Changes in Choriocapillaris and Retinal Pigment Epithelium (RPE) in Age-Related Macular Degeneration

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Retinal pigment epithelial cells (RPE) and the choriocapillaris are on opposite sides of Bruch’s membrane and control transport in and out of the retina. In age-related macular degeneration (AMD), they may also be responsible for deposition of material in and on Bruch’s membrane and the formation or regression of choroidal neovascularization (CNV). Indocyanine green (ICG) angiography can be used to visualize the choroidal vasculature and CNV. Filling of the choriocapillaris with ICG was delayed in subjects older than 50 years of age, and areas of hypofluorescence were observed in maculas of AMD subjects, often associated with CNV. Laser Doppler flowmetry of the choriocapillaris in the macula demonstrated that choroidal blood flow and volume are reduced in subjects older than 46 years of age and further decreased in subjects with AMD. The human choriocapillaris can be histologically studied in two dimensions by incubating the tissue for alkaline phosphatase activity, flat-embedding it in transparent polymer and sectioning it. Using this technique, choriocapillaris dropout was found to be associated with deposition of material in Bruch’s membrane in diabetic subjects. When RPE are removed from Bruch’s membrane, the choriocapillaris degenerates; the regeneration of choriocapillaris can be blocked by Genistein, a tyrosine kinase inhibitor. Finally, RPE cells may produce substances that both stimulate the formation and regression of CNV in animal models. These studies suggest that there may be a reduction in choriocapillaris flow in AMD, and this loss of choriocapillaris can be associated with the Bruch’s membrane deposits that are hallmarks of AMD. Furthermore, RPE may stimulate the formation and regression of CNV and RPE loss can result in loss of choriocapillaris.

INTRODUCTION

The lesions identified as causing loss of central vision in age-related macular degeneration are detachment of the retinal pigment epithelium (RPE), outer retinal atrophy, and new blood vessel growth between Bruch’s membrane and the retina. The accumulation in Bruch’s membrane of waste products, presumably derived from RPE, is believed to play a major role in the induction of these lesions. Two theories that have evolved to explain accumulation of material in AMD are: (a) deposits accumulate and cause choroidal vascular dysfunction and loss; (b) choriocapillaris dysfunction or dropout initiates the accumulation of waste associated with Bruch’s membrane [1]. It is believed that transport across Bruch’s membrane is reduced when deposits accumulate, resulting in a continuum of waste deposition [2]. Dropout of choriocapillaris could also cause RPE to become ischemic and produce growth factors that stimulate the formation of choroidal neovascularization (CNV) in AMD. Changes in choriocapillaris and RPE and their relationship with Bruch’s membrane deposits and CNV are explored.

EVIDENCE FOR POSSIBLE ICG PERFUSION DEFECTS IN PATIENTS WITH AMD

Indocyanine green (ICG) angiography is a useful tool for detecting choroidal neovascularization in patients with AMD. However, few ICG observations have been reported concerning either aging changes of the choroid or choroidal vascular, other than choroidal neovascularization in patients with AMD. Recently, Yoneya et al reported early dye filling patterns in young, healthy volunteers using a video ICG system (modified TCR 50-IA, Topcon, Tokyo, Japan), which uses a 790 nm diode laser as an emission light [3,4]. Based on these observations, aging changes in the choroid were evaluated using this system. They also investigated changes in patients with AMD and the possible role of choroidal vascular changes in the development of AMD.

Video ICG angiography was performed on 35 eyes from 35 healthy, normal volunteers (21-81 years old, average age 50.5 years old) for 30 minutes after ICG injection. A fixed area of 64 x 64 pixels in the macula was used for evaluating the number of arterioles in the image of the early arterial phase with IMAGEnet (Topcon). The mean intensity of fluorescence in the same area was also quantified with both the early and late phase images. With subjects in their second and third decades, choroidal arterioles began to fluoresce preferentially in the subfoveal area. Subsequently, feeding arterioles and choriocapillaris filled rapidly. Water-shed zones running vertically through the optic disc were often observed. In eyes of subjects over fifty, it took longer to fill the choroidal vasculature with the dye. Eventually, the margin of the water-shed zone became indistinct. Quantitative analysis disclosed that the number of choroidal arterioles and the fluorescence intensities in the macular region were reduced significantly with age (p<0.005 and p<0.001 respectively). In late phase angiograms, the mean intensity of fluorescence in the macula...
increased with subject age, through a time course ending 24 hours after dye injection.

