Expert Opinion

informa
healthcare

2nd Ophthalmic Drug Development and Delivery Summit

San Diego, CA, USA, 19 – 20 September 2006

Michelle Marra, Hovhannes J Gukasyan, Swita Raghava & Uday B Kompella†
†*College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198-5840, USA*

The Second Annual Ophthalmic Drug Development and Delivery Summit was held on 19 – 20 September 2006 in San Diego, CA, US. The 2-day symposium, having a highly focused theme, was packed with cutting-edge science, insightful overviews and networking opportunities. With a total of 11 recognized specialists presenting reviews and recent results in the advancement of ocular drug development and delivery, the invited expert speaking faculty presented the latest preclinical and clinical developments in novel ophthalmic therapies and drug delivery technology. The talks included various case studies from primary investigators and pharmaceutical companies touching upon key topics: updates on current clinical trials, study design issues, sustained delivery to the eye, views of the vitreous space as a drug reservoir, new developments in dry and wet age-related macular degeneration and diabetic retinopathy, formulation for optimal drug delivery, differences and similarities in developing drugs for the eye compared with other targets, pharmacokinetics, novel ocular delivery methods and devices, delivery of proteins and peptides, focal drug delivery, non-invasive drug delivery to the eye, neuroprotection challenges, *in vitro* and *in vivo* models for glaucoma and angiogenesis for early efficacy estimation, and toxicology. Overall, the 2-day annual symposium continues to grow as an efficient platform for fostering discussion on a range of scientific topics and challenges and avenues for building collaborative partnerships in ophthalmic drug development.

Keywords: age-related macular degeneration, controlled release, diabetic macular edema, diabetic retinopathy, disease models, microdialysis, microspheres, nanoparticles, ocular, sustained delivery

Expert Opin. Drug Deliv. (2007) 4(1):77-85

1. Meeting introduction

The symposium began with an introduction by Dr Val Harding (Pfizer, Inc.). Dr Harding highlighted new trends in ocular drug delivery, including the importance of new disease targets emerging with continued growth in understanding of the eye as an organ. Increased focus on understanding risk factors to ocular disease in order to provide early treatment or intervention was also emphasized. From an ocular drug delivery perspective, the development of technologies that would minimize the frequency of treatment or dosage form administration, utilize less invasive routes and methods of reaching target intraocular tissues and ultimately shift disease management towards better overall patient compliance was underlined.

2. Etiology of macular diseases and strategies for clinical studies

Dr Scott Cousins (Duke Center for Macular Diseases) reviewed the etiology of macular degeneration and the importance of understanding disease state complexity to address challenges in retinal tissue pharmacotherapy. Neovascular age-related macular degeneration (AMD) and diabetic retinopathy are the outstanding major illnesses in the ophthalmic therapeutic area. Because the complexity of neovascularization in AMD is routinely underestimated, the ultimate success of a drug may be in its ability to treat multiple aspects of the diseased state. Therefore, it may be beneficial to test drugs of interest against multiple target cells from inflammatory and vascular smooth muscle origin. As the actual cause of vision loss is not the newly formed blood vessels (i.e., choroidoretinal angiogenesis), novel active substances that can more specifically address the biochemical mechanisms of vision loss in combination with anti-angiogenesis may be superior to existing pharmacotherapy. Dr Cousins also highlighted potentially crucial elements that may be missed under the assumption that treating the abnormal anatomy of the eye during these disease states will always translate to improved vision – the primary clinical end point. Existing drug treatments that show 90% anatomical success, often exhibit only a rate of 30 – 40% in vision improvement.

Intravitreal injections of Macugen® (Eyetech Pharmaceuticals, Inc. and Pfizer, Inc.) and Lucentis™ (Genentech) have emerged as the proof-of-concept treatments and delivery methods for macular disease management. However, the injections are very costly and resource intensive. Quarterly administrations of the drugs do not produce positive results, therefore requiring a monthly treatment regimen. The new standard in clinical trials will be to demonstrate equivalent efficacy to ranibizumab, in terms of arresting vision loss with a decrease in dosing administration frequency. Furthermore, depending on the target and indication of the investigative new drug, correct stages of disease onset and progression must be identified when enrolling patients in studies. As alternatives to visual acuity, biological and biochemical end points (e.g., clinical biomarkers) can be incorporated as well. Finally, aspects of combinatorial therapy should be explored and pursued with greater interest.

