Drug Delivery to Posterior Intraocular Tissues: Third Annual ARVO/Pfizer Ophthalmics Research Institute Conference

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The third Annual ARVO/Pfizer Ophthalmic Research Institute Conference was held Friday and Saturday, May 4 and 5, 2007 at the Fort Lauderdale Grande Hotel and Yacht Club, Fort Lauderdale, Florida. The conference, funded by the ARVO Foundation for Eye Research through a grant from Pfizer Ophthalmics, provided an opportunity to gather experts from within and outside ophthalmology to develop strategies to address drug delivery to posterior intraocular tissues—a topic of great interest, as the major route of drug delivery is via intravitreous injection.

A working group of 33 participants, focused interdisciplinary contributors, 19 observers from ARVO/Pfizer, and clinical and basic ophthalmic researchers convened to identify (1) unmet patient needs regarding current drug delivery, novel treatment of retinal diseases, the potential for novel drug design opportunities, drug delivery methods for targeted localized and sustained release, and delivery of macromolecules (siRNA, DNA); and (2) to evaluate the usefulness of nanoparticles, microbeads, and implants for drug delivery, as well as physical means of drug delivery (ionophoresis, electroporation, and microneedles).

Session I: Unmet Needs and New Drug Opportunities in Treating Disorders of the Posterior Segment

Session II: Animal Models of Posterior Ocular Diseases

Session III: New Drug Design and Delivery Systems: What Do Experts See Beyond the Horizon?

Session IV: Ocular Drug Delivery Using Nanoparticles, Microbeads, and Microneedles

Session V: Transscleral, Intravitreous, and Suprachoroidal Drug Delivery

Session VI: Ocular Tissue Dissection, Modeling, and Ocular Tissue Assays

Session VII: Iontophoresis, Electroporation, Electrophoresis, and Photo-acoustic Delivery

Each session began with a 10-minute introduction followed by a 30-minute lecture by a distinguished expert. Allan S. Hoffman, ScD, Professor of Bioengineering at the University of Washington, Seattle, presented “Design of Polymer Carriers for Intracellular Delivery of Biomolecular Drugs” and Mansoor Amiji, RPh, PhD, Professor and Associate Chairman of Pharmaceutical Science at Northeastern University (Boston, MA), presented “Nanotechnology for Advanced Drug Delivery.”

During the remainder of each session, participants, and attendees discussed pertinent questions, voiced opinions, and identified unanswered questions. These discussions confirmed current and future needs for ocular drug delivery to the posterior segment.

OCULAR DRUG DELIVERY OVERVIEW

Paul Sternberg, Jr, MD, summarized the clinical perspective of ocular drug delivery. Traditional means of drug delivery to the eye have involved topical medications, applied either in eyedrop or ointment form, supplemented with systemic medications such as antibiotics or corticosteroids. To achieve higher intraocular penetration, physicians began using subconjunctival and then sub-Tenon injections. However, it was demonstrated in the 1980s that adequate levels of antibiotics to treat endophthalmitis were achievable only with intravitreous injection. The Endophthalmitis Vitrectomy Study showed that supplemental use of intravenous antibiotics did not offer additional benefit. With the emergence of infectious retinitis associated with AIDS and the need for chronic antiviral therapy, investigators developed the Vitrasert (Bausch & Lomb, Rochester, NY), the first FDA-approved device for sustained delivery of intraocular medication. Many chronic ocular diseases require long-term therapy (glaucoma, AMD, diabetic retinopathy, uveitis, intraocular malignancy); review of the recent literature reveals a dramatic increase in studies evaluating novel methods for drug delivery.[1–3]

Clinicopathologic considerations of drug delivery for posterior segment diseases was summarized by Hans Grossniklaus, MD, MBA. Posterior segment diseases may generally be classified as inflammations, degenerations, and neoplasms. Drug delivery should be targeted to a particular coat (layer) of the eye, including the outer coat (sclera/cornea), middle coat (uveal tract), inner coat (retina), and vitreous. Posterior segment drug delivery for inflammatory conditions, both noninfectious and infectious, should minimize collateral damage, and duration should be timed in accordance with the acute or chronic nature of the disease. In proliferative diabetic retinopathy, drugs may be delivered via and/or targeted to neovascular tissue. In choroidal neovascularization (CNV), such as occurs in age-related macular degeneration (AMD), the growth pattern and stage of the CNV should be considered when designing drug delivery strategies. For instance, occult CNV grows between the retinal pigment epithelium (RPE) and Bruch’s membrane; thus, transscleral or transuveal delivery may be desir-

