Technology needs for tomorrow's treatment and diagnosis of macular diseases

Gisèle Soubrane

Department of ophthalmology, Intercommunal Hospital, Paris XII University, Créteil, France

ABSTRACT

Retinal imaging is the basis of macular disease's diagnosis. Currently available technologies in clinical practice are fluorescein and indocyanin green (ICG) angiographies, in addition to optical coherence tomography (OCT), which is an in vivo "histology-like" cross-sectional images of the retina. Recent developments in the field of OCT imaging include Spectral-Domain OCT. However OCT remains a static view of the macula with no direct link with dynamic observation obtained by angiographies. Adaptative optics is an encouraging perspective for fundus analysis in the future, and could be linked to OCT or angiographies.

Treatments of macular disease have exploded these past few years. Pharmacologic inhibition of angiogenesis represents a novel approach in the treatment of choroidal neovascularization in eyes with age-related macular degeneration. The major action explored is the direct inhibition of the protein VEGF with antibody-like products. New anti-VEGF drugs are in development aiming at the VEGF receptors or synthesis of VEGF. But various components of the neovascular cascade, including growth factor expression, extracellular matrix modulation, integrin inhibition represent potential targets for modulation with drugs.

Intra-vitreal injections are nowadays the main route of administration for these new treatments but they are potentially responsible of side effects such as endophtalmitis. Development of other routes of treatment would require new formulation of used drugs. The improvement of retinal imaging leads to a better understanding of macular disease mechanisms and will help to develop new routes and targets of treatment.

KEYWORDS

Age-related Macular Degeneration
Fluorescein angiography
Indocyanine green angiography
Optical Coherence Tomography
Choroidal neovascularization
Anti-angiogenesis
Intravitreal injection

1. INTRODUCTION

Retinal imaging is the foundation of disease diagnosis and management. Since D. Gass[1] described fluorescein angiographic features of choroidal neovascularisation associated to age-related macular degeneration (AMD), the technique of fluorescein

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angiography was widely used for macular degeneration but also for all retinal diseases. The development of digital technologies and indocyanine green dye completed the improvement of angiographic techniques, allowing to individualize more precisely clinical features of choroidal neovascularisation of AMD. However, the introduction of Optical Coherence Tomography (OCT) in the late 1990s allowed to observe retinal morphologic changes in an axial plane and took naturally an indisputable place for macular diseases management. Nowadays, the progresses of retinal imaging are focusing obviously on the improvement of OCT technologies but development of new capabilities of angiographic imaging too. Finally, the availability of adaptive optics would probably open a new way of macular imaging.
New therapeutics and routes of treatment have definitely emerged in parallel with a better understanding of the physiopathology of the disease. The improvement of retinal imaging will help the clinicians to define more precisely the indications of these new treatements.

2. LIMITS OF ACTUAL RETINAL IMAGING

Fluorescein angiography was first reported in 1961[2], but its fundamental role in the diagnosis of retinal and macular diseases was evidenced in 1967 by D. Gass, who described for the first time that the disciform lesion in AMD was due to choroidal neovascularisation identify with fluorescein angiography. Fluorescein angiography allows to obtain dynamic representation of physiologic and pathologic circulation of dye inside retinal and choroidal vessels at different timepoints. An effraction of the external (retinal pigment epithelium) and internal retinal blood barrier can be also appreciated. However, fluorescein angiography provides two-dimensional anatomy of the retina and the choroids in an en-face imaging. The size of lesion can easily be evaluated but the localisation in depth of an abnormal fluorescence is extrapolated from our knowledge of the retinal structure and its different layers (figure 1).

Indocyanin green[3] (ICG) angiography developped in conjunction with digital imaging in the 1990s. ICG dye permits to identify more precisely the network of choroidal vessels and newvessels and provides further information in addition to fluorescein angiography. ICG angiography requires digital capture methods and post-image acquisition enhancement.

Both fluorescein and ICG angiographies are complementary. In one hand, fluorescein dye is particularly useful to individualize and follow up retinal vascular diseases. On the other hand, ICG dye is helpful in choroidal abnormalities, and especially choroidal neovascularisation of AMD. Fluorescein and ICG angiographies can be realized in the simultaneously with some devices such as the Scanning Laser Ophtalmoscope (SLO) in which low energy laser beams are scanned across the retina, providing a composite image.

The main limits of angiographic imaging are related to the incapacity to provide three-dimensional approaches of physiologic and pathologic aspects of the retina. Optical Coherence Tomography (OCT) resolves partially this limitation by providing an “histology-like” axial representation of the different layers of the retina without the use of dyes but it remains a static view of the retina. The interpretation of OCT depends of indirect signs such as a cystoid macular edema or a serous retinal detachment to determine a breakdown of the blood-ocular barriers.

2.1. Technology improvement in angiographic imaging

2.1.1. Improving post-acquisition manipulation of the images.Digital technology is used to capture,store and transfer the angiographic images.

2.1.2. Developing a variety of tools of digital measurements of lesions size (autofluorescence, atrophy or leakage) and quantitative analysis for drusen. Diagnostic and therapeutic outcomes should certainly improved with the ability to quantify the data[4].