Imaging technology is going to play a critical role in the design, execution and interpretation of clinical trial data for posterior segment disease treatment. With high-resolution deciphering of retinal anatomy, target tissues in AMD have emerged as the retinal pigmented epithelium (RPE), Bruch's Layer and the choroidal capillaries. New targets for the treatment of retinal diseases are emerging that will venture beyond VEGF, bFGF, PGE_2, and angiopoeitin. Fluorescein angiographic classification of choroidal neovascularization (CNV) is the major technique used clinically for the past 20 years. Optical coherence tomography (OCT) has recently emerged as a more quantitative method to investigate clinical cases of retinal disease. Dr Cousins made a strong case for using multiple imaging modalities for anatomical measurements by showing examples of diseased human tissues using aforementioned imaging methods. With fluorescein, the commonly used dye in angiography, only well-defined capillaries can be imaged. However, with indocyanine green (ICG), other strata emerge from the background, not usually visible when using flourescein. The commonly imaged subretinal space is increasingly becoming known as only the tip of the iceberg when considering posterior neovascularization. Dynamic high speed imaging with ICG allows visualization of the choroidal space, in addition to the subretinal vasculature. Furthermore, preliminary images indicate that ICG may be able to resolve between multiple types of neovascularization. In summary, Dr Cousins suggested that at certain clinical end points, it may be beneficial to combine multiple imaging techniques for prognosis, such as ICG angiography and OCT imaging.

3. Therapeutic targets and animal models

Dr Cathy Thut (Merck & Co., Inc.) focused on current and future approaches to neovascular AMD and diabetic retinopathy therapeutics. Disease states encompass two blood supplies of interest, choroidal and retinal, and these are each affected differently during AMD. The retina seems to be more affected in the diabetic disease state, and the choroid is the largely afflicted tissue in wet AMD. An overview of ocular antiangiogenic targets, animal models of ocular angiogenesis, early diabetic retinopathy targets and biochemical pathways, and animal models for diabetic retinopathy research were presented. Some popular ocular antiangiogenic therapies include VEGF inhibitors, receptor tyrosine kinase (RTK) inhibitors, steroids, growth hormone inhibitors and others (i.e., integrin inhibitors, Sdf1/CXCR4 pathway inhibitors, nACh receptor antagonists and pigment epithelium-derived factor gene therapy). Animal models for ocular angiogenic diseases include laser-induced CNV (an inflammation model), rodent retinopathy of prematurity (ROP) (a hypoxia model), corneal neovascularization (another inflammation model), ectopic growth factor addition and vein occlusion/ischemia (hypoxia/ischemia model).

Several observations were presented for ocular antiangiogenic targets. Many recent therapies have focused on the VEGF pathway, but there are still potential improvements to be made in this pharmacotherapeutic area in terms of dosing frequency and efficacy. Evaluation of small molecule multi-RTK inhibitors is important because of characteristic specificity towards other kinases, and, therefore, potential inhibitory activity in multiple disease pathways resulting in increased efficacy. From a drug delivery perspective, sustained release will be required for these molecules, as they are likely to display a short ocular half-life. Although the steroid class shows some potential, they have well-established undesirable side effects, such as ocular hypertension and cataract formation. Furthermore, due to additional known systemic side effects with steroids, local controlled delivery would be required. Other non-VEGF targets that show antiangiogenic

activity include integrin inhibitors, Sdf1/CXCR4 pathway inhibitors, pigment epithelium-derived factor gene therapy, and less characterized mechanisms involving squalamine and combrestatin 4-phosphate. Potential for improved therapies in the future is vast.

Several key messages regarding animal models of ocular angiogenesis were presented. At disease onset, mechanisms triggering neovascularization in AMD are unclear. In the diabetic disease scenario, degenerating capillaries send biochemical signals to nearby blood vessels, triggering them to proliferate. As a result of recent positive pharmacotherapy readouts in both the laser-induced CNV model and the clinic, there is now stronger evidence that some preclinical models are better predictive of certain human pathological features. There is less confidence in the O_2-induced retinopathy model due to anatomical result discrepancies in potential drug candidate activity when using either rat or mouse readouts. Current earlier-stage compounds in clinical trials have the potential to validate the utility of pre-existing and newer animal models for posterior segment disease within the next few years.