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able. However, classic CNV grows between the RPE and neurosensory retina; thus, transvitreal or subretinal delivery may be desirable. Typical CNV in patients with AMD has both sub-RPE and subretinal components. Primary intraocular large cell lymphoma grows within the retina around vascular channels and in the sub-RPE space, where it receives nutrition from the choriocapillaris. Lymphoma cells become apoptotic at approximately 90 to 110 μm external to the retinal vessels in the vitreous and choriocapillaris in the sub-RPE space, thus limiting the utility for local drug delivery. Similarly, retinoblastoma typically becomes necrotic at the same distance from its vascular supply, thus enabling the efficacy of systemic chemoreduction therapy in combination with local therapy. The exception in retinoblastoma is intravitreous seeding, which is notoriously unresponsive to chemoreduction and local therapy. Local carboplatin injection for intravitreous seeds may lead to ischemic optic neuropathy, thus indicating that advances in local drug delivery are necessary. In most instances, uveal melanoma may be locally controlled with radioactive plaque brachytherapy, proton beam irradiation, and/or TTT. However, exceptions include plaque failures, collateral radiation, retinopathy/optic neuropathy, and TTT failures, thus favoring the utility of local drug delivery. Local drug delivery must be superior to currently available treatments, cause minimal collateral damage, and be based on the pathobiology of the disease.

2007 Third ARVO/Pfizer Ophthalmics Research Institute Working Group Participants

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UNMET NEEDS AND NEW DRUG OPPORTUNITIES IN TREATING DISORDERS OF THE POSTERIOR SEGMENT

George Williams, MD, emphasized the need for advances in drug delivery to treat retinal disease. Diseases of the posterior segment represent the leading cause of visual impairment and blindness in the United States. Until recently, treatment of most posterior segment disorders has been primarily surgical: laser photocoagulation for retinal vascular disease and vitrectomy for vitreopathies. Over the past few years, improved understanding of the pathophysiology of many retinal diseases has led to development of effective novel drug therapies, which in some diseases have replaced surgical therapies and in others, complement surgery. Increasingly, the combination of surgery and drug-based therapy addresses retinal disease at both the anatomic and molecular level, resulting in improved visual outcomes.

Despite the relative success of these novel drugs, important problems and new issues related to drug delivery remain. The explosion in the use of intravitreous drugs carries the potential of ocular and even systemic complications. The need for repeated intravitreous injections over the course of months and years creates a significant treatment burden for patients and their families. Improved drug delivery technologies that provide optimal pharmacokinetics, dose intervals, and less invasive routes of administration are needed.

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Imaging technologies such as ocular coherence tomography now provide a reproducible, quantitative method of assessing therapeutic response to drug therapy at the anatomic level. Unfortunately, anatomic improvement commonly does not correlate with improved visual function. Better comprehension of the mechanisms underlying poor visual function after anatomically successful treatment is necessary for the development of novel drug therapies that will enhance efficacy and improve safety.

Novel drug treatments for retinoblastoma were reviewed by Joan O'Brien, MD. Local delivery of drugs to the eye is a goal for all ocular therapies that demonstrate significant systemic toxicity when delivered intravenously. Avoiding intraocular injection is especially important in the management of retinoblastoma, in which violation of the ocular barriers has resulted in disease dissemination. Data were presented on two chemotherapeutic agents, carboplatin and topotecan, delivered in fibrin sealant for treatment of transgenic (LHβ-Tag) retinoblastoma-bearing mice. Both agents demonstrated significant therapeutic efficacy in this murine model, but did so through distinctly different mechanisms of action.

Tim Stout, MD, PhD, MBA, reviewed current mechanisms of control of VEGF gene expression. Identification of factors that regulate transcription of the vascular endothelial growth factor (VEGF) gene may help us understand the etiology and progression of neovascular diseases. Stout and his colleagues have studied in in vitro and in vivo models the mechanisms through which hypoxia controls VEGF gene transcription. Hypoxia-inducible factors 1α and 1β stimulate VEGF gene expression via "hypoxia responsive elements" within the VEGF promoter. Alternative splicing of RTEF transcripts is stimulated by hypoxia; the resultant RTEF isoforms profoundly affect transcription via SP elements within the VEGF promoter. Positive and negative regulators of VEGF gene transcription are of therapeutic interest.

ANIMAL MODELS OF POSTERIOR OCULAR DISEASES

This session was focused on rodent models of ocular disease, with discussion expanded to large animal models, including pig and dog.

John Heckenlively, MD, posited that mouse disease models are extremely useful in understanding human retinal conditions. Human and mouse genomes have high homology, with some estimates of up to a 95% overlap, such that genetic pathologic findings in mice are likely to have parallels in humans. Mice have fast generation times and aging, thus reducing maintenance costs. Possibly most important, custom and controllable mutations can be created in mice, thus increasing their utility and relevance as models. Heckenlively and collaborators at The Jackson Laboratory (JAX; Bar Harbor, ME) have identified and characterized over 110 naturally occurring mouse mutants with various heritable ocular diseases or transgenic mice that were imported by JAX for nonocular conditions but on screening were found to have hereditary retinal degenerations. Inbred mouse strains and stocks are screened with indirect ophthalmoscopy, histology, and electroretinography for visual system diseases. Techniques for monitoring treatment effects include electroretinography, sweep VEP, multifocal ERG, VECP, and histology.[4,5]

A critical point made by Heckenlively is that most murine diseases found to date have congenital or early-onset manifestations in their human counterpart. The current methodology of checking younger mice has skewed results. Funding agencies have shown great resistance to supporting the use of house mice in aging studies (e.g., 2 years). However, when Dr. Bo Chang of JAX ran a pilot study for 2 years, observing 20 inbred strains that were regarded as having normal retinas (at younger ages), 15 of those strains developed retinal degenerations by 18 months.[6] Heckenlively suggests that with more patience, allowing investigations of appropriate mouse strains for at least 2 years, a great deal more can be learned about aging diseases from mouse models.