2.1.3. New angiographic dyes should be developed. Fluorescein and ICG dyes are presently the only dyes employed for retinal and choroidal angiography. Fluorescein provides visualisation of retinal vessels and ICG allows better definition of choroidal vascular circulation. However, some limits still remains.

2.1.3.1. Fluorescein can be responsible in some cases for allergic and rarely anaphylactic reactions and the only alternative is ICG which is not able to identify retinal capillaries. Despite improvements continue to be made in the processing and the fabrication of sodium fluorescein, the profile of this dye can not be considered to be totally safe.

2.1.3.2. Sodium verteporfin (Visudyne®) is a medication given intravenously and used as a photosensitizer for photodynamic therapy. It accumulates specifically in abnormal newvessels. Another dye could target specifically abnormal newvessels (with abnormal endothelium) and behave as a marker to differentiate active newvessels from fibroglial or atrophic scars after treatment either on angiography or either with other diagnostic devices.

2.2. Technology improvement of Optical Coherence Tomography imaging

Optical Coherence Tomography[5] (OCT) is a high-resolution non-invasive imaging of the eye analogous to ultrasonic imaging but using light instead of sound to create the image. OCT has completed diseases diagnosis in vitreomacular diseases such as macular hole, diabetic macular edema and epiretinal membrane, but for AMD diagnosis and follow-up too, especially after treatment. The principles of Time-domain OCT (TD-OCT) are based on low-coherence interferometry which detects echo time of light. It requires time for mechanical movement of a scanning mirror to obtain scan informations.

2.2.1. Spectral-domain OCT

Two-dimensional TD-OCT is available since 1996, some improvement happened, in the software with Stratus-OCT (Carl Zeiss Meditec) which allowed better visualisation of the different retinal layers. Recently, new generation of OCT based on Fourier or Spectral-domain technology has been developed and a large number of different new systems are presently commercially available. Spectral-domain OCT (SD-OCT) uses low-coherence interferometry. But it uses a spectrometer and CDD camera to detect all light echoes *simultaneously* and axial scan information is obtained by calculating Fourier transform of interference spectrum. Thus it is possible to obtain greater speed acquisition (25 000 axial scans/second versus 400 axial scans/second in TD-OCT) and decrease many artefacts due to eye motion during time examination. It provides not only more detailed images (Figure 2) and good definition of the different retinal layers, but it can produce 3-dimensional images thus making possible to calculate the volume of the subretinal fluid for example.

Another possibilities of the SD-OCT 3-D are the creation of the summed voxel-projection (SVP) which is helpful for the orientation of individual OCT scans. Summing pixels allows to calculate a single representative pixel intensity along each line in the projection[6]. The SVP image can also be used to orient the SD-OCT scans to a color fundus image, an angiogram, an autofluorescence image or a microperimetry.

2.2.2. Limits of OCT imaging

Despite that real improvement happened in OCT technology, increasing resolution or post-acquisition manipulation, it remains a static view of the retina, contrary to angiographic imaging. The presence of choroidal newvessels are suspected at best by the visualisation of an hyper reflective area but mostly by indirect signs such as serous retinal detachement, pigment epithelium detachment or intra retinal fluid accumulation. OCT is able to identify the consequence of leakage. Thus it could be sometimes difficult to distinguish cystoid macular edema from a choroidal.neovascularisation or from a cystoid degeneration of the retina for example. Thus the activity of the disease cannot be determined.

The other problem is to whether SD-OCT imaging of a small foci of fluid would be relevant and would change the decision of the physician to treat or not. Even 3-D OCT constitutes a technological improvement, it does not supply a tool for macular diseases diagnosis and need the confrontation with angiographic imaging.. Its interest and its advantages in the clinical practice in comparison to TD-OCT should be precised. On the other hand, OCT is only capable to provide images in the axial dimension. It will be a challenge in the future to integrate dynamic information in the OCT data such as the origin of the leakage or the identification of biomarkers.

Further improvement of OCT are expected, possibly by coupling by adaptative optics.

2.3. Adaptative optics

In the coming next few years, adaptative optics may lead to major advances in retinal imaging. This old new technique comes straight ahead from astronomy for which the optical resolution is a fundamental purpose. As far as1953, Babcock suggested that adaptative optics could allow astrophysicists to improve images obtained from ground-based telescopes.

Adaptive optics technology enhances the efficacy of optical systems by reducing the effects of rapidly changing optical distortion. While the technique was theoretically understood for some time, it was only advances in computer technology during the 1990s that finally made the technique practical. The simplest form of adaptive optics is tip-tilt correction, which corresponds to correction of tilts of the wavefront in two dimensions (equivalent to correction of the position offsets for the image). This is performed using a rapidly moving tip-tilt mirror which makes small rotations around two of its axes. A significant fraction of the aberration introduced by the atmosphere can be removed in this way. Tip-tilt mirrors are widely used in night time and solar telescopes, to correct the aberration introduced by the atmosphere on the light path and improve image quality over what would be possible according to the atmospheric seeing.

