·Review·

Pharmacological therapy in age-related macular degeneration

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Received:2008-07-19 Accepted:2008-08-10

Abstract

• Age-related macular degeneration (AMD) is the leading cause of legal blindness in individuals aged over 65 in the United States and other industrialized nations. Till now, we have limited choices of treatment for this kind of disease. Treatment available can be grouped into two major categories: physical and pharmacological therapies. The former received extensive attention with little success whereas the latter attracted new attention with great hope of success. The pharmacological therapies include photodynamic therapy (PDT), steroids, vascular endothelial growth factor (VEGF) inhibitors, extracellular matrix(ECM) modifiers, gene therapy, nutrition supplements, choroidal blood flow facilitators and the like. PDT treatment is the only available effective treatment for certain forms of neovascular AMD. Anecortave acetate, as a synthetic derivative of cortisol, might stabilize vision in patients with predominantly classic subfoveal choroidal neovascularization (CNV) for up to 6 months through subtenon juxtascleral depot application. Intravitreous injection of VEGF aptamer stabilized or improved vision in 87.5% of patients with subfoveal CNV 3 months after treatment. Malfunction of choroidal blood flow is found in early stage of AMD. Elevation of intravascular pressure is the crucial hemodynamic factor in age-related macular degeneration, resulting in a decrease of the blood flow of choriocapillaries. Chain reactions are triggered which lead to retinal pigment epithelium(RPE) degeneration, Bruch's membrane breakdown,CNV formation,AMD and blindness in the end.Therefore, specific drugs that can increase the choroidal blood flow could be very useful to prevent AMD from developing and worsening.Although most of them are still in the experimental stage,it is hopeful to find a way to treat AMD at the early stage and to prevent the disease to be triggered and developed.

• KEYWORDS: age-related macular degeneration; pharmacological therapy; photodynamic therapy; vascular endothelial growth factor; anecortave acetate; choroidal blood flow

Zou YH, Chiou GCY. Pharmacological therapy in age-related macular degeneration. *Int J Ophthalmol* 2008;1(3):264-272

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of legal blindness in individuals aged over 65 in the United States and other industrial ized nations [1,2]. Till now, the precise etiology is poorly understood despite intensive researches. Thus, we have limited choices of treatment for this kind of disease. Available treatment can be grouped into two major categories: physical and phar macological (chemical) the rapies. The former received extensive attention with little success whereas the latter attracted new attention with great hope of success. The physical therapies include laser photocoagulation, transpupilary thermotherapy, radiotherapy and surgical intervention. The pharmacological therapies include photodynamic therapy, steroids, vascular endothelial growth factor (VEGF) inhibitors, extracellular matrix (ECM) modifiers, gene therapy, nutrition supplements, choroidal blood flow facilitators and the like.

PHYSICAL THERAPY OF AMD

Laser photocoagulation is the earliest, widely tried treatment for AMD and it remains the treatment of choice for 'classic' juxtafoveal and extrafoveal choroidal neovascularization (CNV). However, only less than 1/4 of CNV are eligible for laser photocoagulation treatment according to the Macular Photocoagulation Study criteria and of these at least half persist or recur within 2 years [3-6]. Besides, coagulation necrosis is not tissue-specific and results in collateral damage to the overlying retina.

Transpupillary thermotherapy (TTT) involves the use of a long-pulse, 810nm near-infrared diode laser irradiation. Although the exact mechanism is unknown, near-infrared irradiation is well-suited for the treatment of macular disease because it has high tissue penetration and minimal

ocular media absorption. In addition, it is poorly absorbed by hemoglobin and xanthephyll, allowing transmission through preretinal and subretinal hemorrhage and reducing nerve fiber layer damage. Several retrospective reviews showed TTT might stabilize visual acuity in a majority of patients with occult subfoveal CNV secondary to AMD [7]. A prospective, double-masked, randomized trial is currently under way to directly compare TTT with the natural history of occult CNV[8].