Jayakrishna Ambati, MD, addressed mouse models of AMD. He suggested that the major criterion for usefulness of an animal model is whether it predicts novelties that spark development of novel diagnosis or clinical treatment approaches. He highlighted the convergence and divergence of AMD models to and from the clinical phenotype and addressed the importance of the reproduction of clinical features in fashioning therapeutics. He noted that identification of the *ABCR* gene mutation as causative in Stargardt disease[7] led to the development of the rim protein knockout mouse.[8] This mouse had an unexpectedly moderate phenotype, but it provided great insight into the function of rim protein and the understanding of lipofuscin A2E. This finding in turn led to further study of retinoids and their role in decelerating the loss of dark adaptation.[9,10,11] These studies resulted in a clinical trial to test retinoid treatment in patients with AMD. Ambati noted that many other mouse models continue to provide insight into disease and gene function and potential therapies, concluding that the last decade has witnessed an explosion of rodent models that capture many salient features of the human condition. These include various laser injury CNV models[12–19] combining risk factors (e.g., aged mice or mice subjected to high fat intake) with genetic lesions,[20] iron transport mutant mice,[21] *ELOVL4* mutant mice,[22–26] and the *SOD1* mouse.[27]

Ambati also discussed his work with mouse strains that are deficient in monocyte chemoattractant protein-1 (MCP-1, Ccl-2) or its chemokine receptor-2 (Ccr-2). These animals accumulate lipofuscin in and drusen beneath RPE and exhibit photoreceptor atrophy and CNV.[28] Using this model, his group was able to show that drusen could be cleared ex vivo by wild-type monocytes (macrophages). More recently, he found that complement activation is present in AMD drusen and not incidental drusen (thus, it is a possible biomarker). Further, if activation signaling of complement components is blocked in experimental CNV, neovascularization is reduced.[29] Ccl-2 can be expressed in Ccl2-deficient mice after AAV-mediated gene delivery. Expression is followed by infiltration of monocytes into Bruch's membrane and reduction of large sub-RPE deposits. Thus, it seems possible to cause the regression of drusen by using gene therapy (Kleinman ME, et al. *IOVS* 2007;48:ARVO E-Abstract 2354).

Steven Bernstein, MD, PhD, spoke about the development of models of anterior ischemic optic neuropathy (AION). AION is an optic nerve (ON) stroke, resulting from sudden ischemia-induced functional disruption in the anterior portion of the ON near the ON-retina junction. There are two forms of AION: (1) arteritic (AAION), involving autoimmune-mediated thrombosis of the short posterior ciliary arteries and vessels supplying both the retina and ON, and (2) nonarteritic (NAION), apparently involving only the vascular supply to the anterior ON. In AAION, outer retinal blood flow and function are affected, whereas in NAION, ON dysfunction is isolated.

An earlier AAION model involved destroying the short posterior ciliary arteries, which requires major orbital surgery, with variable changes in choroidal flow. Bernstein's team recently generated the first rodent and primate models of NAION, which closely resemble the human condition and involve selective photothrombosis of the ON capillaries without significant compromise of inner or outer retinal circulation (Bernstein SL, et al. *IOVS* 2007;48:ARVO E-Abstract 4410).[30–33] Because ON is actually a white matter central nervous system (CNS) tract, this newer model is the first in vivo isolated CNS white matter stroke model, with the added advantage that it enables direct, precise evaluation of neuroprotective mechanisms and drugs to treat the common condition of white matter infarcts, not just the eye.[32] The models include mice, rats, and nonhuman primates. The mouse models allow genetic analysis of specific gene contributions to AION susceptibility, progression, and resistance. There are considerable similarities between the rodent and primate NAION models, but also some significant differences that may offer different treatment windows.[32] Gene expression studies have revealed several potential intervention points for treatment. These have been further evaluated using stereologic (statistically driven cell quantification) analyses.[31] Early inflammatory changes are important and may set the stage for success or failure of attempts at axonal repair and regeneration.

In the general discussions that followed, participants noted that large-animal models are becoming more viable and clearly are necessary to bridge the gap between rodent models and humans. Gustavo Aguirre, DVM, discussed the many dog strains that he and collaborators and other colleagues have developed into useful animal models of ocular diseases, some with profound impact on clinical treatment.[34,35] Timothy Olsen, MD, discussed the advantages of a pig model for studying pharmacokinetics. The pig sclera is close to the thickness of the human sclera, and the lens is smaller than the rabbit and more closely resembles the human lens. Choroidal blood flow and retinal pigment epithelium are similar. There are cone cells and an area centralis (a macular analogue). Porcine retinal vasculature is more analogous to the vasculature of humans than is that of the rabbit. Systemic pharmacokinetics are similar because of the body size. Finally, pigs are inexpensive to purchase. Disadvantages include the size of the animal for housing, high per diems, and lack of inbred strains. In addition, recovery after some surgical procedures can be difficult because of inflammation.[36,37]

NEW DRUG DESIGN AND DELIVERY SYSTEMS: WHAT DO THE EXPERTS SEE BEYOND THE HORIZON?