To examine the retina, ophthalmologists have to deal with many structures that can be more or less transparent, inhomogeneous or unstable. The lachrymal film, the cornea, the optic lens, the vitreous may constitute a barrier for an accurate observation. One of the major problems in the diagnosis and treatment of human retinal diseases is the considerable loss of resolution when imaging the living retina. The loss of lateral and axial resolution, which is brought about by the dynamic aberrations introduced by the optics of the eye, makes the detection and progression of certain retinal anomalies associated with particular diseases harder or impossible to detect.

At the end of the nineties, Williams applied adaptative optics to ophthalmology, producing the first in vivo image of the retina, at a cellular scale. Various techniques have been developed to characterize microscopic structures in the living human eye. Of the various cell types in the retina, scientists have focused on imaging the cone photoreceptors (although the cones make up only 5 percent of the total number of photoreceptors in the retina). Many debilitating retinal diseases are reported to primarily affect the cones, and in normal vision, the cone mosaic places constraints on our spatial and color vision. The ability to visualize cones in vivo has thus been an attractive goal for many researchers. Like a tiny fiber optic, cones act as waveguides-collecting incoming light over some fixed acceptance angle and funneling the light along the length of the cone until it is reflected back through the cone [by the retinal pigment epithelium (RPE)] towards the pupil center. Cones can thus be seen even without complete correction of the eye's aberrations. The correction of lower order aberrations enabled single cones to be visualized with a fundus imaging system, although only in eyes with excellent optical quality. But routine visualization of the cone photoreceptors only became possible with the appearance of adaptive optics.

This technology has revealed the topography of all three classes of cones and the angular tuning of individual cones in the living human eye.

Human colour vision depends on three classes of receptor, the short- (S), medium- (M), and long- (L) wavelength-sensitive cones. These cone types are interleaved in a single mosaic so that, at each point in the retina, only a single class of cone samples the retinal image. The proportion of L to M cones is remarkably different in two subjects, each of whom has normal colour vision. The mosaics of both subjects have large patches in which either M or L cones are missing.

As mentioned above, the waveguide properties of the cones make them easy to visualize in adaptive optics imaging. Using off-axis illumination, Roorda and Williams found that the cone photoreceptors demonstrate remarkable precision in the alignment of their optical axes, with cones pointing to an area of the pupil that is less than 0.15 mm. A curious feature observed in high resolution retinal images of the cone mosaic is that there is variation in the reflectance between different cones. Moreover, there is temporal variation in the reflectance of individual cones on scales ranging from a few seconds to a few days. In an effort to determine the cause of this spatiotemporal variability, Pallikaris et al. monitored cone images obtained with adaptive optics over a 24-hour period. They found that the spatial variation was not caused by changes in cone directionality but was likely mediated by intrinsic changes within the cone or the cone-RPE interface[7]. The authors proposed that at least some of the temporal variation might be related to the process of disc shedding of the outer segments of the cone. If this is true, it could provide a metric with which to monitor the health of individual cones in vivo, in that cones that are unhealthy may show abnormal rates of disc shedding.

It is possible to combine adaptative optics with other technologies, as Scanning Laser Ophthalmoscopy. A scanning laser ophthalmoscope (SLO) creates an image of the retina by raster scanning a laser beam into the eye and detecting the reflected light point by point. The main advantage offered by the SLO over traditional imaging is improved efficiency in light collection (through the use of more sensitive detectors) and video-rate imaging capabilities. Additional benefit is obtained when confocal detection is implemented. The benefit of confocal detection is two-fold: resulting images have higher contrast, and through axial scanning of the focal spot, axial slices of the retina can be obtained with ~300 nm resolution. Confocal SLOs, which have been commercially available for over 15 years, have been an important tool for studying the coarse features of retinal disease. Some groups have used confocal SLOs to obtain retinal images in which information about the

photoreceptor mosaic could be obtained through extensive image processing. The combination with adaptive optics to correct the eye's monochromatic aberrations further increases the lateral and axial resolution of a confocal SLO. Recent integration of a real-time adaptive optics system (that corrects the higher order aberrations of the eye) with an SLO by Roorda et al. enabled the first high-resolution SLO images of the retina, in which axial sectioning is greatly improved (~100 μm axial resolution) and cells as small as 3 μm can be readily visualized.

During the year 2006, Drexler and al. have used the Spectral Domain Optical Coherence Tomography (SD-OCT), combined with adaptive optics technology patented by Imagine Eyes, to develop a new imaging system designed to provide early diagnosis and improve the possibilities for treatment of retinal diseases. Beginning in the fall, the team unveiled the first three-dimensional images of retina microstructures obtained in vivo with high definition and run without the slightest risk to the subjects of the study.

What distinguishes the work of Drexler from other projects in the same area is the ability of its deformable mirror to image retina in very various subjects, including those whose eyes have important optical defects which usually prevent any functioning of adaptive optics. One of the areas of most interest in this new approach is the early detection of age-related macular degeneration (AMD), which is one of the major causes of blindness in the world: about 20 million patients are affected by AMD in Europe.

