbesartan has yet to be tested experimentally. PPARγ activation has beneficial effects by lowering hyperglycemia and improving the metabolic profile in individuals with type 2 diabetes and the metabolic syndrome. The fact that both AT1-R blockade and PPARγ activation by telmisartan had independent synergistic effects in the murine model of laser-induced CNV is an important finding [40]. It would be useful to test whether irbesartan has effects similar to those of telmisartan in animal models of PDR and CNV/AMD [28, 31–34, 40], as both ARBs similarly attenuate inflammation, proliferation, and improve the metabolic syndrome [111, 112]. Also, unlike TZDs, telmisartan (but not valsartan) increases caloric expenditure and protects against weight gain and hepatic steatosis [113]. With its high lipid solubility, large volume of distribution, and other favorable pharmacokinetic properties [86–88], telmisartan may be effective when administered orally. If oral delivery proves therapeutically ineffective, the drug may be formulated for administration via implant or transscleral application for local delivery to the posterior segment [114–116].

5. CONCLUDING REMARKS

Hypertension, insulin resistance, dyslipidemia, and risk for atherosclerosis and atherogenesis, all components of the metabolic syndrome, comprise significant epidemiologic risk factors for neovascular, proliferative retinopathies [6, 9, 12, 117, 118]. Photodynamic and anti-VEGF therapy, current treatments for CNV/AMD are cost-intensive. Treatments for PDR are limited to surgical options in advanced disease when the visual function is irreversibly affected [3–6, 14–16]. Therefore, alternative, low cost, prophylactic and/or palliative pharmacotherapeutic approaches are attractive and desirable. The currently approved antidiabetic TZD, rosiglitazone (a full PPARγ agonist), and the antihypertensive ARB, telmisartan (a partial PPARγ agonist) have both shown promise in animal models of proliferative retinopathies. The potential efficacies of irbesartan in proliferative retinopathies remain to be determined. Administration of TZDs may, in patients with AMD, slow the progression to CNV, and in patients with diabetic retinopathy attenuate the progress to PDR, provided that: (1) their risk of macular edema is low, (2) they lack symptoms of CHF or cardiomyopathy, and (3) are not taking insulin or nitrates. The efficacy and safety limitations of the TZDs are well understood [119–123] and their use would require careful benefit-to-risk analysis. Because these drugs have been in use clinically for a decade, well-designed retrospective analyses in carefully selected patient populations may reveal useful information regarding their clinical potential.

Several SPPARγMs currently which are under development for treating type 2 diabetes [124] could be screened in animal models of PDR and CNV to determine their potential efficacy for treating proliferative retinopathies. Long-term, prospective clinical trials are needed to demonstrate the efficacy of currently approved TZDs and ARBs (Table 3). Notably, three large prospective phase III trials are underway to evaluate the effect of the ARB, candesartan on retinopathy in normotensive type 1 and type 2 diabetes patients, the diabetic REtinopathy candesartan trials (DIRECTs) Programme [125]; estimated study completion date: June 2008. These studies will provide important insight into the potential efficacy of ARBs in general in the treatment of DR. With their capacity for activating PPARγ and improving the metabolic profile, the clinical efficacy of telmisartan and possibly irbesartan could be evaluated in patients at risk for developing PDR and CNV, especially those with deficiencies in carbohydrate and lipid metabolism. Moreover, with their unique structure/activity profile, these compounds may provide a drug discovery platform for designing therapeutic agents for treating proliferative retinopathies.

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Review Article

PPAR-γ, Microglial Cells, and Ocular Inflammation: New Venues for Potential Therapeutic Approaches

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The last decade has witnessed an increasing interest for the role played by the peroxisome proliferator-activated receptor-γ (PPAR-γ) in controlling inflammation in peripheral organs as well as in the brain. Activation of PPAR-γ has been shown to control the response of microglial cells, the main macrophage population found in brain parenchyma, and limit the inflammation. The anti-inflammatory capacity of PPAR-γ agonists has led to the hypothesis that PPAR-γ might be targeted to modulate degenerative brain diseases in which inflammation has been increasingly recognized as a significant component. Recent experimental evidence suggests that PPAR-γ agonists could be exploited to treat ocular diseases such as diabetic retinopathy, age-related macular degeneration, autoimmune uveitis, and optic neuritis where inflammation has relevant role. Additional PPAR-γ agonist beneficial effects could involve amelioration of retinal microcirculation and inhibition of neovascularization. However, PPAR-γ activation could, in some instances, aggravate the ocular pathology, for example, by increasing the synthesis of vascular endothelial growth factor, a proangiogenic factor that could trigger a vicious circle and further deteriorate retinal perfusion. The development of new in vivo and in vitro models to study ocular inflammation and how to modulate for the eye benefit will be instrumental for the search of effective therapies.

1. INTRODUCTION

The peroxisome proliferator-activated receptor-γ (PPAR-γ) is a ligand-inducible transcription factor that belongs to a large superfamily comprising the nuclear receptors for steroids, thyroid hormones, and retinoids. The PPAR-γ and the two closely related PPAR-α and PPAR-δ (also known as β, NUC-1, or FAAR) are activated by naturally occurring fatty acids and act as sensors that regulate whole body metabolism in response to the dietary intake by controlling lipid and carbohydrate metabolism and lipid storage [1]. All three PPARs, once agonist-activated,form heterodimers with retinoic X receptors and regulate specific target gene transcription by binding to specific DNA regions (peroxisome proliferator response elements, PPREs) or by a mechanism independent of PPRE binding, termed transrepression, which begins to be unravelled [2].

Because of their role in the regulation of genes involved in lipid and carbohydrate metabolism, PPARs deeply affect lipid homeostasis and insulin sensitivity [3, 4]. The serum glucose lowering activity of PPAR-γ has lead to the development of specific PPAR-γ agonists for the treatment of type-2 diabetes and the metabolic syndrome [5]. PPAR-γ agonists such as thiazolidinediones (TZD), including pioglitazone (Actos) and rosiglitazone (Avandia), increase insulin sensitivity thereby improving glycaemic control, but also modify lipidemic profile and decrease blood pressure [6–9]. On the other hand, fibrates, which are PPAR-α agonists, are prevalently antilipidemic drugs, and therapeutic benefits of PPAR-α and PPAR-γ activations, which only are minimally overlapping, have generated interest in dual agonists that target both receptors, thus offering improved insulin sensitivity and lipidemic control in the same molecule [10, 11]. This would provide a therapeutic tool against diabetes and the metabolic syndrome.

The three PPARs share a high homology, but differ for tissue distribution and ligand specificity. PPAR-α is mainly expressed in tissues with high catabolic rates of fatty acids, such as the liver, muscle, and heart, whereas PPAR-δ shows a much wider distribution. PPAR-γ is highly expressed in

adipose tissue, where it plays a central role in the regulation of adipocyte differentiation [12], and in cells of the immune system, including lymphocytes and macrophages. In peripheral monocytes, PPAR-γ expression is induced during the process of extravasation from blood vessels into the tissues, and in the course of activation by pro-inflammatory stimuli, suggesting that PPAR-γ is important for promoting monocyte-macrophage differentiation and activation and, thus, controlling inflammation [13–16]. As for macrophages of peripheral tissues, PPAR-γ regulates the activation of microglial cells, the main macrophage population found in brain parenchyma, and increasing evidence indicates that PPAR-γ might modulate brain inflammation and neurodegeneration [17] and be exploited as valuable therapeutic target in neurological diseases [18]. Indeed, brain inflammation is increasingly viewed as a target for treating neurological diseases, not only in classical infectious and immune-mediate disorders such as meningitis or multiple sclerosis, but also in stroke, trauma, and neurodegenerative diseases that were not originally considered to be inflammatory [19, 20].

In a similar way, inflammation could represent an important target to treat ocular diseases. In the study of ophthalmology, the classical subdivision of pathology textbooks in metabolic, inflammatory, hemodynamic, and degenerative disorders appears artificial and does not reflect the complexity of conditions, where inflammation, dysmetabolic and hemodynamic disorders, and neurodegeneration often conspire to the development of diseases. Paradigmatic example is diabetic retinopathy (DR), where a metabolic derangement (hyperglycemia) triggers a pathologic pathway, characterized initially by inflammation (leukostasis, enhanced retinal vascular permeability, Muller cell, and microglial activation), followed by microvasculature alterations and ischemia (proliferative DR), eventually leading to degeneration of neural retina and visual loss. To this complexity, a simplicity in the natural history may correspond and the course of different retinal diseases may at a certain stage converge toward a similar evolution. For example, pathologic neovascularization may be the same and ominous outcome of DR, age-related macular degeneration (AMD), and autoimmune uveitis, conditions that are very far from each other from the point of view of etiology.

In the present article, we will first briefly review the immune cells that participate to the ocular inflammation, mainly microglia, and the role of PPAR-γ in controlling their functions. In a second part, we will consider three conditions, where inflammation has a relevant function, microglia is involved, and the role of PPARs has been taken into consideration: DR, AMD, and optic neuritis (ON).

2. MICROGLIAL CELLS AND OTHER CELL POPULATIONS OF THE IMMUNE RESPOSE IN THE EYE.

Glial cells are the primary participants in the formation of scars in response to retinal or ocular injury and diseases. In addition, under normal conditions, they carry out a variety of supportive functions for the neurons with which they are closely related. Glial cells include astrocytes, oligodendrocytes, the retina-specific Muller-glial cells, and microglia, which are considered the main immune resident cells.

Retinal microglia, like their counterpart in the brain, belong to the myeloid lineage and their myeloid progenitors enter the nervous system primarily during embryonic and fetal periods of development. During embryogenesis, microglial precursors migrate to the retina before retinal vascularization and differentiate into ramified, quiescent microglia typical of adult healthy retina. A second population of phagocytes, which express macrophage markers, invades the retinal later through the developing vasculature and remains associated with the blood vessels (see below). In the adult retina, microglia are distributed through most of the retinal layers, including outer plexiform layer, outer nuclear layer, inner plexiform layer, ganglion cell layer, and nerve fiber layer. Engraftment experiments have shown that they display some proliferative capacity and have a slow turnover in respect of other macrophage populations [21]. Disturbances in the number or distribution of these cells disrupt the normal development of the eye and its related structures. Ritter and collaborators [22] have recently reported that myeloid progenitors migrate to vascular regions of the retina where they differentiate into microglia and facilitate the normalization of the vasculature, thus underlining a main role of microglial cells in promoting and maintaining retinal vasculature during development.

Microglia show particular capacity of interaction with retinal cells, supervising the immune environment (see [23] and references therein). As for microglia in the brain parenchyma, retinal microglial cells are immunocompetent cells, able to remove the debris created during normal eye development or degenerative conditions by phagocytosis and to mount an inflammatory and immune response against ocular injury, infection, and disease.

Under normal conditions, microglia are characterized by a downregulated phenotype when compared to other macrophage populations of peripheral tissues. The maintenance of microglia in this "inhibited" state is crucial for the regulation of the immune state of the retina, which has to maintain tissue homeostasis while preventing the destructive potential of inflammatory and immune response. The complexity of the several intraocular structures on which the correct vision is dependent renders the eye particularly vulnerable to the reactions of the immune system against invading pathogens or ocular injury. To prevent that a defensive reaction can transform into a threat to vision in itself, the eye is equipped with several regulatory mechanisms, which contribute to make the eye an "immune-privileged" site [24]. As recently described for the brain parenchyma [25], the immune privilege is not an absolute or an immutable state, but rather it is the result of the active interplay among specialized cellular elements and specific microenvironment characteristics, and it can be overcome in several instances. Among the main features that account for the ocular immune privilegeare is the presence of blood-ocular barriers (the blood-aqueous barrier and the blood-retinal barrier), which are physical barriers between the local blood vessels and most parts of the eye itself, and the peculiar characteristics of the resident immune cells, namely, microglia, which are largely dependent on the presence of immunomodulatory factors

Table 1: Retinal pathologies characterized by microglial activation.