In this session, we concentrated on the delivery of drugs by two unique delivery systems—polymer carriers and catalytic nanoparticles—and considered transscleral drug delivery.

Allan Hoffman, ScD, is well known for his studies on nanoparticles. His contributions involve the design of effective polymer carriers for intracellular delivery of biomolecular drugs, such as peptides, proteins, and nucleic acid drugs; this last category includes plasmid DNA (pDNA as used in gene therapy), antisense oligodeoxynucleotides (AS-ODNs), and the latest and “hottest” drug, silencing RNA (siRNA)—also called RNA interference (RNAi). Hoffman and colleagues are focused on enhancing the effectiveness of intracellular delivery of siRNA, which remains a major barrier to its use. The group has developed a family of acid-sensitive polymers that become membrane-disruptive within the acidic environment of the endosome and thereby enhance the escape of the drug to the cytosol. The “biomimetic” design of these polymers is based on similarities with fusogenic peptides in the protein coat of some viruses, which fuse with endosomal membranes at the low pH of the endosome, disrupting the membrane and allowing the genomic cargo of the virus to escape to the cytosol. The endosomal membrane-disruptive polymers are incorporated into polymeric micelles for carrying the siRNA into the cytosol of target cells. A polymeric micelle is formed from a block copolymer of two different polymers linked together. In this case, the first block is the endosomal membrane–disruptive polymer (hydrophilic at pH 7.4, and acting as a stabilizing outer corona of the micelle) linked to a second block, which is a cationic polymer; this block forms a polyion complex with the siRNA and thereby forms the insoluble core of the micelle in which the drug is entrapped. Thus, the polymeric micelle can carry the siRNA and release it in the cytosol of targeted cells (where the targeting ligand is conjugated to the first block). Hoffman's group is also working with micelle designs in which the siRNA is conjugated to the second block by a degradable bond, such as a disulfide bond that will be reduced by glutathione in the cytosol of the target cell. In that case, the second block is hydrophobic rather than cationic. These innovative and exciting models hold great promise.

James McGinnis, PhD, described the use of a unique type of nanoparticle for scavenging reactive oxygen species in the eye. Specifically, these oxide (CeO_2) nanoceria particles are nontoxic and nonimmunogenic, are protective at dosages in the parts-per-billion range, and have the potential to improve quality of life dramatically for individuals with retinal degeneration or other neurodegenerative diseases. Unlike other nanoparticles used to deliver DNA, RNA, protein, or drugs, nanoceria particles are themselves the therapy, as they directly scavenge the reactive oxygen species. The particles have been shown to provide protection in vivo in a light-damage animal model. The use of nanoceria particles as a direct therapy for neurodegenerative diseases represents a novel strategy for protection of the eye against the generation of reactive oxygen species.

Michael Robinson, MD, lectured on barriers to delivery of drugs by a transscleral route. Transscleral delivery of drugs into the vitreous using subconjunctival injections may be a safer alternative for reducing the sight-threatening complications of direct intravitreous injections. However, subconjunctival injections have demonstrated low and poorly sustained vitreous and retinal drug levels in animal studies. Transport barriers have been categorized as static, dynamic, or metabolic barriers, to improve understanding of the clearance mechanisms of drugs in the subconjunctival space. Static barriers are tissues that the drug diffuses through to reach the retina (i.e., the sclera, Bruch's membrane, and the retinal pigment epithelium [RPE]). Traditionally, in vitro models have been used to study the static barriers and measure drug permeability. Two-chamber in vitro models have demonstrated reasonable permeability of a several compounds across the sclera and RPE/choroid mounts. However, subsequent pharmacokinetic studies in live animals typically show low drug concentrations in the retina for short durations. Tissue permeabilities measured ex vivo do not take into account the effects of dynamic or physiologic barriers that are present in vivo. In vivo studies are necessary to examine the dynamic barriers, which include clearance through lymphatic and blood vessels, bulk fluid flow, and the active transport mechanisms of RPE transporter proteins. Recently, imaging techniques, such as ocular fluorophotometry and dynamic contrast-enhanced magnetic resonance imaging (MRI), have been used to assess the relative contribution of each barrier in the eyes of live animals. The primary dynamic barriers to transscleral drug delivery are the conjunctival lymphatic/blood vessels and the choroid.[38] Both lower the potential for effective drug delivery to the retina. Surgical techniques can selectively eliminate drug clearance by the conjunctival vessels and/or the choroid and have been combined with imaging techniques in vivo to improve understanding of the clearance abilities of the dynamic barriers.

Further development of in vivo models and imaging techniques will improve understanding of transscleral drug transport. A clear understanding of the dynamic barriers is essential for successful transscleral drug delivery systems for the treatment of retinal diseases.