Early-stage diabetic retinopathy targets and models were also discussed. Anti-VEGF therapy is beneficial in late diabetic retinopathy, but not in early stages of the disease. Early-stage diabetic retinopathy disease-modifying approaches include: aldose reductase inhibitors (sorbinil, failed Phase III testing); AGE inhibitors (aminoguanidine, successful in Phase III, but has tremendous side effects), antioxidants (α-tocopherol, failed in Phase III) and PKC-β inhibitors (most effective, e.g., ruboxistaurin mesylate). It is critical to remember that animal models target different phases of diabetic retinopathy. For example, streptozocin-induced diabetes and genetic rodent models (*db*/*db* mouse, Akita mouse, Zucker diabetic fatty rat) are best applied in research for early diabetic retinopathy pathology. In contrast, the rat and dog galactosemia model is suitable for both early and late pathology research. Other laboratory models focusing on VEGF include VEGF-induced vascular leakage. In attempts to find treatments for diabetic retinopathy, some molecules were designed to address biological consequences of elevated glucose, such as the stimulation of the polyol pathway, PKC activation, increased generation of reactive oxygen species and increased AGE production. All of the noted pathways have been considered in clinical trials with unsuccessful results. Therefore, a shift towards addressing the consequences of elevated glucose, including altered growth factor expression, vascular inflammation, and impaired vascular repair capacity is apparent. Current research efforts are aimed at addressing the above end points.

The many animal models of diabetic macular edema (DME) include biologically true diabetic models, whereas others focus on specific aspects of the retinopathy. There is often a trade-off between the duration of the model versus the model breadth of applicability. For example, the genetic models of diabetes such as *db*/*db* mouse, Akita mouse and Zucker diabetic fatty rat are highly relevant in disease context, but the downside is that they are chronic models that require longer/sustained dosing. A galactose-induced microvascular damage model of the beagle dog is one of the better mimics of the retinal disease, but also has duration caveats and may not be a good way of evaluating drug dosage form performance. Although the VEGF-induced leakage model is more acute in nature, it only addresses specific VEGF-induced ocular vasculature leakage. Dr Thut pointed out the difficulties in making conclusions at this point regarding preclinical/clinical correlation due to the lack of substantial readouts from clinical trails so far.

Dr Jeffrey Edelman (Allergan, Inc.) focused his presentation on the wide applicability of steroids, especially dexamethasone, in the management of retinal diseases. The mechanism of steroid action might be a specific interference with VEGF signaling, or a general blockade of the inflammatory response. Dexamethasone blocks the secretion of cytokines, such as IL-1β and TNF-α (IC_{50} = 2 nM) and prevents $VEGF_{165}$-mediated retinal blood vessel vasodilation and blood retinal barrier breakdown. It also suppresses VEGF expression and inflammatory responses downstream of VEGF receptors. Multiple inflammatory mediators are involved in retinal vascular disease, including growth factors, cytokines, adhesion molecules and others. Data from mouse and primate animal models suggest that anti-VEGF and steroidal therapy are equally effective against retinal disorders. Evidence of dose-dependent dexamethasone-mediated inhibition of experimental CNV in mice was shown, while intravitreal implants of the drug completely blocked experimental CNV in model rats. In addition, dexamethasone vitreal infusion suppressed experimental CNV in primates. Although highly effective in the short-term, certain long-term side effects of glucocorticoids are cataract formation and ocular hypertension. It will be critical to minimize these side effects in the future.

The effect of dexamethasone on VEGF-mediated pathology for both retinal edema and AMD was discussed by Dr Edelman. Utilizing a rabbit model for retinal edema, evidence for mechanisms of glucocorticoid action in which a complete blockade of pathological responses downstream of VEGFR-1 and -2 (VEGF receptors) occurs, were shown. In addition, dexamethasone blocked a vasodilation response suggesting an orthogonal intersection of the pathways. Differential potency of dexamethasone is a common phenomenon depending on the target considered due to the multidimensional biological activity of this drug. When considering these inhibitory pathways, Dr Edelman highlighted data showing involvement of VEGFR-1 as a critical factor, in addition to the more commonly accepted VEGFR-2.