Radiotherapy aims to exploit the potential for ionizing radiation to selectively inhibit proliferating endothelium. But the Radiation Therapy for Age-related Macular Degeneration (RAD) Study Group showed no advantage of radiotherapy over sham treatment at 1 year [9]. While for patients with minimally classic or large 100% occult lesions where there is no other treatment option, it may be a choice[8].

Submacular surgery may offer an approach to evacuate submacular hemorrhage, to excise CNV with relocation of the fovea to an adjacent area of intact retinal pigment epithelium (RPE) or pigment epithelial transplantation. But randomized clinical trials are needed to determine whether it is safe and effective[8].

PHARMACOLOGICAL THERAPY OF AMD

In 1995 the International Age Related Maculopathy Study Group published the international classification and grading system for age-related maculopathy (ARM) and AMD [10]. ARM is a degenerative disorder involving the RPE, choriocapillaries and retina which primarily, but not exclusively, affects the macular region. The clinical hallmark of AMD is the appearance of drusen, localized deposits lying between the retinal pigment epithelium and Bruch's membrane. AMD has been categorized into two forms. The exudative form ('wet' form) characterized by subretinal hemorrhage, detachment of RPE, CNV, or retinal scarring and the 'dry' form which includes geographic atrophy. CNV is responsible for almost 90% of cases of severe visual loss[11].

Nutrition and Medication Supplements The lack of effective treatment modalities, coupled with evidence supporting an oxidative pathogenesis, has increased interest in the potential prevention role of nutrition supplementation [12]. The two major carotenoids in the human macula and retina are lutein and zeaxanthin. Lutein and zeaxanthin are deposited at an up to 5 fold higher content in the macular region of the retina as compared to the peripheral retina. Several functions of these pigments have been hypothesized and these include limitation of the damaging photo-oxidative effects of blue light through its absorption, reduction of the effects of light scatter and chromatic aberration on visual performance, and protection against the adverse effects of photochemical reactions because of the antioxidant properties of the carotenoids. So it has been further hypothesized that dietary supplementation with lutein and/or zeaxanthin might protect the retina and/or delay the progression of AMD [12]. Serum lutein increased rapidly after supplementation in individuals, but macular pigment density increased only after several weeks of supplementation[13-15]. Recently, the evidence of a higher incidence of cancer among cigarette smokers who received beta-carotene supplements in two studies was reported [16, 17].Data from the Age-Related Eye Disease Study (AREDS) suggest that supplements that contain carotenoids,anti-oxidant vitamins A, C, and E, and minerals, such as zinc, showed a 25% decrease in the rate of progression to aggressive AMD among high risk patients [18]. The findings of the Lutein Antioxidant Supplementation Trial (LAST), a prospective, 12-month, randomized, double-masked, placebo-controlled trial, also support a possible therapeutic role of lutein in AMD [19]. However, the controversial evidence also exists. The information available provides an indication that the carotenoids, lutein and zeaxanthin, may play a role in modulating the course of AMD, yet critical evidence of the beneficial effect has not been found, and crucial information for the most effective design of clinical trials is needed.

Antiangiogenesis Treatment Angiogenesis is the development of new capillaries from preexisting network. The growth of CNV in AMD patient is a process of angiogenesis. Although this procedure is integral to embryonic development, somatic growth, and tissue repair, it destroys normal ocular architecture. The schematic diagram of angiogenesis in choroidal neovascularization was shown in Figure 1. A lot of factors are involved in this procedure, such as VEGF, fibroblast growth factor (FGF2), angiopoietin, pigment epithelium-derived factor (PEDF), nitric oxide (NO), extracellular matrix, etc. Therapy aimed at the angiogenic process underlying CNV possesses the unique advantage of addressing the most destructive feature of AMD. Sustained and effective anti-angiogenic therapy would not only halt and reverse CNV, but also allow freedom from recurrences and prevent the development of neovascularization [20]. However, an inherent disadvantage common to all these drugs is the inhibition of wound healing and appropriate