Pathology	References
Diabetic retinopathy	[26–29]
Glaucomatous optic nerve degeneration	[30–33]
Human retinitis pigmentosa	[34]
Age-related macular degeneration	[34, 35]
Retinal ischemia and reperfusion injury	[36, 37]
Retinal degeneration	[14, 20, 38]

in the aqueous humor and on the cross-talk between microglia and retinal cells. Several "ligand-receptor-" type interactions between retinal cells and microglia contribute to maintaining microglia in a nonactivated state. Among these, the glycoprotein CD200, which in the retina is extensively expressed in neurons and endothelial cells, and the cognate ligand CD200L on microglia [39], and the neuronal chemokine fractalkine (or CX3CL1) and its microglial receptor CX3CR1 [40].

In spite of their apparent "dormant" state, resting microglia actively monitor the surrounding microenvironment with extremely motile processes and protrusions, entering in contact with other cellular elements and sensing alterations in the nearby environment, to which they rapidly react. Microglial activation comprises morphological changes, such as cellular hypertrophy, retraction of processes, and expression of surface markers, as well as functional changes, including proliferation, migration, phagocytosis, and production of bioactive molecules. Activated microglia have been described in several forms of retinal injury or disease (see Table 1), in which they are believed to play major roles, either protective or detrimental. Indeed, activated microglia can, on one side, remove the degenerating neurons and contribute to re-establish tissue integrity; on the other side, they can secrete proinflammatory cytokines such interleukin (IL)-1β, IL-3, IL-6, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ, which can be toxic to neurons and photoreceptors [41, 42] or to other cellular targets such as oligodendrocytes [43, 44]. In addition, several of these microglial products can up-regulate the expression of vascular cell adhesion molecules and chemokines [45–47], thus promoting the recruitment of lymphocytes and macrophages, and enhancing the immune-mediated tissue damage [23, 48]. In this context, molecules that can enable the control of microglial activation represent valuable tools to counteract the detrimental effects of inflammation and immune response while fostering those necessary for healing.

In addition to microglia, other cell types contribute to the immune response in the eye. The perivascular macrophages reside outside the blood-ocular barrier, in the space that separates the endothelium of the retinal capillaries and retinal pigment epithelium (RPE). Because of their anatomical location, they escape the tight control to which retinal microglia are subjected and their morphology and immunophenotype are very similar to those of macrophages of peripheral tissues. In close proximity, but separate from perivascular macrophages are the pericytes, which are believed to be essential as structural support in microcirculation. In addition, together with astrocytes and Muller glia, they are considered to play a major role in maintaining the inner blood-retinal barrier [49]. These cells, of mesodermal origin, are enclosed within the basal lamina on the abluminal surface of endothelial cells and contain contractile proteins. Pericytes have been shown to control vessel constriction and retinal blood flow [50], and are involved in several pathological conditions, including hypoxia, hypertension, and DR. Their activation, since the very early phases of disease, is thought contribute to the disruption of the blood-retinal barrier [51]. Finally, the RPE cells are important in ocular immune response and in maintaining the eye immune privilege. These cells form a monolayer between the neuroretina and the choroids and are the essential component of the outer blood-retinal barrier. One of the main characteristics of RPE cells is the presence of tight junctions at the apical side of their lateral membrane, which render the monolayer impermeable for macromolecules and limit access of blood components to the retina. In addition to several important supportive functions, including regulation of transport of nutrients to the photoreceptors, phagocytosis of damaged or old rod outer segments, and production of growth factors, RPE cells contribute to the immune and inflammatory response of the retina by expressing major histocompatibility complex (MHC) antigens, adhesion molecules, and a variety of cytokines, which may either promote or enhance immune responses or down-regulate them [52].

In addition to the cell types so far described, a novel population of dendritic cells has been recently reported in normal mouse retina, distinguishable by the cell types by the extent of specific surface antigens and anatomical tissue location [53].

3. DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is one of the most serious complications of diabetes and the leading cause of blindness among working-age adults. DR symptoms are mostly due to the vascular alterations that affect the retina. The early events are increased blood flow and abnormal vessel permeability, due to the impairment of blood-retinal barrier. They are caused by hyperglycemia and the other metabolic consequences of excess glucose disposal. As the disease progresses, retinal vasculopathy develops, showing loss of pericytes, smooth muscle and endothelial cell death, and microaneurysm formation, resulting in areas of ischemia in the retina. At this stage, up-regulation of proangiogenic factors in ischemic retina, such as vascular endothelial growth factor (VEGF), initiates a vicious circle of neovascularization (proliferative DR), characterized by enhanced vascular leakage and formation of new, weak, and prone-to-break blood vessels, which further deteriorates retinal perfusion, worsens ischemia and eventually leads to visual loss.

Although the pathogenetic cascade connecting these events is still unclear, evidence suggesting a role for inflammation in DR is accumulating, supporting the involvement of both chemical mediators and inflammatory cells in the pathogenesis of the disease [54]. Elevated levels of

proinflammatory cytokines, such as IL-1β, IL-6 and IL-8, and TNF-α and vascular cell adhesion molecule-1, have been found in the vitreous of patients with proliferative DR [55–57]. Increased VEGF and IL-6 levels were detected in the aqueous humor of diabetic patients with macular edema [58]. TNF-α was found in epiretinal membranes of proliferative DR [59]. Data from experimental models are in line with these observations. In streptozotocin (STZ)-induced diabetic rats, changes in retinal blood vessel permeability, which characterizes the early phases of DR, are paralleled by increase in the level of the intercellular cell adhesion molecule-1 (ICAM-1), which facilitates the trafficking of leukocytes [60], and pro-inflammatory mediators, such as TNF-α and cyclooxygenase-2 (COX-2) [61, 62]. In the same animal model, an increased level of IL-1β has been observed and put in relation to upregulated inducible nitric oxide synthase (iNOS) [63]. Mice deficient in the leukocyte adhesion molecules CD18 and ICAM-1 demonstrate significantly fewer adherent leukocytes in the retinal vasculature after induction of diabetes with STZ [54]. According to some authors, VEGF could be responsible for the initiation of the inflammatory cascade, as its administration in vivo was found to induce retinal ICAM-1 and endothelial NOS (eNOS) expression [64, 65]. As far as inflammatory cells are concerned, microglia seem to be mostly involved. Microglial activation appears early in the course of DR, before the onset of overt neuronal cell death [62]. In STZ-induced diabetic rats, hypertrophic microglia were observed one month after the onset of diabetes [66], with significant increase also in cell number [67]. In mice with alloxan-induced diabetes, changes in microglial cell morphology were the first detectable cellular modifications, apparently preceding ganglion cell apoptosis and increase in blood barrier permeability [68]. Treatment of STZ-induced diabetic rats with minocycline, a semisynthetic tetracycline that counteracts microglial activation, besides decreasing the expression of proinflammatory cytokines, decreased caspase-3 levels [62], suggesting a potential neuroprotective anti-apoptotic effect of inhibition of microglial activation.

Considering the role of inflammation in the pathogenesis of DR, it has been suggested that PPAR-γ ligands exert therapeutic effects also as modulators of inflammation, besides providing glycemic control [69]. In diabetic patients, PPAR-γ agonists reduce several markers of inflammation, such as serum levels of C-reactive protein, IL-6, monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1, soluble CD40 ligand, and matrix metalloproteinase-9 [70–75]. In addition, they have been shown to induce the suppression of activated NFκB and decrease ROS generation in blood mononuclear cells [70, 73].

Modulation of the inflammatory process has also been studied in DR in in vivo models. In streptozotocin-induced DR, rosiglitazone was shown to inhibit both retinal leukostasis and retinal leakage [76]. The effect was not accompanied by downregulation of proinflammatory cytokines, such as TNF-α, although the adhesion molecule ICAM was found reduced. Nitric Oxide (NO) of endothelial origin regulates ocular blood flow. In the endothelial dysfunction, which characterizes the early stages of DR, a reduction in the bioavailability of NO may contribute to impairment of ocular hemodynamics [77]. In bovine aortic endothelial cells, troglitazone increased NO production in a dose- and time-dependent manner with no modifications in eNOS expression [78]. A study focused on NO production in pericytes showed that PPAR-γ is constitutively expressed in retinal pericytes and that troglitazone increases NO production and iNOS expression in a PPAR-γ-dependent manner, an effect which is opposite to what observed in cultured microglia [79, 80]. This study suggests that PPAR-γ agonists, in addition to improving insulin sensitivity, might also improve retinal microcirculation in early DR [81]. However, NO is a double-edged sword. Overproduction of NO by neuronal NOS is supposed to contribute to retinal injury in ischemia [82, 83]. Thus, although in DR early phase an increase in NO may contribute to the improvement of retinal microcirculation, in proliferative DR a beneficial effect is doubtful. A further reason of concern is represented by TZD effects on VEGF. Several in vivo and in vitro studies have reported increased expression of VEGF in response to PPAR-γ ligands. TZDs have been found to upregulate VEGF in human vascular muscle cells [84], in 3T3-L1 adipocytes [85], in cultured cardiac myofibroblasts [86]. In bovine aortic endothelial cells treated with troglitazone, NO increase was accompanied by upregulation of VEGF and its receptor, KDR/Flk-1 [78]. Administration of pioglitazone [87] and troglitazone [85] also significantly increased plasma VEGF levels in diabetic patients. Considering the role played by VEGF in the development and progression of DR, caution has been suggested in the use of PPAR-γ ligands in patients with advanced disease [85, 87]. However, in partial disagreement with the results above reported, antiangiogenic properties of PPAR agonists have been shown both in in vitro and in vivo models [35, 88–90]. In neonatal mice, where ischemia was used as a model of retinal neovascularization, intravitreous injection of rosiglitazone or troglitazone inhibited development of new retinal vessels [91]. In the same study, TZDs have been found to inhibit retinal endothelial cell proliferation, migration, and tube formation in response to VEGF treatment [91]. Further studies are therefore required to clarify the issue.

4. AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration (AMD) is the leading cause of vision loss in the elderly in the western world. It is characterized by degeneration of the macula, the central area of the retina with the highest concentration of cone photoreceptors, responsible for visual acuity and color vision. Histopathologically, the early phase of AMD is characterized by formation of drusen, deposits of lipid and cellular debris that are found between the RPE cells and Bruch's membrane, possibly as a result of RPE degeneration or, as recently proposed [92], microglial infiltration and transformation in foam cells. As the disease proceeds, photoreceptor degeneration and, in the most aggressive cases, choroidal neovascularization (CNV) intervene, with growth of new blood vessels from the choroids into the subretinal space. Two major clinical phenotypes of AMD are recognized: nonexudative (dry type), and exudative (wet type). The latter more frequently develops into CNV.

AMD is a complex, multifactorial disease and both genetic and environmental factors may contribute at some level. In the pathogenesis of the disease, both altered angiogenesis and inflammation play a role. The study of pathologic angiogenesis in the retina has focused on two main factors: the angiogenic VEGF [93, 94] and the antiangiogenic PEDF [94–96], although a number of other factors are implicated(for a review, see [97]). It is widely agreed that in CNV an imbalance between angiogenic and anti-angiogenic factors takes place, but what disrupts this delicate equilibrium is still unclear. Several lines of evidence point to inflammation as a pathogenetic mechanism. Many risk factors for AMD are related to inflammation, including environmental factors, such as smoking and low intake of omega-3 fatty acid [98, 99], and genetic factors, such as polymorphisms of complement factor H [100–102] and the chemokine receptor CX3CR, which is expressed by microglia and mediates migration and adhesion in response to its ligand fractalkine or CX3CL1 [103]. Increased serum levels of IL-6 and C-reactive protein have been found to be related with progression of AMD [104]. More recently, IL-6 receptor neutralization has shown to lead to decrease in the expression of inflammatory mediators, such as the chemokine MCP-1, the adhesion molecule ICAM-1, and VEGF, and to reduce macrophage infiltration into CNV in in vivo model of the disease [105]. Inflammatory mediators, such as macrophage chemoattractants and activated complement components, especially C3a and C5a, are also found in drusen samples from AMD patients [106–108]. A role for complement in the development of the disease has been suggested [34]. In line with this hypothesis, it has been observed that genetic ablation of receptors for C3a or C5a reduced VEGF expression, leukocyte recruitment, and CNV [109].