Dr Abbot Clark (Alcon Research, Ltd) focused on a review of preclinical models of glaucoma and their translational applicability to humans. This was the only discussion devoted to glaucoma at the conference, as most talks focused on retinal disease. There are three major anatomical sites of glaucomatous damage, which are the trabecular meshwork, optic nerve head and retinal ganglion cells. Dr Clark reviewed glaucoma risk factors, affected ocular physiology as a result of glaucoma, and

current glaucoma treatments. Pharmaceutical therapies include prostaglandin inhibitors, β-blockers, α_2-adrenergic agonists and topical carbonic anhydrase inhibitors. Some patients find themselves on all four types of medications at one point in time if they do not respond to monotherapy. Surgical interventions are also available, such as refined laser therapy procedures. One limitation, even in the developed world, is that an estimated > 50% of glaucoma cases in existence remain undiagnosed. In fact, 40% of retinal ganglion cells have usually died by the time glaucoma is diagnosed in the clinic. Besides diagnostics and monitoring, there are also challenges with treatment and patient compliance. In addition, current treatments only address one major risk factor: intraocular pressure (IOP), and do not directly intervene in ocular neuropathic progression.

In vitro models, such as trabecular meshwork and ciliary muscle cell cultures, provide avenues of rapid evaluation of established cell-type-specific biomarkers that would lead to *in vivo* IOP modulation. In addition, a multitude of *ex vivo* models are available that include perfused anterior segment isolates or whole bovine/porcine eyes, constant pressure/variable flow and constant flow/variable pressure models. Human anterior segments cultured for perfusion studies agree best with clinical results. Many *in vivo* normotensive models including rabbits, cats and primates exist. Hypertensive models, such as the laser-induced glaucomatous primates (in which the trabecular meshwork aqueous humor drain is intentionally damaged), glucocorticoid-induced ocular hypertension and various rodent models, are also available. In this context, human–rabbit correlation is poor in that in many cases, drugs work in man, but not in rabbits, or vice versa. Prostanoid agonists of the prostaglandin F (FP) type receptors and some β-blockers are examples of such drugs. The laser-induced monkey model has been determined over time to mimic human glaucoma disease. Most compounds that are effective in this model lower IOP in humans. Importantly, this translation spans across various pharmacological mechanisms of action, including β-blockers, topical carbonic anhydrase inhibitors, α_2-adrenergic agonists, prostaglandin analogs and 5-HT agonists. Improved techniques for IOP measurement (i.e., 'TonoLab IOP measurement') in conscious, non-anesthetized animal models of mice and rats are also emerging.

4. Drug-delivery systems and barriers to drug delivery

4.1 Overview of delivery systems

Dr Ashim K. Mitra (University of Missouri) surveyed colloidal approaches to drug-delivery systems. These include many established drug delivery approaches such as emulsions, suspensions and microemulsions, and more novel approaches such as liposomes, niosomes, microspheres, nanoparticles, dendrimers, and polymer-based drug-delivery systems. Selected marketed products were discussed that utilize novel colloidal drug delivery approaches. Although the presented marketed products were of non-ophthalmic indications, Dr Mitra suggested that many of the basic principles and existing technology can also be applied to ophthalmic drug delivery.

Liposomes are lipid assemblies that store drugs in their core or bilayer with a few commercial product examples. Poly(DL-lactic-co-glycolic acid) copolymer (PLGA)-based microspheres, also having commercial precedence, are being successfully utilized as biodegradable implants. Nanoparticles are a newer and more desirable medium for some modes of ocular drug delivery. Particulate systems in general stabilize their drug load on two dimensions – chemically and/or metabolically. Dendrimers are monodisperse macromolecules with a regular and highly branched three-dimensional architecture. They also provide multiple advantages in drug delivery design space: for example, they possess cavities for drug loading and storage, with highly tunable surface chemistry, and no known toxicity. Polymeric drug-delivery systems are desirable for intravitreal injection; however, their toxicity or compatibility with ocular tissues over extended-release periods are unknown. Delivery systems for intravitreal injection require many attributes, such as biocompatibility, mechanical strength, comfort, capability of achieving high drug loads, safety from rupture, ease of administration and/or removal, and ease of fabrication and sterilization.