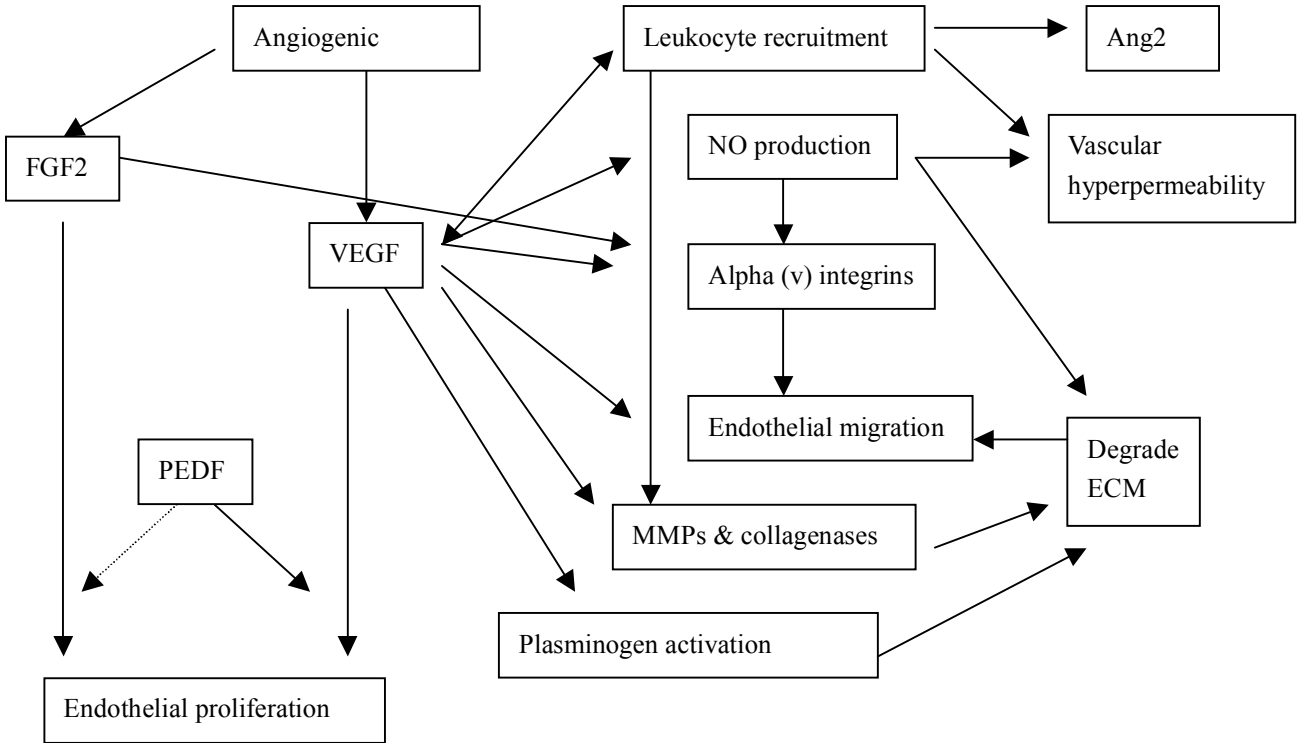

Figure 1 Schematic diagram of angiogenesis in choroidal neovascularization[20]

angiogenesis in situation such as trauma[8].