Activation of microglia and infiltration of macrophages have been reported in the human AMD as well as in experimental CNV [110–112]. In transgenic mice lacking CX3CR1, microglia migrate defectively and accumulate in the subretinal space, evoking morphological and pathological features similar to those observed in human AMD. In addition, laser-induced CNV was exacerbated in these mice [92]. A controversy exists regarding the origin of activated retinal mononuclear phagocytes, that is, whether they are resident microglia [113, 114] or blood-derived bone marrow macrophages [46, 115]. In support of the latter hypothesis, it should be noted that systemic depletion of macrophages using clodronate-filled liposomes blocked neovascularization [116, 117]. However, the role of macrophages is still debated, since some studies suggest an antiangiogenic role for macrophages. For example, mice lacking CC chemokine ligand 2 (CCL2) or its receptor, both involved in chemoattraction of macrophages and/or microglia, show drusen-like deposits and CNV, suggesting that macrophage recruitment may protect against AMD [118]. In addition, mice lacking IL-10, an anti-inflammatory cytokine known to control macrophage/microglia functions, had significantly reduced neovascularization and increased macrophage infiltrates compared to wild type, in a laser-induced model of CNV. In these experiments, prevention of macrophage entry into the eye promoted neovascularization while direct injection of macrophages significantly inhibited CNV.

As mentioned earlier, beside mononuclear phagocytic cells, RPE cells have also a role in the inflammatory and angiogenetic process, as a major source of VEGF and PEDF. In addition, there is a cross-talk between RPE and macrophages. It has been shown that macrophages in CNV are immunopositive for VEGF, TNF-α, and IL-1β [119]. The latter factors can induce the secretion of IL-8 and MCP-1 in RPE cells in vitro [120, 121]. MCP-1 is, in turn, involved in the recruitment of macrophages [122], thus closing the circle. Indeed, in surgically excised CNV specimens, RPE was found to express VEGF and MCP-1 and macrophages were immunolabeled for VEGF [123].

The interest in the role of PPARs in AMD has been mainly focused on their activities as modulators of angiogenesis. PPAR agonists have shown antiangiogenic properties both in in vitro and in vivo models [35, 88, 89]. It has been shown that choroidal ECs and RPE cells express PPAR-γ and that PPAR-γ ligands inhibit their response to VEGF, without apparent toxicity to the adjacent retina, in a laser-induced model of CNV [90]. Decrease in angiogenesis apparently takes place by inhibition of VEGF, since PPAR-α agonists are found to inhibit endothelial VEGFR2 expression [124]. An opposite role has been recently described for PPARδ, which induced endothelial proliferation and angiogenesis in vitro, through a VEGF-dependent mechanism [125]. The natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) was found to protect a human RPE cell line from oxidative stress by elevating GSH and enhancing MAPK activation, but such activity was independent of its PPAR-γ binding activity [126]. The roles of infiltrating macrophages and/or resident microglia in the pathogenesis of AMD open the possibility that PPAR-γ agonists may ameliorate the course of the disease also through the down-regulation of several proinflammatory functions of these cells [8] and reference therein, including TNF-α and iNOS, and MHC-II expression.

However, possible beneficial effects of PPAR-γ agonists in the treatment of ocular inflammation and, particularly, of AMD need to be further verified. It is important to keep in mind that PPAR-γ is involved in the differentiation of macrophages to foaming cells and PPAR-γ ligands can induce expression of adipocyte lipid binding protein (ALBP/aP2), a gene that is highly expressed in vivo in macrophage/foam cells of human atherosclerotic plaques [127]. Moreover, activation of PPAR-γ has been shown to reduce CCR2 expression in monocytes and their chemotaxis in response to MCP-1 [128]. These PPAR-γ mediated activities are of particular interest in the view of the recent finding by Combadière et al. [92], suggesting that subretinal microglial foam cells might be the origin of drusen-like deposits and that accumulation of microglia in the subretinal space may be a driving force in the pathogenesis of AMD.

5. OPTIC NEURITIS AND RELATED DISORDERS

Optic neuritis (ON), an inflammatory, demyelinating disease of the optic nerve, may be the initial symptom of multiple sclerosis (MS) or appear in the course of the disease.

Table 2: PPAR agonists and EAE.

Agonists	Biological activity	Receptor	References
Troglitazone	Amelioration of clinical symptoms. Reduced expression of proinflammatory cytokines, IL1β and TNF-α	PPAR-γ	[129]
Ciglitazone, 15d-PGJ_2	Decrease of severity and duration of clinical paralysis. Decrease of CNS inflammation and demyelination. Decrease of IL-12 production	PPAR-γ	[130]
15d-PGJ_2	Delay in the onset and decrease in the severity of disease. Reduction of Con A- and MBP Ac1–11-reactive, IFN-α- and IL-4-secreting cells	PPAR-γ	[131]
Pioglitazone	Decreased mRNA levels of iNOS and the chemokines MIP1 and RANTES in the central nervous system	PPAR-γ	[132]
Gemfibrozil and fenofibrate	Dose-dependent suppression of lymphocyte proliferation. Promotion of IL-4 production and inhibition of IFN-γ production	PPAR-α	[133]
GW0742	Improvement of clinical recovery. Reduction of glial activation	PPAR-δ	[134]
Ciglitazone, 15d-PGJ_2	Amelioration of clinical and pathological symptoms. Inhibition of neural antigen-specific T cell proliferation	PPAR-γ	[135]
Gemfibroil	Reduction of incidence and clinical signs. Inhibition of the infiltration of inflammatory cells into the CNS. Reduced expression of proinflammatory molecules such as iNOS, IL-1, IL-6, and TNF-α	no PPAR-γ	[136]
Pioglitazone	Prevention of relapse episodes and reduction of mean clinical scores during the treatment period. Decrease of IFN-γ levels	PPAR-γ	[137]

In any event, nearly half of MS patients develop ON during the course of the disease. An idiopathic demyelinating disorder of the optic nerve also occurs as NeuroMyelitis Optica (NMO) or Devic's disease, which is characterized by the coexistence of usually bilateral and severe optic neuritis with spinal cord involvement and the presence of a highly specific serum autoantibody marker (NMO-IgG), recognizing the transmembrane channel Aquaporin 4 [138, 139]. The boundaries between NMO and MS are, however, rather imprecise, from both the clinical and pathologic points of view and it is still a matter of controversy whether NMO should be considered a variant of MS or a separate entity [139, 140].

Considering their role in inflammation, the possible therapeutic efficacy of PPAR-γ agonists has been studied in experimental autoimmune encephalomyelitis (EAE), an animal model of the disease where the autoimmune reaction against myelin is induced in animals by active sensitization with myelin components. Although several criticisms have been moved towards this model, EAE still provides a valuable tool for improving our understanding on the pathogenesis and treatment of MS. EAE is also considered a model relevant to the study of demyelinated diseases of the optic nerve [141, 142]. An additional animal model is represented by T cell receptor transgenic mice specific for myelin oligodendrocyte glycoprotein (MOG). These mice develop isolated optic neuritis either spontaneously or after sensitization with suboptimal doses of MOG [143]. Therapeutic efficacy of PPAR-γ ligands has been demonstrated in terms of suppression or amelioration of clinical symptoms and decrease of inflammatory signs (see Table 2). Although the anti-inflammatory activities of PPAR-γ agonists are complex and multifaceted, evidence has been provided suggesting a direct action of PPAR-γ agonists on microglia/mononuclear phagocytic cells. Indeed, taking part in both innate and adaptive immune responses, microglia and mononuclear phagocytes are deeply implicated in the complex inflammatory cascade associated with MS. Their role has been recently and extensively reviewed [144, 145]. The PPAR-γ natural agonist 15d-PGJ_2 [146] and the PPAR-α agonist gemfibrozil [133] were found to significantly reduce macrophage infiltration in the lesions. A decreased number of IL-1β-positive cells were found in EAE brain of mice treated with GW0742 and a PPAR-δ agonist and this observation was considered indicative of a reduction of glial activation [134]. PPAR-γ inhibition of microglial cell activation is also supported by in vitro experiments [8, 79, 80, 147–152].

Notwithstanding the amount of data regarding a therapeutic activity of PPAR agonists in EAE, clinical studies are lacking and report on their clinical use in MS or ON is still anecdotical [153]. Clinical trials are however in course with pioglitazone and rosiglitazone.

6. CONCLUSIONS

The promising results obtained in experimental models of ocular diseases and the recent advancements in the knowledge of the pathogenic mechanisms driving ocular damage and vision loss strongly point to PPAR-γ as a valuable target to control inflammation and treat invalidating diseases such as DR, AMD, and ON. Given the complexity of the

phenomena that can be influenced by PPAR-γ activation, involving not only inflammation but also retinal microcirculation, neovascularization, and transformation of activated microglia in foam cells contributing to drusen-like deposits, further studies are mandatory for a correct evaluation of pro and cons of using PPAR-γ agonists in ocular disease treatment. The PPAR-γ agonists could also find other important applications in controlling the adverse effects of inflammation that can put at risk the eye integrity and the correct vision. As an example, some of the adverse reactions described after liquid artificial vitreous replacement use in vitreoretinal surgery are a consequence of inflammatory reaction and activation of mononuclear phagocytic cells [154], suggesting that the use of PPAR-γ agonists could be very advantageous in controlling the inflammatory response to biomaterials.

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Review Article

Role of Peoxisome Proliferator Activator Receptor γ on Blood Retinal Barrier Breakdown

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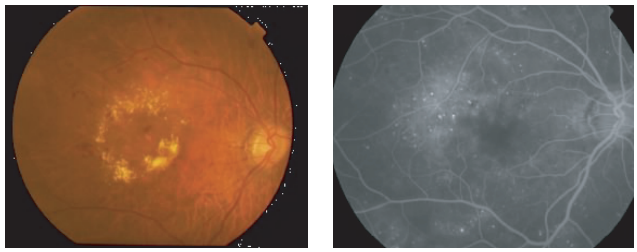
The retinal vessels have two barriers: the retinal pigment epithelium and the retinal vascular endothelium. Each barrier exhibits increased permeability under various pathological conditions. This condition is referred to as blood retinal barrier (BRB) breakdown. Clinically, the most frequently encountered condition causing BRB breakdown is diabetic retinopathy. In recent studies, inflammation has been linked to BRB breakdown and vascular leakage in diabetic retinopathy. Biological support for the role of inflammation in early diabetes is the adhesion of leukocytes to the retinal vasculature (leukostasis) observed in diabetic retinopathy. PPARγ is a member of a ligand-activated nuclear receptor superfamily and plays a critical role in a variety of biological processes, including adipogenesis, glucose metabolism, angiogenesis, and inflammation. There is now strong experimental evidence to support the theory that PPARγ inhibits diabetes-induced retinal leukostasis and leakage, playing an important role in the pathogenesis of diabetic retinopathy. Therapeutic targeting of PPARγ may be beneficial to diabetic retinopathy.

1. BLOOD RETINAL BARRIER (BRB) BREAKDOWN IN DIABETIC RETINOPATHY

The retinal vessels have a barrier consisting of the tight junction of the retinal pigment epithelium and the retinal vascular endothelium. Each barrier exhibits increased permeability under various pathological conditions. This condition is referred to as blood retinal barrier (BRB) breakdown. Clinically, the most frequently encountered condition that induces vascular permeability is diabetic retinopathy [1]. BRB breakdown causes retinal edema. Clinically, the retinal edema often affects macula, the highly sensitive area of the central retina, and often severely affects vision (Figure 1). The frequency of diabetic macular edema ranges from 2% to 13.3% of all diabetic patients, and 6.7% to 62% of insulin-dependent diabetic patients, and its incidence is 1.3% to 5.1% over a four-year observation period [2]. Due to the enhanced retinal vascular permeability, endothelial cell damage and capillary nonperfusion are aggravated. Much effort has been directed toward establishing effective treatments, and recent clinical studies have found that laser photocoagulation, pars plana vitrectomy, and antivascular endothelial growth factor (VEGF) therapy might be effective in ameliorating macular edema [3–6], but the treatment efficacy is limited and the results of the preliminary clinical investigation will have to be confirmed by further studies.