Retinal imaging by OCT present a three-dimensional resolution of 15 μm x 15 μm x 10 μm, thus photoreceptors that activate the vision remain hidden. The electromagnetic deformable mirror patented by Imagine Eyes, combined with its detection technique in the spectral field is able to increase the performance of the OCT by a factor of 80 and get a resolution stunning 3-μm x 3 μm x 3 μm.

Imagine Eyes is currently developing the commercial version of a retinal camera, which uses adaptive optics technology. The prototype of this instrument previously scheduled for 2007, will for the first time allow ophthalmologists to benefit from adaptive optics in an actual clinical environment, to explore the cellular level and offer new ways of care and treatment for their patients.

Although our understanding of the genetic basis of retinal disease is advancing at a rapid pace, the physical manifestation of disease is often more difficult to assess. One of the more exciting applications of adaptive optics retinal imaging has been towards providing earlier detection and improved diagnosis of retinal diseases. While promising results have been obtained with conventional adaptive optics systems, the realization of adaptive optics imaging as a tool to study retinal disease will no doubt involve other imaging technologies.

One approach that has already demonstrated enhanced capability to image non-photoreceptor cell types is fluorescence imaging. Images of ganglion cells in living rat and primate retina where obtained using an intravitreal injection of an Annexin 5-bound fluorophore that only becomes active when a cell is undergoing apoptosis. Thus tracking in real-time individual ganglion cells that where dying was possible. The key to human imaging will be delivery of contrast enhancing agents so that with the appropriate laser source and filter set, cells that were otherwise invisible in reflectance imaging can be imaged noninvasively[8].

Commercial confocal SLOs have been used to study changes in autofluorescence in retinal disease, and the future application of AOSLO imaging to obtain high-resolution autofluorescence maps of the retina will help elucidate the role of increased autofluorescence in disease progression. AOSLO provide an attractive, noninvasive alternative for studying blood flow dynamics in vivo and will serve as a stepping-stone for future work in studying diseased eyes. The fact that adaptive optics is compatible with so many other methods to improve retinal imaging opens many new doors for future research. The most exciting applications of adaptive optics for retinal imaging are probably yet to come.

3. ANTIANGIOGENIC TREATMENTS

Angiogenesis is characterized by a complex cascade of events. Initially, cytokines lead to vasodilatation of existing vessels and increase vascular permeability, followed by degradation of the surrounding extracellular matrix, which facilitates chemotactic migration and proliferation of newly formed endothelial cells. Behind the advancing front of proliferating endothelial cells is an area where the endothelial cells stop proliferating and join to form a lumen that becomes a new capillary tube. Finally, these vessels fuse and generate a network in which blood circulates in a newly vascularized region.

This new vascular network subsequently matures and undergoes remodeling to form a stable vascular network. The successful execution of this angiogenic cascade requires the carefully balanced interplay of growth-promoting and growth-inhibiting angiogenic factors. There are several activators of angiogenic factors including hypoxia. Angiogenic factors released from local tissue may activate endothelial cells and provide signals for cell migration, proliferation, and differentiation and may induce capillary formation. Conversely, inhibitors of angiogenesis including thrombospondin, angiostatin, endostatin, and pigment epithelium-derived factor (PEDF) balance the pro-angiogenic factors. Identified activators of angiogenesis include vascular endothelial growth factor (VEGF), the family of fibroblast growth factor (FGF), transforming growth factor (TGF), angiopoietin-1, and angiopoietin-2. Although there are several potential regulators of angiogenesis, it appears that VEGF-A signaling represents a critical rate limiting step. The better understanding of angiogenesis has led to the development of many therapeutic agents mostly in antitumoral research. Some have been tested to inhibit the formation of choroidal newvessels in AMD, such as pegaptanib and ranibizumab that bind directly to VEGF. The angiogenesic cascade can be blocked at many levels. Some of these therapies are currently under clinical trials.

3.1 Current treatments

Verteporfin photodynamic therapy has opened a new area in therapeutic care of choroidal neovascularization in AMD since 1999. Indeed, a photosensitizer verteporfin injected intravenously, striggers a cascade of reactions when activated by a suitable infrared light wavelength leading to an occlusion of choroidal newvessels. But some lesions are not indication of dynamic phototherapy and do not allow stabilization of visual acuity

The recent availability of anti-VEGF revolutionized the therapeutic results of the treatment of neovascular AMD. The role of VEGF in the pathogenesis of choroidal neovascularization was highlighted in several animal models. The VEGF-A was first identified as a potent inducer of vascular permeability before its pro-angiogenic growth factor activity It presents six isoforms whose isoform 165 seems mainly involved in the neovascular process.

The pegaptanib sodium or Macugen ® is an aptamer with a high affinity for the isoform 165 of VEGF-A thus inhibiting its binding to its receptors on endothelial cells. Intravitreal injection of 0.3 mg of pegaptanib sodium every 6 weeks stabilizes visual acuity at 1 and 2 years for all types of new vessels (VISION study).

The ranibizumab or Lucentis ® or rhuFab V2 is the Fab portion of a monoclonal VEGF antibody binding to all isoforms VEGF-A. Marina (minimally or occult new vessels) and Anchor (predominantly classic new vessels) studies show after an injection per month for 24 month a stabilization in visual acuity in 90 % of eyes (loss of less than 15 letters) and an improvement in visual acuity (greater than or equal to 15 letters) in 40.3% of cases.