Photodynamic Therapy Photodynamic therapy (PDT) is the most popular treatment in recent years. Through injection of a photosensitive agent, e.g. visudyne, light with specific wavelength excites the photosensitizer at the CNV area and leads to formation of free radical intermediates. They cause direct endothelial cell damage and secondary platelet adhesion and result in localized vascular thrombosis and occlusion [8]. According to the report of Zacks *et al* [21], 24 hours after PDT treatment on CNV rat model, lesions were defined as closely based on fluorescein angiography (FA) analysis. Vacuolization of the endothelial cells and collapse and closure of the vascular channels were confirmed by histology study [21]. Angiography analysis in AMD patients showed that CNV size in early FA and indocyanine green angiography (ICGA) reached its minimum 1 day after PDT while an immediate massive exudation occurred with a continuous increase in hyperfluorescence originated from the CNV with a maximum in leakage area. At 1 week, PDT induced exudation was slowly resolved. This indicates that in human, occlusion of the CNV lesions occurred 1 day after PDT treatment while a breakdown of vascular barriers was caused initially [22]. Several phase III randomized clinical trials recommended PDT in the treatment of patients with predominant classic subfoveal CNV or purely occult subfoveal AMD lesions that were presumed to have progressed recently [23-25]. Lesion size may be an important predictor of the magnitude of treatment. Treating small rather than large neovascular lesions, likely will result in a better level of visual acuity [26]. Verteporfin therapy in Age-related Macular Degeneration (VAM) Study Group reported a low incidence of adverse events among 4 435 enrolled patients. Totally 6.8% experienced an adverse event associated with treatment, including 2.6% with abnormal or decreased vision, 0.6% experienced acute severe visual acuity decrease, 0.3% with transient infusion-related back pain, and 0.05% photosensitivity reaction despite a 24-hour photosensitivity protection[27]. The main limitation of PDT is the need for multiple treatments with concomitant fluorescein angiograms and the high cost of the photosensitizers. The current recommendation is re-treatment every 3 months until cessation of fluorescein leakage[28]. Till now, PDT treatment is the only available treatment for some forms of neovascular AMD. Under these consumptions, PDT can be considered moderately cost effective for those with reasonable visual acuity[29].

Steroids Steroids broad-spectrum suppression of inflammation often translates into anti-angiogenic activity. Laser-induced CNV in rats was inhibited by systemic delivery of dexamethasone or intravitreous injection of triamcinolone

acetonide[30, 31]. The anti-angiogenic effect of corticosteroids has a dual mechanism. Not only do corticosteroids inhibit inflammation, but they also affect vascular endothelial cell extracellular matrix (ECM) turnover [32, 33]. Similarly, corticosteroids decrease RPE cellular migration and proliferation by effecting a diminished enzymatic degradation of ECM components. While RPE cell proliferation may be a salutary phenomenon in enveloping CNV to prevent subretinal fluid leakage and possibly induce regression[34]. So it is possible that steroids may have an adverse impact on preexisting CNV.

Triamcinolone is a synthetic glucocorticoid. Pre-clinical studies have shown that intravitreal triamcinolone reduces the incidence of experimentally induced subretinal neovascularization in rats and monkeys [31,35]. And a single intravitreous injection of triamcinolone acetonide stabilized vision in patients with subfoveal recurrence of CNV after laser photocoagulation of extrafoveal CNV in an uncontrolled case series [36]. The improvement of acuity and the lack of fluorescein leakage was also found when intravitreal triamcinolone acetonide (iTAAC) injection was used as an adjunctive treatment to photodynamic therapy (PDT) with verteporfin in case series reports[37]. However, 12-month data from the largest RCT to date do not support the suggestion that a single injection of triamcinolone reduces the risk of severe vision loss [38]. And a significant disadvantage is the adverse effects of cataract development and raised intraocular pressure, so further investigation is needed.

Anecortave acetate is a synthetic derivative of cortisol. Its specific and irreversible chemical modifications to the cortisol structure have resulted in the creation of a potent inhibitor of blood vessels growth with no evidence of glucocorticoid receptor-mediated bioactivity. Significant anti-angiogenic activity was observed in several neovascular models (rabbit corneal neovascular models, hypoxic retinal neovascularization in rats, murine uveal melanoma) [39]. A significant reduction in the severity of abnormal retinal neovascularization was observed in the steroid treated eyes compared with vehicle injected eyes in retinopathy of prematurity rats [40]. Clinical trials showed subtenon juxtascleral depot application of anecortave acetate might stabilize vision in patients with predominantly classic subfoveal CNV for up to 6 months. At 12 months, anecortave acetate (15mg) posterior juxtascleral administered at 6-month intervals prevented severe vision loss, and inhibited subfoveal CNV lesion growth [41]. No clinically relevant safety issues were noted related to either anecortave acetate or the administration procedure for up to 4 years when anecortave acetate was administered as a posterior juxtascleral depot every 6 months[42].