2. THE ROLE OF INFLAMMATION IN BRB BREAKDOWN

In recent studies, inflammation has been linked to vascular leakage in diabetic retinopathy [7]. Biological support for the role of inflammation in early diabetes is the adhesion of leukocytes to the retinal vasculature (leukostasis) observed in both experimental diabetic retinopathy in rats and in human diabetic retinopathy [8, 9]. Increased adhesion of leukocytes to the retinal vasculature is considered to promote vascular leakage. Thus, leukostasis is considered to be a critical event in the pathogenesis of diabetic retinopathy. Clinical investigations have demonstrated that the vitreous level of VEGF protein is higher in patients with diabetic macular edema than in patients with other conditions [10]. Ample evidence suggests that the adhesion of leukocytes to the retinal capillaries is controlled by vascular endothelial growth factor (VEGF), and focal adhesion molecules such as the intercellular adhesion molecule



(a) (b)

Figure 1: Macular edema in diabetic retinopathy. (a) Macular edema in diabetic retinopathy. (b) Increased vascular permeability is observed by fluorescin angiography. Note the leakage of the fluorescent dye showing the blood retinal barrier breakdown. Although the retinopathy is mild, this patient has a visual acuity of 20/200 due to severe macular edema.

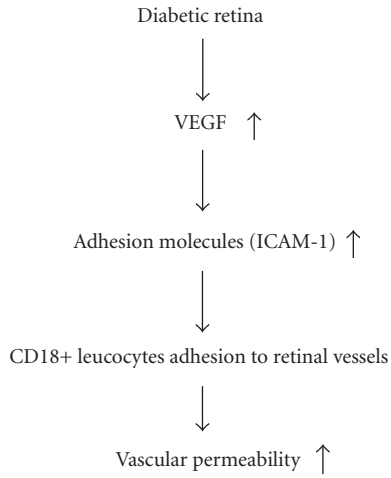


Figure 2: Schematic representation of the molecular mechanism of macular edema. VEGF drives the expression of ICAM-1 in the retinal vessels, which subsequently makes CD18+ leukocytes adherent to the retinal vessels. Adhesion of leukocytes to the retinal vessels leads to increased vascular leakage, subsequent endothelial cell damage, and capillary nonperfusion.

1 (ICAM1) [11]. It is a commonly accepted molecular mechanism of leukocyte adhesion that VEGF drives the upregulation of the ICAM-1 molecule in the retinal endothelial cells [12, 13], and that this upregulated ICAM-1, together with upregulated leukocyte integrin CD18, triggers adhesion of leukocytes to the retinal vessels [14]. Indeed, CD18(−/−) and ICAM-1 (−/−) mice demonstrate significantly fewer adherent leukocytes in the retinal vasculature after the induction of diabetes with streptozotocin (STZ) [15]. It is, however, not only VEGF but also several other molecules that are involved in the expression of ICAM-1. NF-κB molecules, activated by inflammation, also drive ICAM-1 expression [16]. Furthermore, blockage of the bioactivity of VEGF or ICAM-1 or inhibition of inflammatory pathways leads to decreased retinal leukocyte adhesion and reduced vascular leakage [17]. Thus, it is generally assumed that the upregulation of the adhesion molecule, triggered by VEGF and other inflammatory stimuli, is important in the leukostasis (Figure 2).

3. PPARγ AND INFLAMMATION

PPARγ is a member of a ligand-activated nuclear receptor superfamily and plays a critical role in a variety of biological processes, including adipogenesis, glucose metabolism, angiogenesis, and inflammation [18]. Synthetic ligands of PPARγ, that is, thiazolidine derivatives such as rosiglitazone and pioglitazone, are used as oral antihyperglycemic agents for the therapy of non-insulin-dependent diabetes mellitus. In addition, recent studies have shown that PPARγ ligands modulate the production of inflammatory mediators [19]. Actually, it has been reported that PPARγ ligands, such as rosiglitazone and pioglitazone, suppress inflammatory diseases such as adjuvant-induced arthritis [19]. Importantly, some evidence suggests that PPARγ is involved in the regulation of adhesion molecules. Previously, it has been demonstrated that PPARγ ligand suppressed ICAM-1 expression in a murine model of intestinal ischemia-reperfusion injury [20] and in human umbilical vein endothelial cells in vitro [21]. Some of these anti-inflammatory functions are mediated through the inhibition of NF-κB activation (Figure 3). Considering the close link between inflammation and diabetes, it is rational to consider that PPARγ ligand therapy may also improve diabetic retinopathy.

4. PPARγ IN BRB BREAKDOWN

We investigated the effects of a synthetic PPARγ ligand, rosiglitazone, on an experimental diabetic model [22]. Additionally, heterozygous PPARγ-deficient (+/-) mice were used in an experimental model to determine whether endogenous PPARγ played a role [22]. Experimental diabetes was induced by intraperitoneal injection of STZ. This model is considered to destroy pancreatic beta cells completely [22] Retinal leukostasis quantification was performed by counting the number of adherent leukocytes after fluorescein-isothiocyanide (FITC)-Concanavalin A lectin (Con A) perfusion. A retinal leakage assay was performed by evaluating the retinal concentration of FITC-dextran after the animals were perfused. The results showed the PPARγ agonist, rosiglitazone, inhibited both the retinal leukostasis and retinal leakage observed in the experimental diabetic rats and that the decreased expression of the endogenous PPARγ in mice leads to the aggravation of retinal leukostasis and retinal leakage in diabetic mice. Together, these findings support the theory that the PPARγ signaling pathway inhibits diabetes-induced retinal leukostasis and leakage. In addition, it was demonstrated that PPARγ ligand suppresses ICAM-1 expression, but not VEGF expression, raising the possibility that NF-κB mediated ICAM-1 is suppressed by PPARγ ligand (Figure 4).

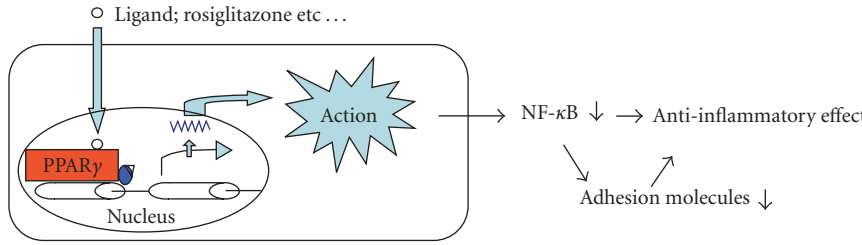


Figure 3: PPARγ exerts anti-inflammatory effects. Schematic representation showing molecular pathways mediating the anti-inflammatory effects of PPARγ ligands.

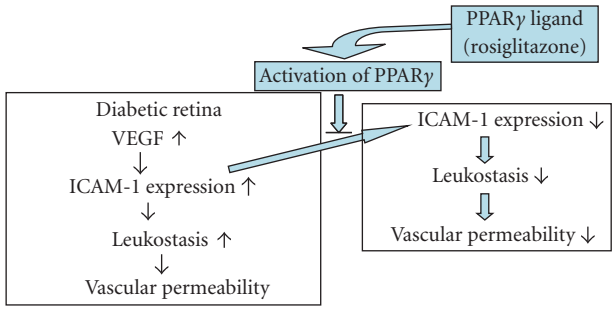


Figure 4: Involvement of PPARγ ligand and its receptor system in retinal leukostasis and vacular permeability. Schematic representation showing the role of PPARγ system in the retinal leukostasis and vascular permeability in diabetic retinopathy.

These results provide strong evidence to support the theory that PPARγ activity plays an important role in the pathogenesis of diabetic retinopathy and introduce the novel possibility that the therapeutic targeting of PPARγ may be beneficial to diabetic retinopathy.

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Review Article

Regulation of Lymphocyte Function by PPARγ: Relevance to Thyroid Eye Disease-Related Inflammation

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Thyroid eye disease (TED) is an autoimmune condition in which intense inflammation leads to orbital tissue remodeling, including the accumulation of extracellular macromolecules and fat. Disease progression depends upon interactions between lymphocytes and orbital fibroblasts. These cells engage in a cycle of reciprocal activation which produces the tissue characteristics of TED. Peroxisome proliferator-activated receptor-γ (PPARγ) may play divergent roles in this process, both attenuating and promoting disease progression. PPARγ has anti-inflammatory activity, suggesting that it could interrupt intercellular communication. However, PPARγ activation is also critical to adipogenesis, making it a potential culprit in the pathological fat accumulation associated with TED. This review explores the role of PPARγ in TED, as it pertains to crosstalk between lymphocytes and fibroblasts and the development of therapeutics targeting cell-cell interactions mediated through this signaling pathway.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily that bind to DNA as heterodimers formed with retinoid X receptors (RXRs) [1].These heterodimers control gene expression by binding to a specific *cis* acting DNA element known as the peroxisome proliferator response element (PPRE) found in the promoter or enhancer regions of target genes. PPRE binding can occur in the presence or absence of ligand and can either induce or repress gene transcription in a cell-specific manner. The ability of PPAR-RXR heterodimers to transactivate genes results not only from their binding to DNA, but also from their association with transcriptional coactivators or corepressors. Usually, agonist binding to these receptors inhibits corepressor and promotes coactivator binding, resulting in increased transcription of target genes.

Three PPAR subtypes, PPARα (NR1C1), PPARβ/δ (NUC1, NR1C2), and PPARγ (NR1C3), are encoded by separate genes [2]. Three isoforms of PPARγ, PPARγ1, PPARγ2, and PPARγ3 are generated by alternative splicing of the same mRNA [3]. PPARs are differentially expressed in a variety of tissues and are important to the regulation of lipid and carbohydrate metabolism, energy homeostasis, cellular differentiation, apoptosis, and immunity and inflammatory responses [2, 4–6]. The physiological functions of PPARα and PPARγ have been well characterized, whereas the physiological function of PPARβ/δ is poorly understood although the protein is widely distributed [3]. PPARα is expressed in brown adipose tissue, liver, kidney, heart, and skeletal muscle, but is also detected in cells of the vasculature and the

immune system [1, 3, 7–10]. Its activation affects transcriptional expression of many genes involved in fatty acid oxidation, lipid metabolism, and inflammation [8, 11]. PPARα agonists (including the fibrates) have been reported to increase levels of high-density lipoproteins (HDL), lower those of triglycerides and decrease weight gain [12, 13]. They also induce adipogenesis in fibroblasts in vitro through the induction of genes such as high-mobility group AT-hook 2 (*HMGA2*) and leptin [8, 14–18].

PPARγ is highly expressed in adipose tissue, colon, retina, and in cells of the immune system, including platelets [1, 3–5, 19–25]. The PPARγ1 isoform is the more widely expressed, while PPARγ2 is mainly found in adipose tissue and liver [3, 26]. PPARγ3 mRNA is detectable in mouse macrophages, but little is known about the protein expression and functional significance of this isoform [3, 27]. Synthetic PPARγ agonists, including drugs of the thiazolidinedione (TZD) family (e.g., ciglitazone, pioglitazone, rosiglitazone and troglitazone), have potent insulin-sensitizing properties [3, 28, 29]. Because of this, rosiglitazone and pioglitazone are often prescribed for the treatment of type 2 diabetes mellitus [3]. These and naturally occurring PPARγ ligands, such as lysophosphatidic acid [30], nitrolinoleic acid [31], prostaglandin D_2(PGD_2), and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2(15d-PGJ_2) [32, 33], are also potent promoters of adipogenesis [3, 28, 34–37]. PGD_2 and 15d-PGJ_2 are derived from arachidonic acid by the catalytic activities of the cyclooxygenase-2 (Cox-2) and prostaglandin D synthase enzymes [28, 32, 33]. PGD_2 spontaneously undergoes a series of dehydration reactions to form the PGJ family of prostaglandins, including 15d-PGJ_2, and 15d-PGD_2, which can also transactivate PPARγ and induce adipogenesis [28, 38–41]. Many of the genes under PPARγ control are important to glucose uptake, lipid metabolism and storage, as well as adipogenesis, explaining the ability of PPARγ ligands to increase insulin sensitivity and to trigger the differentiation of fibroblasts to adipocytes [8, 42–44]. Others act to dampen inflammation by decreasing TNFα, IL-6, and IL-8 production, suggesting potential therapeutic applications in chronic inflammatory diseases [45]. It has been suggested that the adipogenic action of PPARγ could serve as another of its anti-inflammatory functions because remodeling of inflamed tissue to fat may render it more quiescent [28]. Others would argue that adipogenesis is a proinflammatory action because an increase in fat mass would result in increased release of proinflammatory adipocytokines [36]. In any case, increased adipogenesis may lead to disease, even if it serves to attenuate active inflammation. Thyroid eye disease (TED) provides a cogent example of such a circumstance. This review will explore the role that PPARγ and lymphocytes play in advancing pathological tissue remodeling in TED and how PPARγ may be exploited as a target for therapeutic strategies.