Bevacizumab or Avastin ® is a humanized monoclonal antibody from mice, intended for intravenous use, inhibiting all VEGF isoforms, and developed for the systemic treatment of metastatic colorectal cancers in humans. The efficacy of treatment in exudative AMD by intravenous bevacizumab was evaluated in many non-randomized studies on small series of patients. Neither its efficacy, nor its safety has been demonstrated.

3.2 Next generation of therapies for exudative AMD currently undergoing clinical evaluation (phase III)

3.2.1 SiRNA

RNA interference (also called "RNA-mediated interference", abbreviated RNAi) is a mechanism for RNA-guided regulation of gene expression in which double-stranded ribonucleic acid inhibits the expression of genes with complementary nucleotide sequences. Conserved in most eukaryotic organisms, the RNAi pathway is thought to have evolved as a form of innate immunity against viruses and also plays a major role in regulating development[9] and genome maintenance. Synthetic RNAs of 21 and 22 nucleotides in length are able to mediate cleavage of target mRNAs. SiRNA can inhibit the VEGF angiogenesis pathway

Bevasiranib (Acuity pharma- Opko) is a siRNA which mediates cleavage of VEGF mRNA. An open label, pharmacokinetic, dose escalation phase I study showed a good safety up to 3.0 mg/eye. Phase II studies (CARE and RACE) have shown that approximatively 78% of patients lost < 15 letters at the 12-week endpoint. Visual acuity and lesion size were stable through the week 18 visit (12 weeks after the last dose of bevasiranib). Most commonly reported adverse events consisted of procedure-related findings, such as conjunctival injection, subconjunctival hemorrhage, ocular pain, floaters. No cases of endophthalmitis, rhegmatogenous retinal detachment, or other sight-threatening adverse events were reported. Five cases of iritis/uveitis at the 3mg dose, which were associated with transient reductions in vision. Based on these results, phase III

study (COBALT study) evaluating the efficacy of bevasiranib (2.5mg) at 8 and 12 weeks intervals, after pre-treatment with 3 injections of ranibizumab and initiation of bevasiranib treatment at 2 and 6 weeks is in progress.
SiRNA technology can be used to develop many drugs against others growth factors. Sirna-027 (Sirna therapeutics-Merck) targets VEGF Receptor 1 mRNA leading to decrease in production of VEGF R1[10]. Phase I study shows that a single ascending dose of Sirna-027 was safe and well tolerated. Three months after a single injection, 24 of 26 patients (92%) showed visual acuity stabilization, with four of 26 patients (15%) experiencing clinically significant improvement in visual acuity; only two of 26 patients (8%) experienced a reduction in visual acuity of three lines or more. A phase II study, « The small Interfering RNA In Macular Degeneration Study » (SIRIUS) is in progress.
RTP801i-14 is a siRNA drug candidate designed to inhibit the expression of the hypoxia-inducible gene RTP801. The gene RTP801 was initially identified by its dramatic up-regulation response to hypoxia and/or oxidative stress both in vitro and in animal models. Since expression of RTP801 is then rapidly up-regulated, it represents a unique gene target that may regulate hypoxia-induced pathogenesis by a mechanism that is independent of growth factors such as VEGF. This hypoxia-inducible gene promotes neuronal cell apoptosis and the generation of reactive oxygen species in vitro. In both genetic (RTP801-knockout) and therapeutic mouse and primate models of laser-induced choroidal neovascularization (CNV), inhibition of RTP801 expression leads to inhibition or reduction of CNV and vessel leakage following intravitreal injection of RTP801i-14. It is also anti apoptotic and thus may also be useful for the treatment of dry AMD. RTP801i-14 is in Phase I/IIA clinical study.

3.2.2 VEGF trap

The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related placental growth factor (PlGF). The VEGF Trap-Eye is a specific and highly potent blocker of these growth factors
In preclinical experiments, the VEGF Trap has been shown to prevent the development of abnormal blood vessels in the eyes of animals with conditions resembling diabetic retinopathy and AMD. Preclinical studies have shown that VEGF Trap inhibited the growth of new blood vessels when given intravenously, as well as when administered directly into the eye
Preliminary results from phase I trial in 21 eyes with the neovascular form of AMD were positive after a single intravitreal injection of 0.05, 0.15, 0.5, 1, 2, or 4 milligrams (mg) of VEGF Trap. Patients were followed for 6 weeks at which time they were permitted, according to the study protocol, to receive other available treatments. Single doses of VEGF Trap were generally well tolerated at all dose levels tested (0.05 to 4 mg), with no systemic or serious adverse events reported. • Of the 20 patients evaluable for efficacy, 95 percent had stabilization or improvement in visual acuity, defined as < 15 letter loss on the Early Treatment of Diabetic Retinopathy Study (ETDRS) eye chart. •
In a double-masked, prospective, randomized, multi-center Phase 2 trial, 157 patients were randomized to five groups (monthly or quarterly doses from 0.5 to 4.0mg) and treated with the VEGF Trap-Eye in one eye. All five groups showed an improvement in retinal thickness and an increase in mean letters read versus baseline at all time points through week 12. There were no drug-related ocular or systemic serious adverse events reported. The most common adverse events were those associated with intravitreal injections. Preliminary week 16 results showed that retinal thickness for all groups combined continued to improve with a mean decrease of 159 microns versus baseline ($p<0.0001$). The mean change from baseline in visual acuity also continued to improve (all groups combined, increase of 6.6 letters versus baseline, $p<0.0001$). Quarterly dosing improved retinal thickness and visual acuity versus baseline at 12 and 16 weeks, but the effect was not as robust as with monthly dosing. .
In the first Phase 3 trial currently enrolling, the VEGF Trap-Eye using four- and eight-week dosing intervals will be directly compared with ranibizumab administered every four weeks according to its label.