VEGF Inhibitors VEGF is an attractive target in anti-CNV therapy because it has a high degree of selectivity to endothelial cells, reciprocal oxygen regulation, diffusable to its target through extracellular secretion, and affecting multiple components of angiogenesis (endothelial cell proliferation, survival, migration) as well as vascular permeability[20]. There is a lot of evidence showing a putative role of VEGF in CNV formation. First, VEGF is overexpressed in the RPE of autopsy eyes with AMD and in transdifferentiated RPE cells of surgically excised CNV membranes [43, 44]. Second, intravitreous injection of VEGF induced proliferation of choroidal endothelial cells in non-human primates [45]. Third, adenovirus transfection of a VEGF gene into the RPE of rats led to development of CNV[46, 47]. So it is not surprising that intravitreous injection of oligonucleotide targeted to the VEGF sequence inhibited laser-induced CNV in rats [20], intravitreous injection of rhu-Fabv2 (the active fragment of a humanized monoclonal antibody to VEGF) inhibited development of laser-induced CNV in cynomolgus monkeys [48]. Intravitreous injection of VEGF aptamer, a synthetic RNA compound specifically designed to bind to extracellular VEGF, stabilized or improved vision in 87.5% of patients with subfoveal CNV 3 months after treatment. No significant safety issues related to the drug were reported [49, 50]. Double-masked random clinical trials are currently under way to investigate the safety and efficacy of 6-week intravitreal injections administrated for 1 year.

However, elimination of VEGF threatens the normal survival of choriocapillaries, which is the trigger of AMD to begin with. Thus, VEGF inhibitors are double-blade swords, which make the control of VEGF level during the treatment of AMD rather difficult.

Extracellular Matrix Modifiers Invasion and migration of endothelial cells through the extracellular matrix during angiogenesis are orchestrated by the integrin family of cell adhesion molecules. They facilitate migration by interacting with adhesion proteins in the extracellular matrix (ECM), such as collagen, fibronectin, fibrinogen, laminin, vitronectin and von Willebrand factor. The process of interacting with adhesion proteins was potentiated by the secretion of matrix metalloproteinases (MMPs), a family of proteolytic enzymes that degrade basement membrane and extracellular matrix proteins, modulated by tissue inhibitors of

metalloproteinases (TIMPs)[20].

Drugs, which can change the construction of ECM or change the balance of MMPs and TIMPs, may have effect on angiogenesis process. The CNV lesions of MMP-2-deficiency mice showed that relative thickness was reduced by 31% compared with wild-type mice after induction with laser treatment [51]. Integrin alpha (v)beta3 is predominantly expressed on endothelial cells in choroidal neovascularization (CNV). Cyclic RGD (Arg-Gly-Asp) peptide is an alpha (v)-integrin antagonist. Cyclic RGD (0.02-200g/L) can inhibit adhesion of bovine choroidal endothelial cells (BCEC) in a dose-dependent manner and intravitreous injection of cyclic RGD inhibited CNV in laser-induced rat model [52]. N-Biphenyl sulfonyl-phenylalanine hydroxamic acid (BPHA) is a synthetic, selective inhibitor of matrix metalloproteinase (MMP)-2, -9, -14. Oral administration of BPHA can reduce experimental laser-induced CNV[53]. The binding of urokinase plasminogen activator (uPA) and its receptor (uPAR) triggers twin cascades of events during cancer research, the first of which is destruction of the extracellular matrix, and the second is intracellular signaling to program gene expression leading to cell migration, cell invasion, metastasis and angiogenesis. Overexpression of uPA/uPAR system has been shown in surgically excised CNV, and in laser-induced CNV. The octapeptide A6 is derived from the non-receptor-binding region of uPA. In a rat model of CNV, with A6 treatment, angiography showed a 37.9% reduction in CNV in 200mg/kg per day and 70.0% in 400mg/kg per day compared with the control. Both CNV thickness and number of endothelial cells were reduced in a dose-dependent manner and significantly less in the control[54].