OCULAR DRUG DELIVERY USING NANOPARTICLES, MICROBEADS, AND MICRONEEDLES

Mansoor M. Amiji, PhD, presented on nanotechnology for advanced drug delivery. He summarized the era of molecular medicine, which has been accelerated by the human genome project and has led to early disease detection through diagnostics and targeted drug and gene therapy. With the development of nanoparticles, barriers to drug delivery may be overcome at the organ, tissue, cellular, and subcellular levels. Nano drug delivery may occur through gold nanospheres and rods, nanowires, nanotriangles, nanostars, nanocubes, and nanorice. The size of these nano configurations varies from 1 to 100 nm. Nanoplatforms include organic nanostructures, polymeric nanoparticles, lipid systems-liposomes, self assemblies-micelles, dendrimers, and carbon nanostructure-nanotubes. Inorganic nanostructures include metal nanoparticles and nanoshells, silicon nanostructure, nanocrystals, and quantum dots. Hybrid nanostructures, combining two to three of those previous listed can also be produced.

Studies were described in which polymeric nanoparticles were used for tumor-targeted delivery to block tumor blood vessels[39] and to mediate in vitro drug delivery of tamoxifen and paclitaxel in human cancer xenograph models.[40,41] Gelatin-based engineered nanoparticles have been used for gene delivery[42] and multifunctional nanoemulsions for oral and intravenous delivery.[43] Gadolinium-loaded nanoemulsion has been used in animals for brain imaging, and this technology could easily be used for imaging within the eye to observe the results of various drug delivery modalities. Finally, gold nanostructures have been developed for OCT imaging along with superparamagnetics from oxide-gold-core–shelled nanoparticles (60-nm iron oxide nanoparticle with 5-nm gold shells) for MRI imaging.

Uday B. Kompella, PhD, spoke about reserved drug and nanotechnology for gene delivery to the eye. Viral vectors, although more efficient in gene transfection compared to nonviral vectors, are associated with side effects and risks that make them less attractive for pharmaceutical product development. Nonviral vectors such as polymer and protein-based nanoparticles offer a viable pharmaceutical alternative. The factors limiting the success of nonviral vectors include poor cellular and nuclear entry, low plasmid loading residual organic solvents, or positive charge of the vector. To overcome limitations of nonviral vectors, conventional, and supercritical fluid technologies have been used to develop pharmaceutically acceptable biodegradable polymers and naturally occurring proteins—bioengineered nanoparticles. Some of the engineered nanoparticles prepared using conventional methods allow enhanced cellular entry and others prepared using supercritical fluid technology allow high plasmid loading and sustained plasmid release. Kompella outlined approaches for preparing nanoparticulate systems by using conventional and supercritical fluid technologies and presented evidence of the usefulness of nanoparticle gene delivery systems for inhibiting corneal angiogenesis and expressing superoxide dismutase in the retina.

Current developments in microneedles for ocular drug delivery were reviewed by Mark Prausnitz, PhD. Traditional methods of ocular drug delivery include topical application, intraocular injection, and systemic administration. However, each method has limitations in efficient delivery of drugs to the back of the eye. The Prausnitz laboratory has adapted microfabrication technology to develop microscopic needles that penetrate only hundreds of micrometers into the ocular tissue via the cornea or sclera to deliver drugs in a minimally invasive manner. Prausnitz described (1) hollow microneedles used for microinfusion of a drug solution into the sclera, (2) solid microneedles coated with drug formulations that rapidly release drug coatings by dissolution within the ocular tissue, and (3) hollow microneedles for intrascleral microinjection. In the latter, a hollow glass microneedle was inserted into human cadaveric sclera for infusions of sulforhodamine solution, nanoparticle suspension, and microparticle suspension.

In the assessment of use of solid microneedles for intraocular delivery, solid metal microneedles coated with sodium fluorescein were inserted into rabbit cornea in vivo. After needle removal, fluorescein concentration in the anterior segment was measured by fluorophotometry for ≤24 hours. Similar experiments were repeated using pilocarpine-coated microneedles, and the rabbit pupil size was monitored.[44]

Hollow microneedles may be appropriate for model drug solutions and nanoparticle suspensions that can be infused into the sclera. Delivery of micrometer-sized particles into the sclera was improved by breaking down tightly packed collagen or GAG fibers using either collagenase or hyaluronidase.

When solid metal microneedles were inserted into rabbit sclera in vivo, sodium fluorescein from the needles completely dissolved within 30 seconds, which resulted in fluorescein concentrations in the anterior chamber 70 times greater than those achieved with topical delivery of fluorescein without microneedles. Similarly, microneedle delivery of pilocarpine caused rapid and extensive pupil constriction. No inflammatory response or other adverse effects were observed when using microneedles. Microneedles were shown to penetrate the sclera in vitro and cornea in vivo and to deliver useful quantities of model drugs into the suprachoroidal space. These studies demonstrate that microneedles may provide a minimally invasive method for the delivery of drugs into the sclera, to treat diseases in the anterior and posterior segment and to avoid the complications associated with intraocular injection and systemic administration.