3.3 Promising next AMD generation therapies in clinical evaluation

3.3.1 Receptor-tyrosine kinase inhibition

Small molecule receptor tyrosine kinase inhibitors that target all VEGF receptors are being developed. For instance, vatalanib (PTK787; Novartis), is a potent inhibitor of all known VEGF receptor tyrosine kinases with a slightly greater

potency against VEGFR1 and VEGFR2. Preclinical studies suggest that PTK787 induces dose dependent inhibition of VEGF4 Daily oral administration of PTK787 (25 mg/kg) completely inhibited the neovascularization that develops in the subretinal space of rhodopsin/VEGF transgenic mice and daily oral administration (50 mg/kg) for 14 consecutive days decreased choroidal neovascularisation by approximately 80% in a laser-induced model of choroidal neovascularization. Vatalanib is in clinical development as an antiangiogenic therapy for patients with cancer. Oral administration once daily in a Phase I study in combination with verteporfin photodynamic therapy in patients with neovascular AMD is evaluated.
A preclinical study of another small-molecule receptor tyrosine kinase inhibitor, AG-013958 (Pfizer), which is a selective inhibitor of VEGF-R and PDGF-R, demonstrated a vascular inhibition development and capillary regression in rats. Preclinical studies in cynomolgus monkeys that received sub tenon administration of AG-013958 found effective choroidal concentrations and minimal systemic exposure of AG-013958. Similarly, preliminary clinical trial results from 21 eyes with subfoveal CNV secondary to AMD using a similar route suggested that AG- 013958 is associated with minimal systemic exposure and mild adverse events (44% of patients experienced Grade 1 adverse events). A Phase I/II, randomized, masked, dose escalation study of AG-013958 is under way in eyes with subfoveal CNV secondary to AMD[11].

3.3.2 Ad PEGF

Pigmented epithelium derivated factor (PEDF) is a member of the serine protease inhibitor family with neuroprotective, neurotrophic and antiangiogenic activities. It is a natural inhibitor of angiogenesis but an ambivalent effect is described: low doses are antiangiogenic, while high doses increase experimental neovascularization.
Preclinical studies show that high levels of PEDF in the eye are achieved after intravitreous or subretinal injection of an E1-, partial E3-, and E4-deleted adenoviral vector carrying a cDNA encoding human PEDF driven by a cytomegalovirus (CMV) promoter (AdPEDF.11, Genvec). This results in strong suppression of ocular neovascularization in different models. Intraocular injection of AdPEDF.11 after CNV is established causes regression of the CNV by inducing apoptosis in vascular cells participating to angiogenesis. Both intraocular and periocular injection of AdPEDF.11 also suppress CNV in pigs, the eyes of which are similar in size and structure to the human eye. These encouraging preclinical data and the demonstration that intraocular injection of up to 109 PU of AdPEDF.11 in primates resulted in only mild toxicity and prompted a phase I clinical trial to investigate the safety of intravitreous injection of AdPEDF.11 in patients with advanced neovascular AMD.
Twenty-eight eyes with advanced neovascular age-related macular degeneration (AMD) were given a single intravitreous AdPEDF.11. No serious adverse events were related to dose. 3 and 6 months after injection, 55 and 50%, respectively, of eyes treated with106–107.5 PU and 94 and 71% of those treated with 108–109.5 PU had no change or improvement in lesion size from baseline.[12]. These data suggest also the possibility of antiangiogenic activity that may last for several months after a single intravitreous injection[13]. One advantage of gene therapy is the large production of the molecule in the target tissue for a long period. Phase IB Study in patients with moderate to advanced exudative age-related macular degeneration has completed enrollment.