Formulation variables for some of the drug delivery approaches were presented. Different physiological stimuli towards which polymer sensitivity can be engineered include pH, ionic strength, thermal changes, electrical stimuli and even ultrasound. Some novel biodegradable polymers for ocular delivery are polyanhydrides and polyesters, which should be considered in comparison with the older known systems of poly(lactides/glycolides) and their copolymers. The presence of low-molecular-weight compounds, processing conditions, annealing, sterilization, storage history, shape, site of implantation and adsorbed and absorbed physiological substances can also influence the biodegradation of these polymers. In summary, environmentally sensitive drug delivery is highly viable with specialized biodegradable polymers – pH and osmolality of environment, chemical species and enzymes, as well as magnetic, thermal, electrical and ultrasound irradiation can all be used to modulate drug release from fabricated polymeric implants.

The challenges associated with topical delivery to the back of the eye, such as corneal barriers and precorneal loss, were briefly reviewed. Ocular tissue barrier properties were also discussed within this context. In addition to the ocular surface physical barriers and anterior elimination pathways, normal intraocular fluid flow gradient is from vitreous to aqueous. This makes compounds that can be successfully delivered to the anterior chamber after topical dosing virtually non-transferable to the vitreous cavity. Dr Mitra stressed that without a breakthrough, it will be very difficult to achieve successful topical delivery to the back of the eye. It was also noted that past attempts for topical delivery to the back of eye using a variety of emulsions, suspensions and microemulsions, have failed.

Other tissues that may serve as barriers for ocular drug delivery were introduced. The sclera is mainly a hydrophilic matrix and is not a significant barrier compared with other physiological membranes. Hence, given that a molecule of interest maintains < 100 kDa in molecular weight, the general understanding is that scleral permeability will not be rate limiting in ocular drug delivery and distribution. Within the posterior segment context, the blood–retinal barrier is similar in structure and function to the blood–brain barrier and, due to the presence of tight junctions, the RPE is also rate limiting for the delivery of some drugs. The conjunctival route may be the only topical penetration pathway enabling posterior drug delivery. The systemic route to the posterior may become a possibility with a better understanding of the blood–retinal barrier and concurrent identification of 'druggable' and ocular-specific targets.

In the last segment of his talk, Dr Mitra provided examples of advances in ocular drug delivery methods. For example, ganciclovir PLGA microspheres have been developed for delivery to the posterior segment of the eye for the treatment of cytomegalovirus retinitis (CMV), which is the most common opportunistic infection occurring in 15 – 42% of AIDS patients. Ganciclovir is the first FDA-approved drug for CMV treatment. The blood–ocular barriers prevent entry of ganciclovir into the vitreous/retina at therapeutic levels. Therefore, intravitreal injections were utilized to achieve therapeutic concentrations in the vitreous. A key limitation for local ocular ganciclovir delivery is its short elimination $t_{½}$ in the vitreous, which necessitates twice-weekly injections. Microspheres made of PLGA polymers are being developed to reduce injection frequency. Ganciclovir release from these prototypical microspheres consists of three phases: an initial diffusion phase, a slow- or no-release phase, and the degradation release phase. The initial phase depends on the diffusivity of the drug within the polymer matrix. The second phase depends on the rate and efficiency of surface hydration, followed by initiation of polymer hydrolysis from the surface towards the interior. The third phase of drug release is directly dependent on the critical molecular weight of PLGA – a threshold value that also governs polymer biodegradation. One challenge for microsphere-mediated ganciclovir delivery has been the need to use various release-enhancing agents to minimize the duration of the second slow- or no-release phase, such that efficacious levels of drug are present at all times. Another challenge resides in the fact that PLGA microspheres do not suspend well in aqueous vehicles, because they are very lipophilic in nature. In addition, there is potential for vision obstruction, which needs to be further evaluated. After intravitreal administration, the microspheres may migrate away from the site of injection causing vision obstruction or retinal irritation.

A sustained-release system consisting of ganciclovir-loaded PLGA microspheres dispersed in thermogelling PLGA-PEG-PLGA polymer gel was recently developed and tested [1]. The gel-based formulation was found to exhibit more restrained drug-release behavior. The microspheres remained entrapped in the gel matrix for up to 2.5 weeks. Mixtures of gel and microspheres in various ratios can be used to attain and tune sustained release of ganciclovir. The thermal settling gel provides an added advantage by slowing down the rate of drug release during initial diffusion phases compared with microspheres alone. To characterize the *in vivo* pharmacokinetic performance of the ganciclovir microsphere formulation, a previously established conscious rabbit model with a linear microdialysis probe was employed [2].