Gene Therapy Subretinal injection of adenoviral or adeno-associated viral vectors has been used to trans form the RPE into a factory for sustained local delivery of a drug or a gene in experimental models of CNV. Angiostatin (act as a VEGF scavenger), TIMP-3, PEDF has been tested and showed inhibition of development of CNV in animal models [20]. A phase I study of intravitreous injection of an adenovirus encoding PEDF has commenced in patients with neovascular AMD[55], but no data were published so far.

Thalidomide Thalidomide has recaptured interest in oncology due to its potent anti-angiogenic properties. Although it showed inhibition of angiogenesis *in vivo* and *in vitro*, clinical trial of thalidomide in subfoveal CNV was hampered by the high dropout rate of older AMD subjects who were unable to tolerate the side effects. Most critically, no significant anti-angiogenic effect was found even in the small group of tolerant patients[20].

Interferon-alpha Interferon-alpha is an endogenous glycoprotein with immunoregulatory, antiviral, antiproliferative and anti-angiogenic properties. It was proved efficacious *in vivo* and *in vitro* studies on angiogenic disorders. However, the Pharmacological Therapy for Macular Degeneration Study Group reported no statistically significant difference in loss of 3 lines of vision between the placebo group and the active treatment groups in a phase III, double-masked RCT. So interferon-alpha treatment is not recommended in the treatment of CNV[8].

Drug Targeting to Choroidal Neovascularization In general, systemically administered drugs may reach not only targeted tissue but also other tissues, resulting in unwanted side effects. Also, in order to maintain therapeutic level of the drugs in targeted tissues, frequent administration for an extended period of time is required. To solve these problems, drug delivery systems targeted to the CNV are being developed.

Anatomic characteristics of CNV tissues resemble those of tumor vasculature, exhibiting enhanced permeability and retension effect. Drug targeting to CNV may be feasible in the same manner as it is to tumors. There are two approaches of drug targeting to CNV: passive targeting and active targeting. Passive targeting controls biodistribution of the carrier by regulating its physical properties, electric charge, or biological properties. The use of drug conjugation with water-soluble polymers prolongs the half-life of the drug in the blood because the polymers are not quickly excreted in urine and are not likely to be entrapped in the retinoendothelial system, e.g. polyethylene glycol (PEG), polyvinyl alcohol (PVA), dextran, etc. Active targeting uses specific molecular recognition of antibodies or receptors. The perfect antibody to target CNV need recognize a high proportion of vascular endothelial cells in the CNV tissues and show no cross-reactivity with vascular endothelial cells or other cells in normal tissues. To date, no antibodies have been found that meet both criteria. However, some antigens are potential candidates because they show preferential expression in vascular endothelial cells of CNV tissuess, e.g. VEGF and its receptor, intercellular adhesion molecule, e-selectin, CD44, and integrin alpha(v)beta3[56].

Renno reported conjugating verteporfin (after isolation from its liposomal formation) to a modified polyvinyl alcohol (PVA) polymer (verteporfin-PVA) followed by linkage to