3.3.3 Nicotinic acetylcholine receptor (nAChrR) antagonist)

Alpha 7 nicotinic acetylcholine receptor (alpha7 nAChR) is widely expressed in the central and peripheral nervous systems, and is also found in several non-neuronal tissues, such as endothelial cells, bronchial epithelial cells, skin keratinocytes and vascular smooth muscle cells. Recent evidence suggests that alpha7 nAChR is involved in angiogenesis[14]. NACHR agonists increase capillary network formation and enhance neovascularization in experimental inflammation, tumor and ischemia models.
ATG3 (mecamyllamine, CoMentis) derived from mecamylamine used to treat hypertension and in smoking cessation, is an antagonist of the nicotinic acetylcholine (nACh) receptor pathway. The drug was developed to effectively penetrate into the retina and choroid following topical eye drop administration.
Phase I study of ATG3 in healthy volunteers showed excellent ocular safety profile. Phase II a double-masked, randomized, placebo-controlled clinical trial designed to evaluate the safety and efficacy of ATG3 in patients with exudative AMD is underway. Approximately 330 patients will be randomized to one of three treatment groups, administered by eye drop twice daily: two different doses of ATG3 (1% and 0.3%) or placebo. One eye per patient will receive the study treatment. All patients will be treated for up to 48 weeks. Patients will be assessed for change in visual acuity and macular thickness using ocular coherence tomography using that convenient route.

3.3.4 Complement cascade inhibition

Complement cascade has been implicated in pathogenesis of AMD. Drusen complement C3a and C5a have been shown to promote CNV[15] and are constituents of drusen in patients with AMD[16,17,18,19]. Their presence, as well as that of the membrane-attack-complex (MAC) C5b-9 and other acute-phase reactant proteins in RPE cells overlying drusen, has supported speculation that drusen biogenesis involves chronic inflammatory processes that either can trigger complement activation and formation of MAC acting to lyse RPE cells or disturb physiological homeostasis in RPE cells. Complement factor H polymorphism predisposes to AMD[20] .

Several molecules are under development to inhibit complement cascade. POT-4 is a synthetic peptide derived of cyclic peptide compstatin, a synthetic small-molecule that binds tightly to complement component C3. Inhibition of C3 effectively shuts down all downstream complement. A prospective phase 1, uncontrolled, dose-escalating study in exudative AMD is underway. Other molecules targeting complement C5, a central component of the complement cascade, are in the pipeline. ARC1905 (Archemix) is an aptamer that inhibits C5. Jerini Ophtalmics developed a small molecule inhibiting C5a receptor antagonist and Eculizumab (Alexion Parmaceuticals) is a fully humanized monoclonal antibody to C5. It would be of major interest to target complement at the early stages of the disease before neovascularization or atrophic complications develop.

3.3.5 Nuclear Factor Kappa inhibition OT-551

NF-κB (nuclear factor-kappa B) is a protein complex which is a transcription factor found in almost all animal cell types. This factor is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. NF-κB plays a key role in regulating the immune response to infection. Consistent with this role, incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection and improper immune development. OT-551 (Othera pharmaceutical, Inc), is a small molecule that acts via multiple pathway. OT-551's mechanism involves the oxidative stress pathways, as well the inflammatory. OT-551 down-regulates the over expression of the protein complex NF-kB. Through these dual pathways, OT-551 exhibits activity against oxidative stress, inflammation, and angiogenesis. OT-551 also possesses excellent bioavailability, penetrating tissues in both the front of the eye (cornea, conjunctiva, lens) and back of the eye (choroid, retina, vitreous) and can be administered with eye drops. Preclinical models show direct activity against oxidative stress and indirect activity against cytokine-induced inflammation and angiogenesis[21]. A phase II study is planned as adjunct to ranibizumab in exudative AMD

3.3.6 Polyamine analogs

Polyamines such as putrescine, spermidine and spermine are naturally occurring, positively charged compounds found in virtually all living cells. These compounds bind to DNA and have been implicated in a number of crucial cellular processes such as cell division, differentiation and membrane function[22]. It is known that the inhibition of polyamine synthesis, or their depletion from cells, stops cell growth. Inhibitors or analogs of these compounds might have potential as therapeutic treatments for cancers or others angiogenic process like neovascularization in AMD. A strategy to inhibit polyamine pathway is to use polyamine analogs that would mimic the natural polyamines but have modified function. While several potential modes of actions of polyamine analogs have been suggested, their ability to displace the natural polyamines from their DNA binding sites is the most likely mechanism of action. This would lead to a cessation of cell growth or cell destruction, since the polyamine analogs would be biologically inactive or have altered function.

CGC-11047 is a polyamine analog. In preclinical studies, a single periocular injection is able to suppress experimental CNV for two to three weeks[23]. Phase I study of 16.5mg CGC-11047 subconjunctival injection once every two weeks versus once every four weeks is currently under way.

3.3.7 Tubulin blockage

Combretastatin A-4-phosphate (CA4P) (OxiGene) is a prodrug derived from the root bark of the *Combretum caffrum* tree occurring structural analog of colchicine. It selectively binds tubulin of the cytoskeleton in endothelial cells of tumor vasculature, inducing change in shape, stopping blood flow through the capillary, causing cell death.