2. THYROID EYE DISEASE

TED is a condition in which intense inflammation leads to remodeling and expansion of the connective and adipose tissues of the orbit, including proliferation and differentiation of fibroblasts to adipocytes, fat deposition, and disordered accumulation of extracellular matrix glycosaminoglycans (GAGs) [8, 46, 47]. Accumulation of GAGs is accompanied by dramatic swelling due to their prodigious water-binding capacity [48, 49]. The increased volume of orbital connective tissue leads to forward protrusion of the eye (exophthalmos), accompanied by nerve and muscle damage [28, 50–56]. In patients with severe TED, the initial inflammation subsides, but infiltration of muscle fibers by fibroblasts leads to fibrosis, potentially limiting their motility [46, 47, 50–52]. In addition to exophthalmos and extraocular muscle dysfunction, clinical features of TED include periorbital edema, eyelid retraction, dry eye, pain, optic neuropathy, double vision, and vision loss [28, 50, 53, 57].

TED is closely associated with Graves' disease (GD), a common autoimmune disorder in which stimulatory autoantibodies against the thyroid-stimulating hormone receptor (TSH-R) cause the thyroid to produce excess thyroid hormone [50, 54, 58, 59]. In addition to the hypermetabolic consequences of hyperthyroidism, clinically apparent TED develops in approximately 50–60% of patients with GD [50, 54–56]. Furthermore, a subset of patients with severe TED develop pretibial dermopathy, a distinctive thickening of the skin, usually occurring on the anterior lower leg [60, 61]. Although the pathogenesis of the hyperthyroid state in GD is relatively well understood, many questions remain regarding the induction and perpetuation of the orbital (and pretibial) disease that develops in some patients. It is likely that the hyperthyroid state does not promote connective tissue accumulation within the orbit. Euthyroid GD patients remain at risk for developing TED [62, 63]. Furthermore, TED does not usually occur in patients with non-Graves' hyperthyroidism [64]. It has been suggested that the orbit is a secondary target of autoimmune attack, involving the same autoantigen (TSH-R), but resulting in consequences distinct from those in the thyroid [50, 58, 65]. However, TSH-R mRNA and protein are expressed widely in many tissues which are unaffected in GD, so the basis for the anatomical restriction of TED remains unclear [50, 66]. Moreover, no convincing evidence currently exists for TSH-R mediating any important biological events in orbital connective tissues.

To date, there are no effective means of preventing the onset of TED or for predicting which GD patients are likely to exhibit extrathyroidal complications. A study by Khoo et al. [67] suggested that the presence of thyroid-stimulating antibodies combined with the absence of antibodies against thyroid peroxidase is a predictor, but other reports contradict these findings [68, 69]. Current treatment options for TED exist, including corticosteroid treatment, external beam radiation, and surgery, but these interventions are aimed only at the consequences of the disease, and they fail to prevent or reverse pathological alteration of orbital tissues [70]. Histological examination of orbital tissue in TED suggests that its development and progression involve interactions between lymphocytes and fibroblasts [28]. Understanding these complex interactions may both lead to the identification of biomarkers predictive of advanced disease and provide effective early treatments. It is thought that autoreactive B lymphocytes initiate the disease state by producing antibodies against self-antigen, such as the TSH-R [58]. Next,

in a poorly understood and likely variable event, autoantibody production results in orbital fibroblast activation [71]. Activated fibroblasts release chemoattractants that recruit T lymphocytes and monocytes to the orbit [28, 37, 50, 72–77]. These bone marrow-derived cells cooperate with the resident fibroblasts and are engaged in a cycle of reciprocal activation which ultimately produces the pathological changes in the orbit characteristic of TED [50].

3. INTERACTIONS BETWEEN LYMPHOCYTES AND FIBROBLASTS

Orbital tissue from patients with TED is infiltrated by T helper type I (Th1) and T helper type 2 (Th2) lymphocytes, B lymphocytes, mast cells, and macrophages [47, 50, 59, 78–82]. It is currently thought that these cells, once recruited to the orbit, generate cytokines which participate in driving tissue reactivity and remodeling. Autoimmune responses, like that found with TED, are governed primarily by the actions of B and T lymphocytes. Lymphocytes are migratory cells that proliferate extensively and develop into activated effector cells when they encounter specific antigen in the proper costimulatory context. Normally, the antigens to which lymphocytes respond are foreign and several tolerance mechanisms act to prevent the development of reactivity to self antigens or autoimmunity [83, 84]; but these tolerance mechanisms sometimes fail and autoimmunity develops. B lymphocytes are key to this phenomenon, as activated autoreactive B lymphocytes produce autoantibodies and are a critical source of support for the function of other immune cells, such as T lymphocytes and fibroblasts [85].

Fibroblasts were once viewed as merely structural bystanders in the cellular microenvironment, producing extracellular matrix components, but otherwise uninvolved in the regulation of tissue homeostasis. Now, it is understood that fibroblasts are a highly interactive cell type, described as "sentinel cells," which are able to detect events that endanger homeostasis, to communicate these dangers to cells of the immune system, and to respond directly to these threats via proliferation and differentiation to effector cells that support tissue integrity [58, 66, 72]. Fibroblasts do not merely respond to immune stimulation, but actively participate in the inflammatory pathway through the synthesis of proinflammatory mediators, including IL-1, IL-6, and IL-8 [28, 73, 74]. They interact with bone marrow-derived cells in the orbit and are key to the pathophysiology of TED [8, 37, 50, 65, 72, 73, 75, 76]. As described earlier, the clinical symptoms of TED result from excess extracellular macromolecular deposition, fibrosis, and fat accumulation in the orbit [48, 57]. Several differences have been identified that distinguish orbital fibroblasts harvested from patients with TED from those derived from normal orbital tissues and nonorbital anatomic sites. Orbital fibroblasts from patients with TED synthesize excess GAGs, including hyaluronan, are unusually proliferative and can differentiate into adipocytes, leading to accumulation of fat [50, 86, 87]. In addition, they do not express IL-1 receptor antagonist at levels found in other fibroblasts. This results in excessively high levels of Cox-2 and PGE_2 in response to proinflammatory cytokines [47, 50, 59, 77, 86, 88–91]. They also display lymphocyte costimulatory molecules such as CD40 [59, 77, 86, 88]. These characteristics suggest that the fibroblast phenotype underlies the selective anatomic distribution of TED-associated inflammation and tissue remodeling [37, 47, 50, 59, 73, 75, 92, 93].

The unique features of orbital fibroblasts provide an environment in which TED might develop, but the disease is characterized also by mononuclear cell infiltration [48, 59, 94]. Substantial data support the concept that infiltrating T lymphocytes interact with fibroblasts, activate them, and result in their proliferation, synthesis of extracellular macromolecules, and differentiation to adipocytes [50, 59]. A summary of this model for the pathogenesis of TED is depicted in Figure 1. It is thought that autoantigen expression by orbital fibroblasts instigates T lymphocyte recruitment to the orbit [48, 95, 96]. The autoantigen may be TSH-R or another protein, such as insulin-like growth factor-1 receptor (IGF-1R) [34, 48, 54, 94–98]. Recruited T lymphocytes stimulate orbital tissue remodeling by initiating fibroblast proliferation and hyaluronan synthesis [50]. They also contribute to the perpetuation of the inflammatory response by (1) stimulating fibroblast production of chemokines, like IL-16 and RANTES, and cytokines, like IL-6, that initiate T and B lymphocyte migration to local environments, and (2) increasing fibroblast presentation of autoantigens [50, 73, 74, 76, 77, 99]. The T lymphocyte-fibroblast interaction occurs via costimulatory molecules, adhesion molecules, and cytokines like IFNγ, IL-1β, and TNFα [50, 99]. One mechanism by which T lymphocytes may communicate with orbital fibroblasts is through the CD40-CD40 ligand pathway [50, 74, 88]. CD40 is a cell surface receptor found on antigen-presenting cells, whereas CD40 ligand (CD40L, CD154) is expressed on T lymphocytes [50]. Ligation of CD40 on B lymphocytes or other antigen-presenting cells is necessary for efficient activation of T-lymphocyte effector functions [100, 101]. Recently, it has been shown that orbital fibroblasts from TED patients express high levels of CD40, which is upregulated in the presence of IFNγ, produced by infiltrating T lymphocytes [74, 76, 77, 99]. Activation by CD40L induces hyaluronan synthesis, IL-6 and IL-8, Cox-2 and PGE_2 [50, 74, 86, 102]. Thus, the CD40-CD40L bridge is one potential pathway through which T lymphocytes could influence fibroblast activation and proliferation in TED [50].

Fibroblasts respond to T lymphocyte-mediated activation by releasing factors that recruit, activate, and promote the proliferation of T lymphocytes, thus participating in the perpetuation of inflammation [35, 50, 103]. In patients with clinically significant TED, even in those whose hyperthyroidism is well controlled, B and T lymphocytes have been shown to display a distinctly activated phenotype different from those derived from control donors [59]. This sustained activation following treatment of hyperthyroidism contributes to orbital inflammation and tissue remodeling observed in late-stage TED. A recent study found that orbital fibroblasts from TED patients may modulate the activity of T lymphocytes through the production of CXCL10 [35]. TED patients with active disease had higher serum CXCL10 levels than patients with inactive disease. CXCL10 release enhances

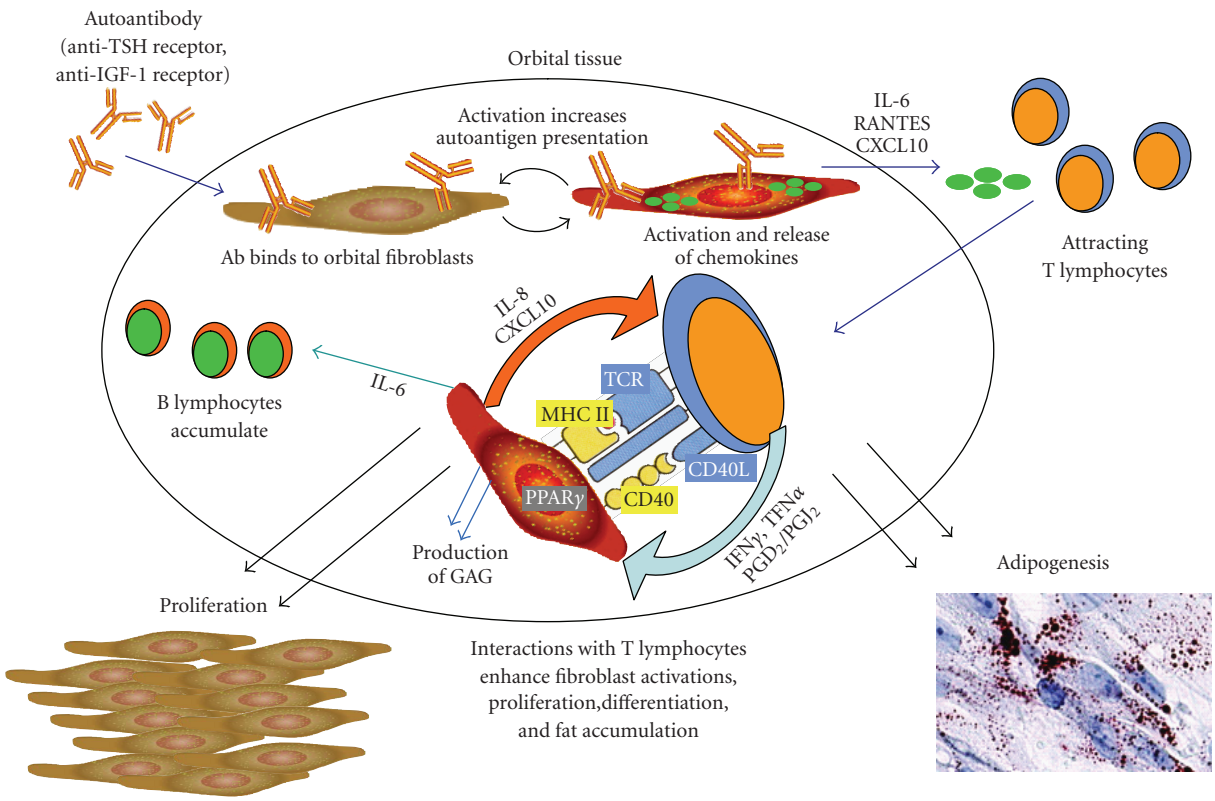

Figure 1: According to one current model, TED is triggered by binding and activation of orbital fibroblasts by autoantibodies. These autoantibodies could be specific for antigens such as TSH-R and/or IGF-1R. Activated orbital fibroblasts release chemokines, including IL-16, RANTES, and CXCL10, which recruit T lymphocytes into the orbit. These lymphocytes then interact with fibroblasts, potentially activating each other, further promoting cytokine production (IFNγ, TNFα, PGD_2, and 15d-PGJ_2) and secretion of T cell-activating factors by the fibroblasts (IL-8 and CXCL10). Fibroblasts are also stimulated to secrete IL-6 (promoting B cell differentiation) and to increase autoantigen presentation, both of which amplify the overall response. The interactions of fibroblasts with T cells result in the deposition of extracellular matrix molecules, fibroblast proliferation, and fat accumulation.

the migration of T lymphocytes into the orbit, where they secrete IFNγ and TNFα. IFNγ levels were higher in TED patients than in patients with GD without orbital involvement. IFNγ and TNFα synergistically induced CXCL10 release by orbital fibroblasts, thereby perpetuating a positive feedback loop [35, 50, 103]. PPARγ activation was found to play an inhibitory role in this process, both in vivo and in vitro [35].