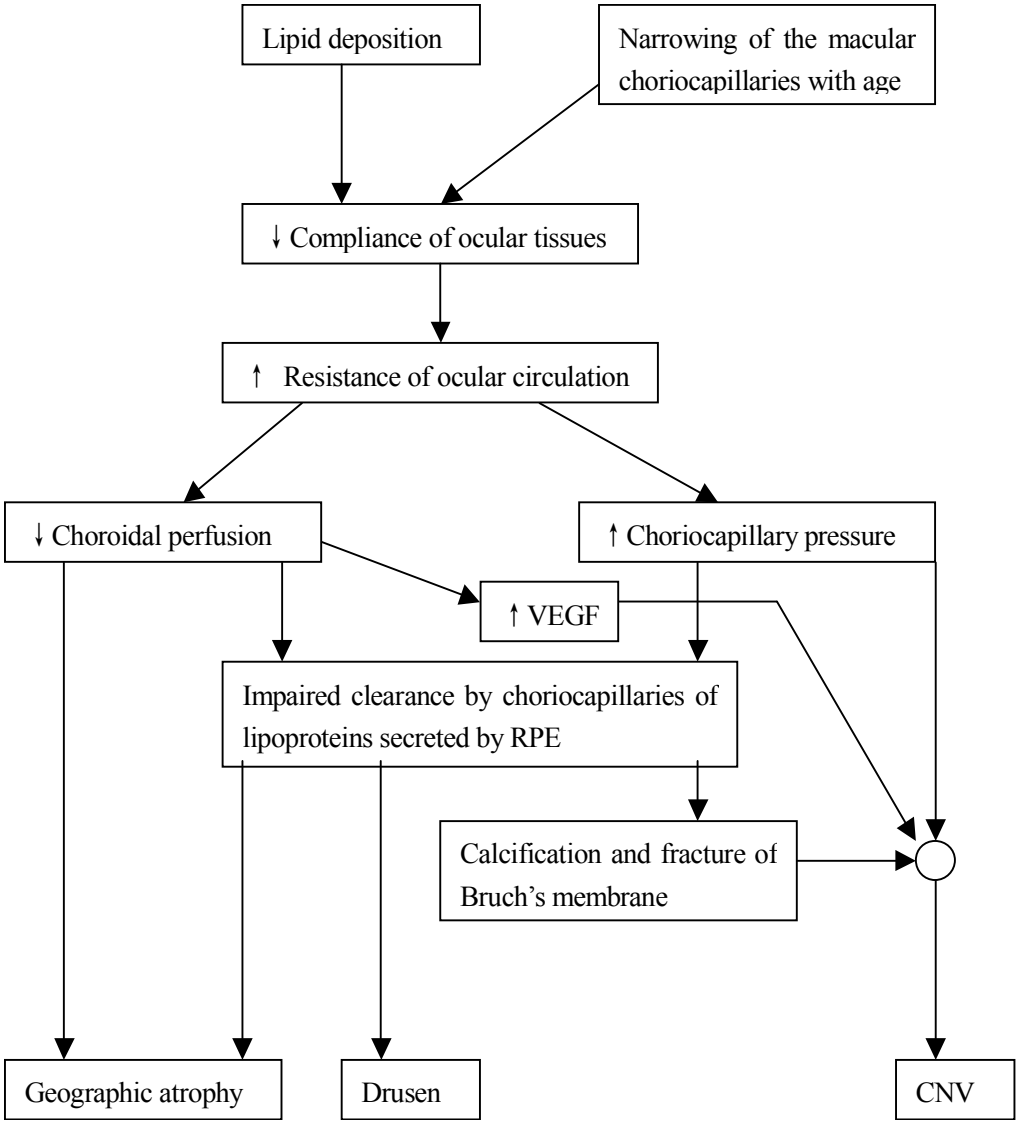

Figure 2 Schematic representation of the vascular model of the pathogenesis of age-related macular degeneration[63]

the peptide ATWLPPR known to bind the receptor for VEGF, VEGFR2. They performed PDT in rat eyes on experimental CNV and normal retina and choroid using verteporfin conjugates. PDT using targeted verteporfin showed angiographic closure of all treated CNV 1 day after treatment. And histological examination after PDT of normal retina and choroid using targeted verteporfin showed minimal effect on RPE and no injury to photoreceptors whereas PDT using verteporfin- PVA resulted in RPE necrosis and mild damage to photoreceptors. So the targeted verteporfin resulted in more selective treatment than the control conjugate or standard verteporfin and may improve current therapy[57].

Triamcinolone acetonide(TAAC)can be effectively deli-vered via long acting sustained release of intraocular microim-plants, TAAC/PVA matrix. In a laser treated rat model, TAAC implants can inhibit fibrovascular proliferation relative to control[58].