TRANSSCLERAL, INTRAVITREOUS, AND SUPRACHOROIDAL DRUG DELIVERY

Anthony Adamis, MD, reviewed current and novel drug treatments for AMD. The ani-VEGF drugs have quickly become first-line therapeutics in wet AMD. Although these drugs have greatly improved visual outcomes, treatment burden remains a major problem.[45–47] Thus, a significant advance in the field will likely involve development of extended release formulations of anti-VEGF drugs, as well as drugs addressing newly identified biological pathways.[17] New targets being addressed in wet AMD clinical trials include PDGF-B,[48] $\alpha5\beta1$ integrin,[49,50] and placental growth factor.[51] In dry AMD, much attention is focused on the complement cascade and its role in inflammation.[52–63] Given the chronic nature of dry AMD, extended release formulations will enhance the viability of pharmacologic compounds.

Dayle H. Geroski, PhD, reviewed transscleral drug delivery. Currently, the treatment of posterior segment eye disease is limited by the difficulty in delivering effective doses of drugs to target tissues in the posterior eye. Traditional routes of local ophthalmic delivery (i.e., topical) do not yield therapeutic drug levels in the posterior tissues of the eye. The use of intravitreous injections and devices has been effective; however, these methods are not always well tolerated by the patient and are not without significant risk. The sclera offers another vector to obtain therapeutic vitreous and retinal drug concentrations. Delivering drugs across the permeable sclera would be safer and less invasive than the use of intravitreous devices and could provide a more effective retinal dose than does systemic or topical delivery. Geroski's laboratory is investigating the potential for delivering drugs across the sclera.[63] The relatively high scleral permeability—compared to the cornea—suggests great potential for development of methods for transscleral drug delivery, especially for compounds that must be administered to the posterior part of the eye. In addition, the sclera provides a large surface area of 17 cm^2; it comprises 95% of the surface area of the human eye. This large area not only provides a large region for transscleral drug absorption, but also offers the possibility of delivering neuroprotective agents, antioxidants, or angiostatic agents to specific regions of the retina.[64]

Previous in vitro permeability studies from this laboratory have shown the sclera to be permeable to a wide molecular weight range of solutes.[65] Solutes traverse the sclera mainly by passive diffusion through the aqueous pathways between collagen fibrils. The porosity of this fiber matrix is the primary determinant of the rate of drug permeation across the sclera. For any given solute, therefore, the molecular size and radius of the solute are the most important determinants of its transscleral permeability. High-molecular-weight compounds (e.g., FITC-dextran, 150 kDa) that would not be able to reach the chorioretinal tissues after intravitreous administration because of the barrier provided by the internal limiting membrane can diffuse through human scleral tissue.[66] Solute diffusion across the sclera can be affected by transscleral (intraocular) pressure.[67] The effects of pressure, however, become a significant consideration in the delivery of high-molecular-weight compounds. Past and ongoing experiments suggest that the sclera, by virtue of its large surface area, accessibility, and relatively high permeability, may indeed provide a useful route for delivering drugs to tissues in the posterior of the eye.

Joan W. Miller, MD, reviewed photodynamic drug delivery. Photodynamic therapy is a treatment modality that relies on a photosensitizer agent delivered locally or systemically that localizes more or less selectively to the target tissue and is activated by light. It results in a cascade of chemical reactions that injure the target tissue.[68,69] Localization has been based primarily on characteristics of the target tissue and the photosensitizer molecule. Leaky neovascularization in tumors or CNV permit photosensitizers to pass through the vasculature.[69] In addition, rapidly proliferating tissues such as the endothelium in CNV has greater LDL receptor expression, and lipophilic photosensitizers associated with serum lipoproteins such as LDL may be taken up selectively by proliferating tissue. Hydrophobic photosensitizers may be formulated for solubility and passive targeting using oil-based emulsions, liposomes, inclusion complexes, organic solvents, and serum lipoproteins. Selective targeting may be accomplished using monoclonal antibodies, antibody fragments, or peptides. Photodynamic therapy, using the benzoporphyrin derivative verteporfin, is relatively selective in treating CNV[70] as a liposomal formulation or as a lipoprotein-associated therapy. Animal studies have demonstrated that this selectivity may be increased through the conjugation of verteporfin with peptide-targeting vascular endothelial growth factor receptor (VEGFR)-2.[71] Selective targeting may improve the effectiveness of photodynamic therapy for ocular neovascularization and other disorders.

OCULAR TISSUE DISSECTION, MODELING, AND OCULAR TISSUE ASSAYS

Martin Friedlander, MD, discussed the need for better means of administration of agents to the retina. Present methods of treatment are grossly inadequate; he proposed that cell therapies in certain instances might be more effective than drug therapies. For example, CNV is substantially more complicated than presently recognized; hence, a therapeutic goal must include substantially more than the obliteration of new blood vessels. Because age-related macular degeneration frequently comprises geographic atrophy as well as subretinal fibrosis, other tissues must also be given equal consideration in tissue destruction. Although well intended, current therapies are not without risk; they could well enhance the progression of destructive retinal diseases. Thus, it is essential that any proposed therapy protect all viable retinal components and at the same time control and/or diminish the pathologic dangers that stem from the development of CNV. To this end, Friedlander proposed a new goal: therapies for CNV should be targeted toward maintenance and improvement of structural integrity of immature blood vessels.