Positive results from an open label, dose ranging phase II study in myopic macular degeneration were reported. Clinical studies regarding two topical ophthalmic formulations (eye drops and ocular mini-tabs) for CA4P in age-related macular degeneration were planned

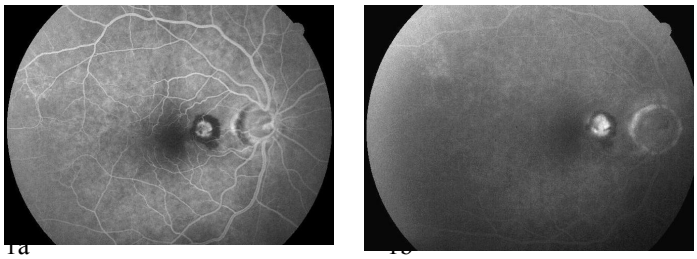
A small molecule OC-10X (Ocucure) is a new class inhibiting selectively tubulin with antiangiogenic and antiangiolytic activity. Indeed, OC-10X both inhibits new blood vessels from sprouting (antiangiogenic) but also causes regression of newly formed immature blood vessels (angiolytic). Preclinical antiangiogenic activity showed reduction of neovascularization of 44% in laser-induced rat CNV model and angiolytic activity with 44% reduction of neovascularization. The animal studies showed that the lead compound penetrates from the front (cornea) of the eye all the way back to the retina in clinically significant concentrations allowing a formulation into topical eye drop. Animal studies indicate the formulations to be nontoxic.

3.4. New routes of treatment in exudative AMD

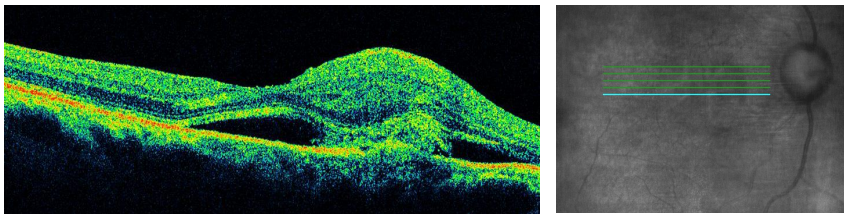
There are several interesting developments in experimental pharmacology that are relevant in the treatment of posterior segment diseases. All these potential treatments are hampered by the problems of drug delivery. Drugs do not reach the posterior eye tissues at adequate levels during eye drop treatment due to the kinetic issues discussed in the previous paragraph. Systemic drug administration is possible, but in this case non-target tissues are exposed to the drug potentially giving rise to adverse effects. Therefore, unless efficient targeting methods are discovered, the local ocular drug administration methods are preferred for the potent drug candidates. Intravitreal drug delivery has gained popularity recently by the advent of the new antibody based treatments of AMD. However, this is invasive and potentially dangerous. Therefore, other less invasive and long acting treatment modalities are needed. Transscleral drug delivery could be a potential solution to the problem. Traditionally subconjunctival, sub- Tenon and retrobulbar injections have been used clinically to administer for example corticosteroids and local anaesthetics, and it is known that higher concentrations in the posterior tissues can be achieved by these injections than by topical eye drops. Relatively high permeability of sclera to macromolecules has revived interest for this route. Delivery of macromolecules is important therapeutically, since anti-angiogenic antibodies, oligonucleotides, growth factors, and trans-gene expression products all are large molecules. Development of controlled release materials and dosage forms may provide means to achieve long duration of action and less frequent drug administration. Transscleral drug delivery, transscleral iontophoresis and controlled release materials are interesting to treat posterior segment disease[24]. However, several topical treatments are in development in exudative AMD: Targen 801, ATG3, OC-10X, OT-551

CONCLUSION

In summary, the management of the macular diseases and especially of the Age-related Macular Degeneration depends on the improvement of the retinal and the macular imaging which are necessary to define precisely the type and the localisation of the lesion, and on the necessity to develop safe and efficiency therapeutics in the future.

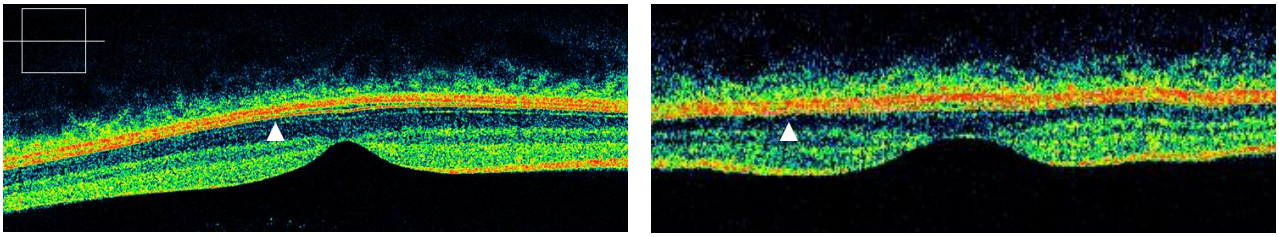


1a



1c 1d

Figure1: Fluorescein angiography shows abnormal extrafoveal classic choroidal newvessels with early fluorescence (1a) and late leakage (1b). Spectral-domain OCT scan reveals abnormal hypereflective lesion upon the retinal pigment epithelium with adjacent serous retinal detachment (1c and 1d).



2a 2b

Figure 2: Comparison between Spectral-domain OCT (Cirrus-OCT, Carl Zeiss Meditec) (2a) and Time-domain (Stratus-OCT, Carl Zeiss Meditec) (2b) resolution. Spectral-domain OCT provides enhancement of details and especially for the visualisation of inner/outer segment photoreceptor junction line (white arrow).

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