One hundred and one eyes, including normal eyes as age-matched control subjects, were used to study choroidal vascular changes in AMD. Early dye filling was observed in 23 eyes and 17 fellow eyes of AMD patients (66.2±11.6 and 67.8±11.2 years old) and in 18 eyes of normal volunteers (62.4±9.4 years old). AMD eyes presenting hemorrhagic RPE detachment or disciform scar were excluded from this study. Initially, large choroidal arterioles outside of the macula began to fluoresce and took a straight course with few branches in diseased eyes. Dye filling of the arterioles was also slow. Eventually, either water-shed or focal hypofluorescence was observed in the macula. Choroidal neovascularization developed in areas of hypofluorescence in all AMD eyes. Focal hypofluorescence in the macula was observed and this turned into negative fluorescence by 24 hours after dye injection. Computer assisted image analysis (performed as above) demonstrated a significant decrease in the number of choroidal arterioles and reduced fluorescence in the macula (p<0.005 and p<0.01 respectively). These observations of Yoneya et al may support the hypothesis that poor perfusion of the choroid in the macula plays an important role in the development of choroidal neovascularization in patients with AMD [5].

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Foveolar choroidal blood flow in aging and in AMD

Grunwald et al [6] investigated the effect of aging on foveolar choroidal blood flow (ChBFlow) and compared foveolar choroidal blood circulation of subjects with nonexudative AMD with that of control subjects. Choroidal blood flow was determined using laser Doppler flowmetry [7]. Twenty-nine normal subject eyes (ages ranged from 15 to 76 years; mean±SD, 42±18 years) were included in the age study. Relative choroidal blood velocity (ChBVel), choroidal blood volume (ChBVol), and ChBFlow were determined in the foveolar region by asking subjects to fixate on the probing laser beam. Measurements were also obtained in eyes of 20 subjects having 10 or more large drusen, visual acuity of 20/32 or better, and no evidence of CNV [8]. Findings obtained in these subjects were compared with those of eyes from 10 age- and blood pressure-matched control subjects with no large drusen.

In normal eyes, significant negative correlations were observed between ChBVol and the subject’s age (R=-0.52, p=0.004) and between ChBFlow and the subject’s age (R=-0.54, p=0.003). No significant correlation was detected between ChBVel and the subject’s age (R=0.07, p=0.70). Significant differences were observed in ChBVol and ChBFlow between younger normal subjects aged 15 to 45 years (mean±SD, 0.48±0.20 arbitrary units [AU] and 18.9±5.8 AU, respectively) and the older normal ones aged 46 to 76 years (mean±SD, 0.34±0.11 AU and 13.3±3.3 AU, respectively; unpaired Student t-test, p=0.04 and p=0.007, respectively). In the second study, no significant differences in average age, blood pressure, or intraocular pressure were observed between subjects with AMD and control subjects. In subjects with AMD, average ChBVol was 0.24±0.08 (±SD) arbitrary units (AU); this value was 33% lower than that of control subjects (0.36±0.1 AU: two-tailed, independent Student’s t-test, p=0.005). Average ChBVel, conversely, was not significantly different from normal (0.44±0.10 AU). Average ChBFlow in subjects with AMD (8.7±3.1 AU) was 37% lower than that of control subjects (13.7±3.5 AU; p=0.0005). Average blood flow pulsatility was 6% higher in subjects with AMD (0.71±0.15) than in control subjects (0.66±0.14), but this difference was not statistically significant (p=0.42). In the subjects studied, foveolar ChBFlow decreased with age [6]. This change was probably related to the decrease in density and diameter of the choriocapillaris that occurs with increasing age. Average ChBFlow in the nonexudative stages of AMD was lower than that of age-matched controls, and the effect was caused mainly by a decrease in ChBVol [8,9]. Throughout the body, decreased circulation can lead to the formation of neovascularization. Further studies are needed to elucidate whether decreased ChBFlow plays a role in the development of choroidal neovascularization, and whether ChBFlow measurements may help identify subjects with AMD at risk for developing choroidal neovascularization.