4. PPARγ LIGANDS AND INFLAMMATION

PPARγ ligands attenuate activity of inflammatory bowel disease in animal models [35, 104–106], experimental autoimmune encephalomyelitis [107, 108], arthritis [21], and psoriasis [109]. Clinical trials have shown that they ameliorate inflammation in patients with mild-to-moderate cases of ulcerative colitis [1, 110, 111]. At least some of the anti-inflammatory effects of PPARγ ligands result from direct actions on cells of the innate and adaptive immune system [23, 112–114]. In macrophages, they inhibit activation and production of inflammatory cytokines such as TNFα, IL-1β, and IL-6 [25, 115, 116]. In addition, PPARγ activation has been shown to skew macrophage differentiation into a more anti-inflammatory phenotype [117]. In dendritic cells, PPARγ agonists downregulate the synthesis of chemokines involved in the recruitment of T lymphocytes [35, 118].

Evidence for a physiological role of PPARγ in regulating B lymphocyte function was generated in studies using PPARγ-haploinsufficient mice [21]. B lymphocytes derived from these mice exhibit increased proliferation and survival, enhanced antigen specific immune response, and spontaneous NF-κB activation [1, 21]. Our laboratory has shown that normal and malignant mouse and human B lymphocytes express PPARγ and that exposure to certain PPARγ ligands inhibits their proliferation and can induce apoptosis [24, 113, 119]. Several anti-inflammatory mechanisms of PPARγ have been suggested, including inhibition of NF-κB, AP1 and STAT transcription factors [120, 121]. A recent study demonstrated that some of these effects are PPARγ-independent [122]. PPARγ also regulates inflammation by blocking gene transcription through "transrepression." Several models of transrepression by PPARγ have been proposed. In one of them, PPARγ-RXR complexes are thought to sequester coactivators, thereby downregulating other transcription factors. A second model suggests that interactions between transcription factors result in mutual antagonism of gene activation [123]. A recent report by Pascual et al.

demonstrated a PPARγ ligand-dependent sumoylation of PPARγ that leads to its recruitment to repressor complexes in the promoter regions of inflammatory genes regulated by NF-κB. This prevents their release and suppresses proinflammatory gene expression [124].

PPARγ also plays a role in T lymphocyte regulation, and its level is upregulated following activation [5, 125]. PPARγ ligands inhibit T lymphocyte proliferation and reduce the production of IFNγ, TNFα, and IL-2 [23, 126, 127]. These inhibitory effects result from the direct interaction between PPARγ and the transcription factor nuclear factor of activated T cells (NFAT) [128]. Recent observations reported by Wohlfert et al. could illuminate yet another mechanism through which PPARγ controls immune responses [129]. They investigated the connection between PPARγ and $CD4^+$ $CD25^+$ regulatory T lymphocytes (Tregs). Tregs have been demonstrated to play a key role in regulating autoimmunity and immune responses [130–132]. There are two different subtypes of Tregs: thymus-derived natural Tregs (nTregs) and inducible or adaptive Tregs (iTregs). nTregs are always present in normal individuals as a functionally mature population constitutively expressing CD25, while iTregs are $CD4^+$ $CD25^+$ T lymphocytes which differentiate from $CD4^+$ $CD25^-$ effector T lymphocytes in the periphery under a specific cytokine stimulation [133, 134]. Wohlfert et al. showed that ciglitazone enhanced the conversion of effector T lymphocytes into iTregs. Moreover, PPARγ expression in nTregs was required for the in vivo effects of ligand treatment in a murine model of graft-versus-host disease. These findings suggest that PPARγ ligands may enhance the activity of regulatory T lymphocytes while dampening the activation of other T lymphocyte subsets. The anti-inflammatory potential of PPARγ may be relevant to TED because this transcription factor is present in orbital tissues from TED patients, its activity may be involved in the regulation of IFNγ-induced chemokine expression, and its activators might attenuate the recruitment of activated T lymphocytes to sites of inflammation [35, 106, 118, 135, 136]. Together, the evidence indicates that PPARγ ligands could interrupt communication between mononuclear cells and fibroblasts [1, 35, 50]. However, PPARγ ligands may also promote T lymphocyte synthesis of IL-8 [137, 138]. Thus, the effects of PPARγ on T lymphocytes are complex and require further study.

End-stage TED can culminate with permanent pathological changes including the differentiation of fibroblasts to adipocytes that contribute to increased connective tissue volume [28]. Adipogenesis is regulated by the interplay of several factors, including PPARα and γ [8, 28, 42, 139]. Natural and synthetic activators of PPARγ are known to stimulate lipid accumulation and the expression and secretion of adiponectin [28, 34, 139, 140]. PPARγ antagonists prevent triglyceride accumulation in orbital fibroblasts exposed to PPARγ agonists. This supports the concept that PPARγ expression and activation are crucial for adipocytic differentiation [28, 35, 36]. PPARγ levels are higher in orbital tissue from patients with active TED than in controls or individuals with inactive TED [35, 135]. Responses of orbital fibroblasts to PPARγ ligands provide an interesting link to T lymphocyte activity. T lymphocytes from patients with GD express constitutively high levels of Cox-2, and produce substantial PGD_2 and 15d-PGJ_2 [28, 141]. We have developed the model depicted in Figure 2, in which T lymphocyte infiltration of the orbit results in adipocytic differentiation of fibroblasts [28, 142]. In fact, coculture of orbital fibroblasts from TED patients with activated T lymphocytes results in cytoplasmic accumulation of lipid droplets in fibroblasts [28].

5. PPARγ AND TISSUE REMODELING

Adipogenesis has been suggested to be a mechanism for stanching chronic inflammation [28]. Alternatively, this process may promote further inflammation by increasing proinflammatory adipocytokine production [36]. Orbital adipocytes express immunoreactive and functional TSH-R [8, 34, 54, 87, 95, 97, 98]. Positive correlation between TSH-R, PPARγ, and other adipocytic differentiation markers has been observed in tissues from TED patients [34]. Upregulation of an autoantigen on the surface of orbital fibroblasts could enhance the recruitment of autoreactive T lymphocytes to the orbit, fueling inflammation [36, 55]. Whether adipogenesis serves to abate or amplify inflammation, the associated increase in orbital tissue mass is undesirable. Thus, despite anti-inflammatory actions of PPARγ, its proadipogenic functions in the orbit might worsen the disease, contraindicating the use of agents activating this pathway in TED [36]. Several case reports have described development of exophthalmos in patients receiving TZD treatment for type 2 diabetes [28, 36, 143]. In particular, a patient with stable and inactive TED experienced aggravated disease with orbital fat expansion following pioglitazone therapy [28, 35, 36].

6. PPARγ AS A THERAPEUTIC TARGET

PPARγ modulators with selective activities would be required if PPARγ function is to be targeted as a TED therapeutic. Identification of selective PPARγ modulators, or SPPARγMs, has been sought as a better therapy for type 2 diabetes [3, 144]. In this context, designing partial PPARγ agonists that display insulin-sensitizing activity but lack adipogenic properties might be attractive [3, 144, 145]. The SPPARγMs take advantage of both the large ligand-binding domain of PPARγ and the complex interactions between PPARγ and its coactivators and corepressors [1, 3, 144, 146]. The ligand binding domain mediates interactions with transcriptional coactivator or corepressor proteins through ligand-dependent conformational changes in the C-terminal activation function 2 (AF2) α-helix [1, 144, 146]. In the absence of ligand, PPARγ functions as an active transcriptional repressor by binding both target genes and transcriptional corepressors [1]. Binding of classical ligands causes the AF2 α-helix to move in such a way that a high-affinity binding site for nuclear receptor coactivator proteins is created while corepressor proteins are dislodged from their binding sites [1, 144, 146–149]. Therefore, the structural change in AF2 resulting from agonist binding serves to both inhibit corepressor interaction and promote coactivator recruitment

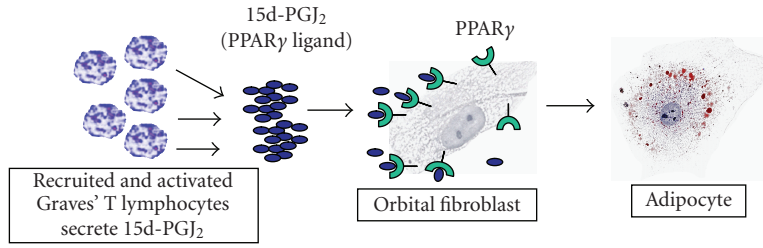

Figure 2: T lymphocytes in TED patients express constitutively elevated levels of Cox-2, one enzyme critical to the production of the naturally-occurring PPARγ ligand 15d-PGJ$_2$. When these lymphocytes infiltrate the orbit, 15d-PGJ$_2$ is secreted in resident fibroblasts result in their differentiation into adipocytes.

[1]. Because the position of the AF2 domain relative to the ligand binding domain determines whether coactivators or corepressors are recruited, ligands that fit into the binding domain without directly interacting with the AF2 helix, such as SPPARγMs, can act as agonists for some receptor functions and as antagonists for others [1, 3, 144, 145, 150–153].

Although not yet clinically available, several SPPARγMs have shown promise as potential glucose-lowering agents in type 2 diabetes. For example, metaglidasen has been shown *in vitro* to act as a partial PPARγ agonist/antagonist, with only a weak ability to recruit coactivators, such as CBP, DRIP205/TRAP220, and p300 [144]. Compared to rosiglitazone, metaglidasen is less adipogenic in primary human adipocytes and mouse 3T3-L1 adipocytes. In rodent models of insulin resistance, both metaglidasen and another SPPARγM, PAT5A, increased insulin sensitivity to levels comparable to those seen with rosiglitazone, with only weak adipogenic potential [3, 144, 154]. Consistent with the preclinical findings, metaglidasen appears to have comparable efficacy to pioglitazone and rosiglitazone in type 2 diabetics, without the undesirable side effect of weight gain [144]. Since developing SPPARγMs to target insulin resistance seems achievable, it is anticipated that the anti-inflammatory properties of PPARγ will be targeted in the future [3].

7. FUTURE PROSPECTS

PPARγ may play an important role in the development of TED. Studies have taken advantage of the availability of orbital tissue from TED patients. Orbital tissues from patients with GD but without TED are far less available. Potential differences between orbital tissues from "normal" and TED patients have not been fully explored. Similarly, few comparisons between tissues from early and late stage TED patients have been possible. Thus, an animal model of TED with fidelity to human disease is critical.

T lymphocytes and fibroblasts exist as multiple phenotypic subsets in the orbit. Aniszewski et al. [82] found that the phenotypes of orbital T lymphocytes in TED patients changed with disease duration. From that report, the T helper lymphocyte Th1subset may predominate early, while Th2 lymphocytes may become more abundant later. Furthermore, as discussed previously, the role of Tregs in TED may differ from that of Th1 and Th2 lymphocytes. Studies comparing PPARγ expression and function in each of these subpopulations may lead to better understanding of the role that this transcription factor plays in TED.