As an alternative to current methods of treatment, Friedlander cited recent work in which mouse or human autologous bone marrow or cord blood-derived hematopoietic stem cells are used to selectively target sites of neovascularization and gliosis where they provide vasculotrophic effects. Moreover, endothelial progenitor cells have been shown by his laboratory to rescue retinal blood vessels that would degenerate under ordinary circumstances. The same therapy also exerted remarkable neurotrophic rescue effects. Freidlander proposed that targeted progenitor cells could well prove useful in a variety of conditions because of their angiostatic and neurotrophic properties. An additional potential use may be work on animal models of retinopathy of prematurity. Freidlander also mentioned a Trojan horse concept in which targeted progenitor cells could carry lethal agents directly to neoplasms.

Matthew LaVail, PhD, presented studies on retinal neuroprotection, focused mainly on the neuroprotective effects of ciliary neurotrophic factor (CNTF). His standard experimental procedure is to subject albino rodents to a light-induced damage protocol. Under this protocol, absent any treatment, widespread photoreceptor degeneration occurs. Intraocular injection of CNTF confers remarkable protection against light-induced damage. Protection is best afforded by pretreatment, as treatment during or after the light-damage protocol begins is remarkably less effective. CNTF also affords some retinal protection in rodents and dogs with an inherited retinal degeneration. Although his laboratory has specialized in single bolus injections, LaVail briefly reviewed the work of others on long-term, sustained administration of CNTF by encapsulated cell technology (ECT) and by gene therapy. In the case of ECT, a specific device containing genetically engineered cells is inserted into the vitreous cavity. The cells are housed within a hollow cylinder with a wall of a semipermeable membrane. Pore size is such that low-molecular-weight proteins can diffuse outward but large proteins such as IgG cannot enter. Devices of this sort have been implanted in Irish setter dogs with an inherited retinal degeneration for greater than 6 months. At the end of that time, dogs so treated demonstrated remarkable retinal protection. When the device was explanted, CNTF production, although somewhat diminished, was still clearly evident.

Even though gene therapy has been used only to a limited extent in animal models of retinal degeneration, several lessons from it are now clear. First, as Jean Bennett, MD, PhD, and her colleagues have shown for dogs with Leber congenital amaurosis (LCA), gene therapy can result in a sustained restoration of vision. Second, sustained production of a rescue agent that results from gene therapy can achieve beneficial results in selected instances where single injection protocols have failed to show such benefit. Several aspects of treatment with CNTF require special mention. First, not all CNTFs are the same; there are remarkable differences in efficacy that are species specific. Second, under certain circumstances CNTF can depress both visual acuity and electroretinographic responses in rodents; thus, there is a potential for toxicity that requires further exploration. Third, the mechanism of action of the agent is not fully understood at present; the best evidence to date indicates that CNTF acts through Müller cells.

Robert Marc, PhD, described methods he has developed for high-resolution imaging of specific classes of retinal neurons. Using immunocytochemical methods on ultrathin tissue sections, his laboratory has pioneered visualization of functional expression of several classes of retinal neurons. Such methodology can be used to map common retinal neurochemicals: taurine and glutamate among them.[72]

Using these technologies, his laboratory has performed quantitative mapping and computational reconstruction to achieve insight into dynamic retinal function.[72] Quoting an original observation by Ann Milam, PhD, that a retina undergoing deafferentation as a result of photoreceptor degeneration begins to remodel, Marc documented with clarity the successive pathologic derangements that occur. In effect, he demonstrated that in the retina, just as in brain, the loss of neuronal input is followed by a series of neuropathologic events that can be visualized in his computational reconstructions. Illustrating this phenomenon with observations on retinal neuropathology in rodent models of retinal degeneration, he has documented "deconstruction of retinal phenotype." Implying that change is widespread, he described studies from the laboratory of Connie Cepko, MD; gene arrays from mutant mouse retina evidence change in the expression of "every" gene.

IONTOPHORESIS, ELECTROPORATION, ELECTROPHORESIS, AND PHOTOACOUSTIC DELIVERY

Daniel Palanker, PhD, Francine Behar-Cohen, MD, PhD, Kevin Li, PhD, and John Nickerson, PhD, described novel technologies for improved drug delivery via electric fields.

It is known that voltages and currents that are too high result in vaporization, burns, and death. Vasoconstriction occurs at relatively low currents, and the duration needed for pooling of blood and thrombus formation after vasoconstriction is not high. However, under control, electrical fields can be used to deliver drugs to specific targets in the cell and subcellular compartments without damage to surrounding tissues.