Choriocapillaris degeneration in the human choroid

McLeod and Lutty have developed a method to study the human choroidal vasculature using alkaline phosphatase (APase) activity. They demonstrated that loss of APase activity in choriocapillaris indicates a loss of viable endothelial cells and, therefore, choriocapillaris degeneration (CCD) and compromise [10]. Lutty and associates have recently used this technique to analyse choriocapillaris dropout in occlusive disorders like diabetes mellitus and sickle cell disease. In diabetic subjects, the area of CCD was more than four fold greater than in the nondiabetic group (p<0.001). The area of CCD in the submacular choroid was 4.9 fold greater in diabetic subjects than in nondiabetic subjects (p<0.001), while the mean age of the nondiabetic subjects was 14 years greater than the subjects with diabetes. The CCD in diabetic subjects was more prominent in the posterior pole than in the peripheral choroid. CCD in diabetics appeared in two different patterns: diffuse CCD (partial loss of APase activity in a poorly defined area) and focal CCD (complete loss of APase activity in a relatively well defined area). The thickness of basal laminar deposits (BLD; a pathologic change in Bruch’s membrane) was correlated with the severity of CCD in the diabetic choroid; focal CCD areas had thicker BLD than diffuse CCD areas [11]. Choroidal neovascularization was associated with diffuse CCD and half of the CNV formations lacked APase and a viable endothelium suggesting they were infarcted.

We have also investigated the association of polymorphonuclear leukocytes (PMNs) with CCD using a double staining technique: APase and nonspecific esterase (NSE) for analysis of the choroidal vasculature and PMNs respectively. The total number of PMNs was increased within the choriocapillaris in diabetic eyes (170.9±12.9 PMNs/mm² of choroid) compared to nondiabetic eyes (84.2±16.9 PMNs/mm²; p<0.001). In the diabetic choroid, increased numbers of PMNs were present in areas of the choriocapillaris with CCD compared with
nonpathologic choriocapillaris (205.1±46.9 PMNs/mm² in pathologic vs 152.3±23.4 PMNs/mm² in nonpathologic areas; p<0.001). PMNs were often queued up within the lumens of capillaries, demonstrating loss in APase activity. Finally, there was a strong correlation between the area of CCD and number of PMNs [12]. In subjects with sickle cell disease, there were also areas of choriocapillaris degeneration which were greater in size than age-comparable normal subjects. The choriocapillaris dropout, however, was not associated with increased numbers of PMNs. The occluded vascular segments instead contained packed red blood cells.

In conclusion, significant CCD occurs in subjects with both diabetes and sickle cell disease, two vaso-occlusive disorders. PMNs may participate in the choroidal vaso-occlusive process in subjects with diabetes due to elevated ICAM-1 and P-selectin in diabetic choroidal vasculatures [13]. RBCs appear to initiate the occlusions in sickle cell subjects. Although subjects with AMD have not yet been studied using these techniques, two pathological features of AMD are associated with choriocapillaris dropout in diabetes; basal laminar deposits and choroidal neovascularization.

THE ROLE OF RPE IN CHOROIDAL NEOVASCULARIZATION (CNV)

Uyama et al hypothesize that RPE cells promote the progression of CNV in the early stage of its development, however, in the late or involution stage of CNV, proliferating RPE enclose CNV and cause its regression [14]. To clarify the role of RPE in the progression or involution of CNV, Uyama et al evaluated CNV lesions histopathologically in experimentally induced CNV. CNV was induced in monkey and rat eyes by interferon (IFN-β) was administered systemically to the animals. To damage the RPE selectively, ornithine was administered intravitreally or sodium iodate was administered intraocularly in diabetic choroidal vasculatures [13]. RBCs appear to initiate the occlusions in sickle cell subjects. Although subjects with AMD have not yet been studied using these techniques, two pathological features of AMD are associated with choriocapillaris dropout in diabetes; basal laminar deposits and choroidal neovascularization.

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Bruch’s membrane and CNV in diseases such as diabetes. Experimental models demonstrate that RPE loss can result in choriocapillaris dropout; as RPE cells repopulate Bruch’s membrane, the choriocapillaris reforms. Finally, RPE cells may produce substances that stimulate both the formation and regression of CNV in animal models. The latter two studies intimate a key role for RPE in maintaining and controlling choriocapillaris and choroidal neovascularization.

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REFERENCES