Novel Choroidal Blood Flow Treatment After age and family history, the epiodemiologic risk factors most consis-tently associated with neovascular AMD are cardiovascular risk factor, including hypertension and smoking[59-61].

It is noteworthy that choroidal blood flow is found to be impaired by every method used to quantify it in the aging eye and in age-related macular degeneration: fluorescein and indocyanine green angiography, color Doppler imaging, laser Doppler flowmetry, and pulsatile ocular blood flow[62].

The vascular model of AMD (Figure 2) suggests that the elevation of intravascular pressure is the crucial hemodynamic factor in AMD. AMD is the result of the accumulation of lipid in the sclera and in Bruch's membrane, progressively increasing the stiffness of these tissues, and increasing the postcapillary resistance of the choroidal vasculature. In addition to decreasing choroidal blood flow,

the increase in resistance tends to elevate the hydrostatic pressure of the choriocapillaries, enhancing leakage and deposition of extracellular proteins and lipids, particularly in the posterior pole. These deposits take the form of basal deposits within Bruch's membrane and of drusen, which can comprise the overlying RPE and cause geographic atrophy of RPE. The progressive deposition of lipid in Bruch's membrane results in the degeneration of elastin and collagen, and ultimately calcification. The combination of elevated choriocapillary pressure, VEGF, and a break in a calcified Bruch's membrane causes CNV in the neovascular form of AMD. Drusen, as well as the decrease in choroidal blood flow may be epiphenomena[62,63].

Vasoactive agents that selectively decrease postcapillary choroidal resistance may prevent the development of CNV. Drugs working in this field may provide a new way for AMD treatment. In our lab, some of the effective drugs, which were tested in ocular blood flow model and ischemia-reperfusion model, were used in laser-induced CNV rat models. The preliminary results showed that some of them could reduce the fluorescein leakage of the lesions effectively. A series of papers are in preparation for publication in the near future.

CONCLUSION

Aging is a chronic process to cause degeneration of cells, tissues, and organs, including choroidal blood vessels, retinal pigment epithelium cells (RPEC) and Bruch's membrane of macula[64]. Most notably, arteriosclerotic aging changes retinal blood vessels, particularly the macular choriocapillaries with a decrease in total capillary membrane and the blood flow. As a result, RPE starts to accumulate lipofusion, alters cells shape, density, pigmentation, lysosomal activity and extracellular matrix formation. Gradually, Bruch's membrane shows thickening and decreased permeability, resulting with breakdown of Bruch's membrane, which allows CNV to appear.

Numerous methods have been used to treat AMD without success. They include, but are not limited to, laser photocoagulation for CNV [65], radiation treatment [66], transpupillary thermotherapy of subfoveal occult CNV [67], submacular surgery [68], limited macular translocation [69], argon laser to drusen[70] and infrared (810nm) diode laser photocoagulation[71]. Therefore, pharmacological treatments have been tried with limited success. For example, photodynamic therapy with verteporfin, visudyne, and benzoporphyrin derivative monoacid ring A (BPD-MA) has been shown to be beneficial for some wet-AMD patients (15%) but not for dry-AMD patients (85%)[72]. More recently, newer agents such as VEGF receptor kinase inhibitors, anti-VEGF antibodies, PEDF, and angiostatin have been tried to prevent the CNV at the very late stage of AMD[73]. They are still in the experimental stage and none have been shown to be efficacious in human patients yet.

The key idea of this review is to treat AMD at the early stage of the disease and to prevent the disease from being triggered and developed. As indicated previously the earliest stage of AMD development is the malfunction of choroidal blood flow, resulting in a decrease of the blood flow of choriocapillaries. Chain reactions are triggered which lead to RPE degeneration, Bruch's membrane breakdown, CNV formation, AMD and blindness in the end. Therefore, specific drugs that can increase the choroidal blood flow could be very useful to prevent the AMD from developing and worsening.

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