Like T lymphocytes, orbital fibroblasts exist in multiple subpopulations. Two major subsets of orbital fibroblast are defined based on their expression of a surface protein known as Thy-1 (CD90) whose function is unknown [37, 73, 155, 156]. The balance between Thy-1 negative and Thy-1 positive populations in the orbit may prove important to normal regulation of inflammation because these subsets exhibit distinct biosynthetic capabilities [73]. However, this balance may also be critical to the development and progression of TED. Depending on the signaling environment and their phenotype, fibroblasts can be stimulated to differentiate into myofibroblasts or lipofibroblasts [37, 157]. Myofibroblasts are important in wound healing, but they may also contribute to fibrosis in late-stage TED patients [158]. The presence of lipofibroblasts is an indication of pathology; in TED, their presence may result in excess orbital fat deposition [28]. Data suggest that the potential for terminal differentiation depends on Thy-1 display. TGF-β triggers differentiation of Thy-1$^+$ fibroblasts into myofibroblasts, identified by their expression of α-SMA [157]. Adipocytic differentiation occurs in the Thy-1$^-$ subset [37, 157]. PPARγ expression or function may differ between Thy-1$^+$ and Thy-1$^-$ subsets, explaining their divergent potential for differentiation.

Finally, TED is one of several pathological conditions in which chronic inflammation leads to tissue remodeling and inappropriate fat deposition. Sjögren syndrome, inflammatory bowel disease, nonalcoholic fatty liver disease, and atherosclerosis are examples [159–162]. PPARγ has been shown to play a major role in the regulation of atherogenesis by countering the inflammation-provoking action of platelet adhesion and activation [3]. Because PPARγ has been implicated in these diseases, it may prove an important determinant in diseases such as TED.

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Review Article

Prevention of Oxidative Stress-Induced Retinal Pigment Epithelial Cell Death by the PPARγ Agonist, 15-Deoxy-Delta 12, 14-Prostaglandin J_2

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Cellular oxidative stress plays an important role in retinal pigment epithelial (RPE) cell death during aging and the development of age-related macular degeneration. Early reports indicate that during phagocytosis of rod outer segments, there is an increase of RPE oxidative stress and an upregulation of PPARγ mRNA in these cells. These studies suggest that activation of PPARγ may modulate cellular oxidative stress. This paper presents a brief review of recent studies that investigate RPE oxidative stress under various experimental conditions. This is followed by a detailed review on those reports that examine the protective effect of the natural PPARγ ligand, 15d-PGJ_2, against RPE oxidative stress. This agent can upregulate glutathione and prevent oxidant-induced intracellular reactive oxygen species accumulation, mitochondrial depolarization, and apoptosis. The cytoprotective effect of this agent, however, is not shared by other PPARγ agonists. Nonetheless, this property of 15d-PGJ_2 may be useful in future development of pharmacological tools against retinal diseases caused by oxidative stress.

1. AGE-RELATED MACULAR DEGENERATION: POSSIBLE INVOLVEMENT OF RPE

Age-related macular degeneration (AMD) is the leading cause of legal blindness in individuals 50 years of age or older in the United States and developed countries. AMD can be divided into two major forms as follows: (i) nonneovascular form, also known as "*dry*" or "*nonexudative*" form; as clinical findings of this form include drusen and abnormalities of the retinal pigment epithelium (RPE) and (ii) neovascular form, also known as "*wet*" or "*exudative*" form, which is defined by the appearance of choroidal neovascularization with subsequent subretinal fibrosis or disciform scarring. Patients with drusen larger than 63 μm in diameter (termed "*soft drusen*") have a high risk of developing choroidal neovascularization [1].

There is evidence that pathological alterations of RPE around macula area may be partially responsible for the development of AMD [2, 3]. Clinical abnormalities of RPE in AMD include clumping and atrophy of these cells. RPE is involved in the ingestion of photoreceptor outer segments and the general health of photoreceptors. As a result, pathological changes of RPE can lead to photoreceptor cell death and visual impairment. Study with human cadaver eyes indicates that there is an age-dependent RPE apoptosis as evidenced by TUNEL staining [4]. A separate study further indicates that eye specimens from patients with AMD show statistically more macular RPE apoptosis than those without AMD [5].

2. POSSIBLE ROLES OF OXIDATIVE STRESS IN AMD

Retina is exposed to a combination of sunlight, high concentrations of polyunsaturated fatty acids, and high oxygen environment. It is proposed that reactive oxygen species (such as hydrogen peroxide, superoxide anion, hydroxyl radicals, and singlet oxygen) are constantly generated in this environment. As a result, oxidative stress is believed to have an important role in RPE apoptosis and in the development of AMD [2, 3].

An increase of oxidative stress in RPE is associated with an increase of cellular catalase, metallothionein [6], and glutathione S-transferase [7], which should serve as a protective mechanism to decrease the cytotoxicity caused by H_2O_2 and other reactive oxygen species. This protective mechanism declines with age. For example, a study analyzing metallothionein levels in RPE of macular region showed a significant (68%) decrease in *aged* donors (mean age = 80-year-old) as compared to those from *younger* donors (mean age = 58-year-old) [8]. A separate report also concluded that there was an age-dependent decrease of catalase activity in RPE [9]. These studies suggest that RPE cells in the elderly are more susceptible to oxidative stress-induced damage.

3. STUDIES OF OXIDATIVE STRESS ON RPE: PREVENTION BY PHARMACOLOGICAL AGENTS

Given the observations that RPE might be the prime targets for oxidative stress, a number of studies are conducted to study this issue. A majority of research use direct oxidative agents, such as hydrogen peroxide (H_2O_2) or t-butylhydroperoxide (tBH), to initiate cellular oxidative stress, as further discussed below. Other conditions of experimental oxidative stress include: intense light [10–12], iron [13], and oxidative metabolites that are toxic to cells, such as A2E [14, 15], acrolein [16], and oxysterols [17–19].

By using H_2O_2 or tBH as the direct source of oxidative stress on RPE, a number of studies focus on strategies to build up cellular defense mechanisms against the insult. Several reports explore the importance of cellular antioxidative enzymes, such as catalase [20], glutathione-S-transferase [21, 22], superoxide dismutase [23], and methionine sulfoxide reductase [24]. Growth factors including lens epithelium-derived growth factor [25], keratinocyte growth factor [26], and pigment epithelium-derived factor [27] are also protective against oxidative stress. Other proteins that can enhance RPE antioxidative mechanism against H_2O_2 include bcl-2 [28], alpha B-crystallin [29], melatonin [30], and poly(ADP-ribose) polymerase [31].

In addition to those protein factors discussed above, many investigators seek the use of small-molecule pharmacological agents to prevent RPE damage caused by H_2O_2 or tBH. Examples of these pharmacological agents include: (R)-alpha-lipoic acid [32], 17-beta-estradiol [33], flavonoids [34], and L-carnitine [35]. The endogenous PPARγ ligand, 15-deoxy-delta-12,14-prostaglandin J_2(15d-PGJ_2), is also very effective in preventing RPE oxidative stress, as further discussed below.

4. PREVENTION OF OXIDATIVE STRESS-INDUCED RPE DEATH BY 15d-PGJ_2

15d-PGJ_2, a prostaglandin derivative, is normally present in tissues at low levels (<1 nM), but can reach high concentrations during infection and inflammation [36]. Under in vitro conditions, it can be induced by chemical [37] or physical [38] stress. It has a very potent anti-inflammatory effect [39]. For example, it is a potent inhibitor of macrophage [40–42] and microglia [43–45] activation.

During RPE ingestion of rod outer segments, there is a generation of H_2O_2 [6, 46] and a 10-fold upregulation of PPARγ mRNA [47]. Based on these observations, it is likely that PPARγ is involved in RPE cellular responses toward H_2O_2. One can hypothesize that PPARγ agonists should modulate cellular defense against oxidative stress.

We reported earlier that the PPARγ agonist, 15d-PGJ_2, protected H_2O_2-induced RPE cell death [48]. With primary human RPE cells, pretreatment of cells overnight with 15d-PGJ_2 dose-dependently prevented H_2O_2-induced cytotoxicity, such that the viability raised from ~25% (H_2O_2 only) to ~80% of control. Maximal protection was observed at ~2 μM 15d-PGJ_2. Similar protection was made in the human ARPE-19 cell line. While H_2O_2 caused significant nuclear condensation, a sign of apoptosis; this was largely prevented by 1 μM 15d-PGJ_2 (see Figure 1). However, it should be mentioned that the protective effect by 15d-PGJ_2 was not shared by other PPARγ agonists, such as ciglitazone, azelaoyl PAF, or LY171883. These results raised the possibility that the protective effect by 15d-PGJ_2 was not mediated through PPARγ activation. This idea was supported by other investigators, as further discussed below.

The cytoprotective effect of 15d-PGJ_2 on H_2O_2-treated RPE was further studied by Qin et al. [49]. These investigators confirmed that 1 μM 15d-PGJ_2 effectively prevented H_2O_2-induced cell death. Other PPARγ agonists, such as AGN195037 or Roziglitazone, had no protective effects. Importantly, reduction of PPARγ by siRNA did not block the protective effect of 15d-PGJ_2. This set of experiments together with those described above strongly suggests that 15d-PGJ_2 protect RPE cells through a PPARγ-independent mechanism. Some properties of 15d-PGJ_2 are independent of PPARγ activation, as reviewed by Straus and Glass [39].

Subsequent studies by Qin et al. [49] indicated that 15d-PGJ_2 could upregulate glutamylcyteine synthetase, the rate-limiting enzyme that regulates glutathione (GSH) synthesis. These investigators reported that 15d-PGJ_2 at 1-2 μM induced GSH levels to ~300% of control. With 1 μM 15d-PGJ_2, the maximal induction occurred at 18–24 hours after treatment. This GSH induction appeared to depend on JNK and p38 pathways because inhibitors of these pathways greatly reduced GSH induction by 15d-PGJ_2. Induction of GSH by 15d-PGJ_2 is also observed in other cell types [37, 50, 51]. Since intracellular GSH is very important in cellular defense against oxidative stress, the induction of GSH should have an important role in the protective effect caused by 15d-PGJ_2 treatment. Even though induction of heme oxygenase-1 (HO-1) was associated with cytoprotective effects of 15d-PGJ_2 in other studies [52], this enzyme had no roles in the protection observed in this experimental system.

If 15d-PGJ_2 greatly induced intracellular GSH, one would expect that this agent should reduce oxidant-induced intracellular reactive oxygen species generation. Indeed, we reported earlier that 15d-PGJ_2 could reduce H_2O_2- and tBH-induced reactive oxygen species in human ARPE-19 cells [53]. For example, pretreatment of cells with 1 μM 15d-PGJ_2 reduced 1 mM H_2O_2-generated reactive oxygen species to ~80% of untreated cells challenged with H_2O_2. Similar reduction was observed in cells challenged with tBH.