Examples were presented that illustrated successful delivery of many different therapeutic drugs currently used in medical practice. Animal studies demonstrated how current and ions flow through the eye. An illuminating study was the use of MRI to monitor iontophoresis in real time.[73] Manganese ions exhibit an MRI signature, making them suitable for monitoring the movement of atomic scale particles in an electric field in eye tissue. In both transcorneal and transscleral iontophoresis, manganese ions moved macroscopic distances in the eye of a live rabbit. Results showed current paths in the living eye and penetration of manganese ions through the sclera and into the vitreous during transscleral iontophoresis. During transcorneal iontophoresis, manganese ions are distributed throughout the anterior chamber.

Examples of iontophoresis were also presented. David Maurice advocates the use of iontophoresis to deliver drugs intraocularly; more than 300,000 patients in Europe have been treated with iontophoresis for eye disease.[74] Antibiotics, antifungals, antivirals, anti-inflammatories, and analgesics have also been delivered by ocular iontophoresis.[75] Parel et al.[76,77] have developed a constant current device that adjusts voltage according to changes in tissue resistance during treatment. They found that transscleral iontophoresis can be safe with a current density up to 50 mA/cm^2 for 5 minutes. This current density is spread out over a large area of the corneal limbus. Their work highlights the change in properties of tissue during iontophoresis. Barrier alteration in a tissue may be the principal mechanism by which drug transport or permeability is increased during and after iontophoresis. Proper electrode placement is important in transscleral iontophoresis, and the pars plana location allows the most drug to be delivered into the vitreous. It remains to be determined whether this location is most susceptible to barrier breakdown or if it simply has the least resistance to start with.[78] Ocular iontophoresis can be used to create a drug depot in the sclera that subsequently undergoes sustained drug release. This strategy reduces the number and frequency of iontophoretic treatments. Li's group delivered triamcinolone acetonide phosphate into the rabbit eye from one electrode and calcium ions from the other at the same time (Higuchi JW, et al. *IOVS* 2007;48:ARVO E-Abstract 5822). Calcium ions and the phosphate moiety on the triamcinolone acetonide analogue precipitated when they came into contact, forming a reservoir of drug in the sclera. The precipitate dissolved slowly and ameliorated symptoms in an experimental model of uveitis.

A strength of iontophoresis is that over short distances, high concentrations of drugs can be delivered in a few minutes. Iontophoresis is the method of choice for charged drugs, which otherwise can be problematic in crossing membranes or hydrophobic barriers.

Weaknesses of iontophoresis include the impracticality of transporting drugs from the anterior surface to the posterior by iontophoresis due to the weak electric field applied across the eye, the low mobility of drugs, and short duration of treatment. Anterior segment structures may be more sensitive to electric field strength than posterior components. For delivery to the posterior segment, it is suggested that the route be transscleral, not transcorneal.

Electroporation has been used most successfully to transfect DNA, RNA, or nucleic acid analogues into eukaryotic and bacterial cells in laboratory experiments. In vitro transfection efficiencies are quite high, 50% to 90%, depending on the cell line, in eukaryotic cells and up to 10^{10} successful transformants per microgram of plasmid DNA in bacteria. Given these high success rates, it seems attractive to test electroporation in vivo in living animals. There are difficulties in translating this technology into clinical practice.

Examples of electroporation include: (1) A reporter protein expressed in RGCs after delivery by intravitreous injection of naked plasmid and electroporation[79]; (2) subretinal injection of a plasmid and electroporation in newborn rats or mice resulted in widespread transduction of retinal cells[80,81]; (3) electroporation pulses administered with a 90° rotation between sets of pulses were more effective than without rotation of the field. The rotation increased the surface area of the cell membrane that was exposed to the electric field[82]; and (4) under typical conditions of electroporation, heat buildup is negligible, but under other conditions the change in temperature can be sharp and so great that the nearby medium vaporizes. Under exceedingly specialized circumstances, this vaporization is advantageous. Rapid vaporization leads to microbubble formation and collapse that initiates a shockwave. The shockwave stretches the plasma membrane of a nearby cell, promoting pore formation. The resulting electric pulse and shockwave are synchronized, which yields increased transfection by 1,000- to 10,000-fold over standard electroporation. This was demonstrated recently in RPE cells in living rabbits.[83]

A strength of electroporation is that reporter gene expression peaks in a tight range of field strengths (100–200 V/cm), while increasing pulse length up to a point increases expression; 100 to 200 V/cm at 20 to 150 ms gives approximately 30% of cells transfected in vivo. In many experiments, reporter expression increases linearly up to maximum dose of plasmid that is tested, indicating that the most effective dosage is not routinely reached. By increasing the concentration of plasmid DNA, the transfection efficiency should be markedly improved.[79-82]

A weaknesses of electroporation is that the maximum safe threshold voltage is reached abruptly. Transfection efficiency seems low compared to some viral delivery systems.

In summary, electric field–tissue interactions are complex and poorly understood, and their applications require optimization for each particular ocular target. The use of electric fields should be given consideration when simpler delivery approaches fail. Electric fields offer the unique advantage of transiently and reversibly breaching any membrane of the cell. Provided that great care is taken to minimize cellular damage by the electric field, iontophoresis and electroporation are delivery approaches worthy of further consideration.

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