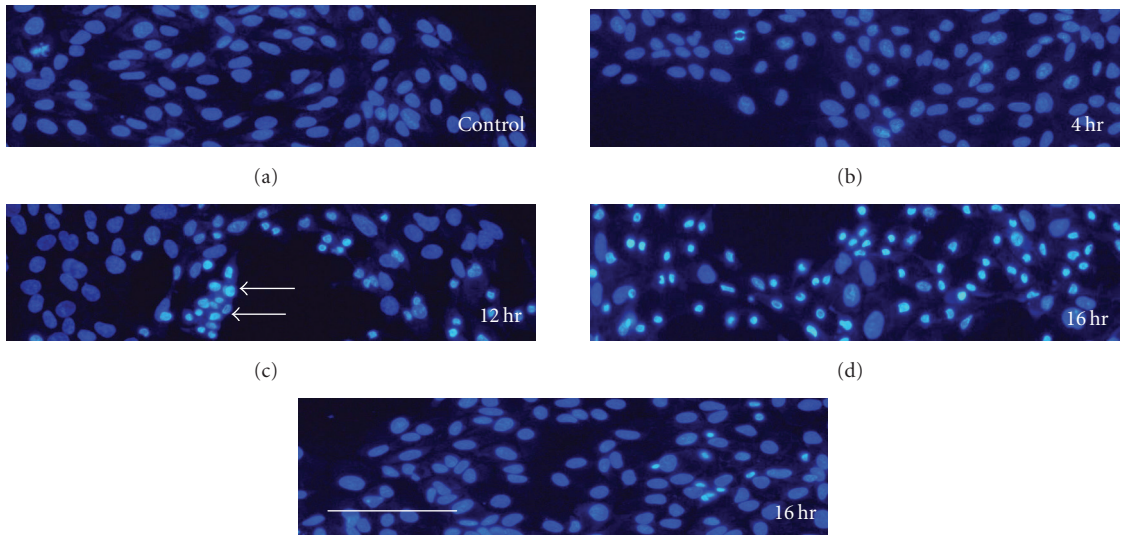

Figure 1: *Prevention of H_2O_2-induced nuclear condensation by 15d-PGJ$_2$.* The human RPE cell line ARPE-19 cells were treated with 1.5 mM H_2O_2 for various periods of time, and then processed for nuclear staining by bisbenzimide (Hoechst 33258) to identify apoptotic cells [48]; (a): untreated cells; (b): 4 hours; (c): 12 hours; (d): 16 hours after treatment. Arrows in (c) point to representative cells with condensed nuclei, an indication of apoptosis. (e): Cells were pretreated with 1 μM 15d-PGJ$_2$ overnight, followed by 1.5 mM H_2O_2 for 16 hours (without 15d-PGJ$_2$). The number of apoptotic cells was greatly reduced by 15d-PGJ$_2$. Scale bar: 100 μm.

This reduction apparently was enough to keep free radical levels under a critical threshold, thus rendering cells survive an otherwise detrimental oxidant insult.

Our study further indicated that 15d-PGJ$_2$ helped RPE cells to maintain mitochondrial integrity [53]. This is significant because mitochondria are intimately involved in apoptosis. Oxidative stress can induce mitochondria dysfunction, which is a critical event that leads to cytochrome c release and subsequent activation of caspases, a group of enzymes that executes apoptosis [54, 55]. An important event associated with mitochondrial dysfunction is a drop of mitochondrial membrane potential ($\Delta\Psi m$), that is, mitochondrial depolarization. This event initiated by oxidative stress was largely prevented by 1 μM 15d-PGJ$_2$ (see Figure 2). This is likely to prevent cytochrome c release and subsequent activation of the apoptosis pathway.

5. CYTOPROTECTIVE VERSUS CYTOTOXIC EFFECTS OF 15d-PGJ$_2$

In addition to those studies described above regarding the protective effect of 15d-PGJ$_2$ against oxidative stress on RPE, this agent is cytoprotective toward other retinal cells. For example, Aoun et al. [56] reported that glutamate could induce oxidative stress and cell death in the rat retinal ganglion cell line, RGC-5 cells. This cell death was prevented by 1–5 μM 15d-PGJ$_2$. Outside of retina, 15d-PGJ$_2$ was effective in preventing glutamate-induced cell death of primary cortical neurons [51]. Both groups attributed the protective effect through the antioxidative property of 15d-PGJ$_2$. In this respect, it should be noted that this agent can also prevent cell death caused by toxic metabolites of oxidative stress. For example, we reported earlier that 15d-PGJ$_2$ prevented cytotoxicity of oxysterols, toxic cholesterol metabolites generated under oxidative stress [57]. The cytoprotective effect of 15d-PGJ$_2$ in other experimental systems were also described in reports by Kawamoto et al. [58] and Itoh et al. [59].

It is clear now that 15d-PGJ$_2$ can induce intracellular oxidative stress [60, 61]. It is likely that this agent at low concentrations (1–5 μM) can cause low levels of oxidative stress, thus inducing the build up of cellular defense mechanisms against oxidative stress. However, at high concentrations, this agent can cause severe oxidative stress and cell death [60, 61]. Induction of apoptosis by this agent was reported in several cell types [62–64]. This interesting bifunctional property of 15d-PGJ$_2$ has been reported [50], and is a subject of review by Na and Surh [65]. This also prompts a recent microarray study analyzing the regulation of prosurvival and prodeath genes by this agent [66].

6. CONCLUDING REMARKS

Oxidative stress is believed to play an important role in RPE cell death during aging and the development of age-related macular degeneration. During phagocytosis of rod outer segments, there is an upregulation of PPARγ in RPE cells. The natural PPARγ ligand 15d-PGJ$_2$ has a potent protective effect for RPE under oxidative stress. This agent can upregulate GSH and prevent oxidant-induced intracellular reactive oxygen species accumulation, mitochondrial depolarization, and apoptosis (see Figure 3). There is also evidence that 15d-PGJ$_2$ can prevent glutamate-induced death of cultured retinal ganglion cells. Current data suggests that this cytoprotection is not mediated through the activation of PPARγ.

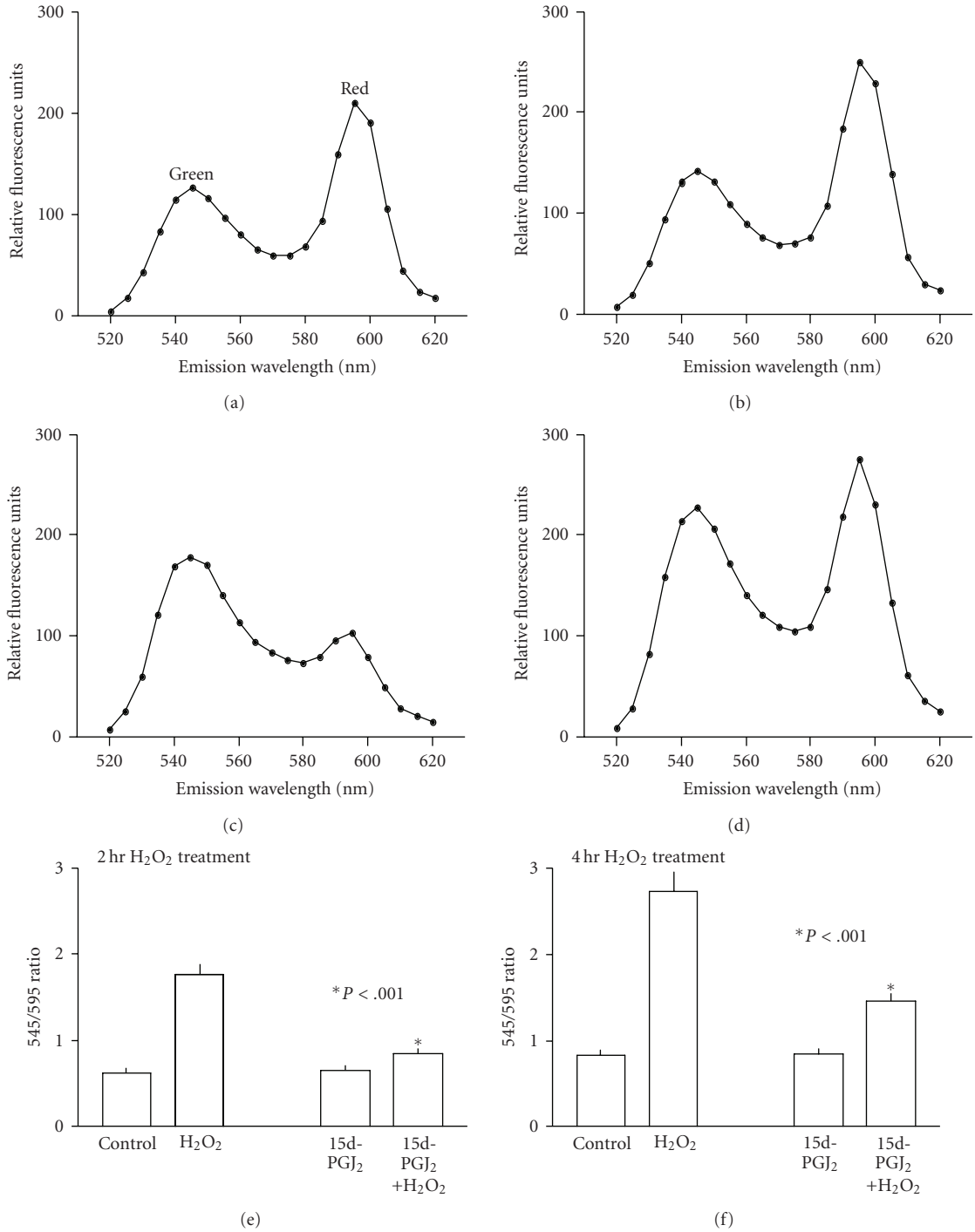

Figure 2: *Prevention of H_2O_2-induced mitochondrial membrane depolarization by 15d-PGJ_2.* Binding of the JC-1 dyes to mitochondria leads to the appearance of two peaks. The green peak (at ~545 nm) represents JC-1 monomers of this dye. The red peak (at ~595 nm) represents JC-1 aggregates, which is caused by the negative charge of mitochondrial membrane. Depolarization of mitochondrial membrane causes a shift in the emission spectrum from red to green color, which can be quantified by a fluorescence plate reader. The relative intensity of these two peaks is a measurement of relative mitochondrial potential such that a higher ratio represents more mitochondrial membrane depolarization. (a)–(d): The JC-1 emission spectra between 520 nm to 620 nm were determined for cells under various conditions [53]; (a): untreated cells; (B): cells treated with 1 μM 15d-PGJ_2 overnight; (c): cells treated with 1.5 mM H_2O_2 for 2 hours; (d): Cells treated with 1 μM 15d-PGJ_2 overnight, then with 1.5 mM H_2O_2 (without 15d-PGJ_2) for 2 hours. Note H_2O_2 caused a shift of the relative intensity of the peaks, and 15d-PGJ_2 pretreatment restored membrane potential to a condition closer to untreated cells. (e)-(f): Cells were pretreated with 1 μM 15d-PGJ_2 overnight, then with 1.5 mM H_2O_2 (without 15d-PGJ_2) for 2 hours (e) or 4 hours (f); then the 545/595 emission intensity ratios were determined. Note in either 2-hour or 4-hour treatment, H_2O_2 caused an increase of the 545/595 emission intensity ratio, indicating mitochondrial depolarization. 15d-PGJ_2 pretreatment restored the ratio to that similar to control value ($P < .001$ between H_2O_2-treated and 15d-PGJ_2+H_2O_2-treated cells in (e) and (f)).

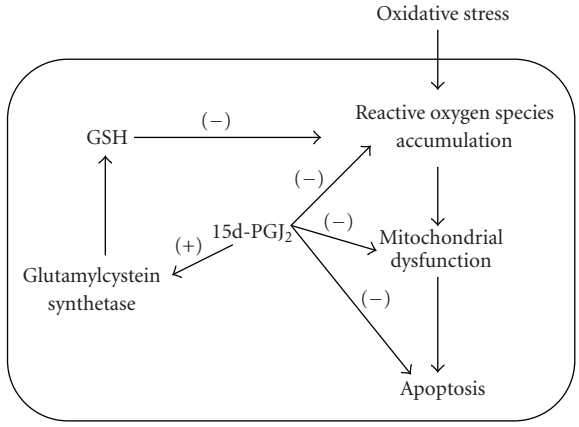

Figure 3: *Protective effects of 15d-PGJ$_2$ against oxidative stress.* Oxidative stress on RPE cells can lead to intracellular accumulation of reactive oxygen species. This can result in mitochondrial dysfunction, which in turn causes activation of the apoptosis pathway. Current data suggests that 15d-PGJ$_2$ can block each of these events. One mechanism that causes this protection is through upregulation of GSH synthesis by activation of the glutamylcystein synthetase. There is a possibility that other cytoprotective mechanisms are also activated that lead to prevention of apoptosis. This remains to be studied.

The antioxidative property of 15d-PGJ$_2$ may be useful in future development of pharmacological tools against retinal diseases caused by oxidative stress.

Finally, based on anti-inflammatory effects of 15d-PGJ$_2$, we would like to speculate that this agent might be effective in the treatment of other ocular diseases such as idiopathic autoimmune anterior uveitis. To confirm our hypothesis, we intend to explore the effect of 15d-PGJ$_2$ on experimental autoimmune anterior uveitis (EAAU) which serves as an animal model of idiopathic human autoimmune anterior uveitis [67, 68].

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