4.2 Implantable systems for transscleral delivery

Dr Karl G. Csaky (National Institutes of Health) discussed what he considered the four critical aspects of successful ocular drug delivery: i) identification of druggable therapeutic targets; ii) integration into the drug discovery process; iii) strategic investment in protection of intellectual property around delivery technology; and iv) global understanding of drug delivery physiology. Important considerations in ocular drug discovery are truly multidimensional. A required high drug activity is critical due to the confined delivery space of the eye. Although the optimal physicochemical space is yet unclear in this emerging field, the importance of molecular charge and size remain true. Finally, to facilitate sustained drug delivery, slow dissolution is another desirable chemical feature that may provide an intrinsic buffer towards rapid clearance from intraocular space.

Dr Csaky reviewed recent work done on reservoir implants with Gilger *et al.* [3]. Silicone mold designs for polymeric reservoir implants allow for good reproducibility during manufacturing. Through a relatively low-technological process, hundreds of implants can be manufactured using minimal human resources with the silicone mold approach. Implants must be engineered to fit into various *in vitro* models, tissues and ocular areas; hence, their rational design and ease of manufacturing are critical. The polymer mold can be adjusted to shape as desired, which has been an advantage for the design and testing of prototypes into various animal models. Drug-release rates from these polymeric implants is essentially modulated by their thickness, making this a parameter that can be fine-tuned to match a desirable profile. Single-action polymers designed solely for sustained release, and dual action polymers which provide both a loading dose followed by sustained release are technically feasible for production and use.

The importance of ocular physiology and lymphatic drainage within the context of treating disease were emphasized. Information describing the impact of live ocular pharmacokinetic monitoring through special imaging techniques, as opposed to sampling preclinical species after termination, was presented. Currently available methods for monitoring drug distribution in preclinical species include radioactivity, MRI and direct drug level measurement. With advantages in sensitivity and selectivity, post mortem animal tissue preparations pose limitations with radioactive-tracing

assays. To illustrate, a rabbit study using MRI, in which a gadolinium-diethylenetramine pentaacetic acid (Gd-DTPA) implant was placed on the episclera, was presented [4]. The live MRI scans showed negligible intraocular drug exposure. However, in sacrificed animals, there was substantially more drug found in the eyes, suggesting that the barriers were not so much anatomical, but more dependent on the circulatory physiology. Although both ocular tissue configurations used in Gd-DTPA distribution studies applied MRI detection, a substantial difference between drug penetration and elimination in live and post mortem samples could be detected. Elimination into the lymph – a relatively unknown clearance pathway – was also apparent with the MRI technique, and would not have been detected by other methods requiring ocular tissue isolation and counting.

The spatial location of implants can also be critical for disease management and pharmacotherapy, as was demonstrated in horses with equine recurrent uveitis receiving ciclosporin A carrying implants [3]. Implants placed episclerally failed to control inflammatory episodes in equine recurrent uveitis, but those placed in the deep sclera adjacent to the suprachoroidal space resulted in high levels of ciclosporin A in target tissues. *In vivo* imaging confirmed that the lymphatics on the surface of the eye played an important physiological role in drug elimination for the former, and not the latter, implant configuration.

4.3 Injectable particulate systems for transscleral drug delivery

Dr Uday Kompella (University of Nebraska Medical Center) provided an overview of sustained-release systems for ophthalmic drug delivery, emphasizing his research on the application of particulate drug-delivery systems for periocular administration as a modality for delivering drugs to the posterior segment of the eye [5]. Sustained-release depot systems reviewed include: intraocular implants, episcleral plugs, polymeric particles and relatively novel encapsulated cell technologies. Furthermore, Dr Kompella reviewed other retinal drug delivery approaches, including light-targeted delivery, iontophoresis and electroporation, and macromolecular carrier conjugation for passive or active targeting.

Based on his research, Dr Kompella provided examples of periocular injections of celecoxib suspensions that provided higher drug levels in sampled ocular tissues of the sclera, retina, vitreous, lens and cornea, compared with intraperitoneal injection [6]. Furthermore, PLGA/PLA microparticles and nanoparticles can sustain levels of drugs in ocular tissues, compared with a simple solution/suspension. Based on a critical particle size, periocularly-injected particulate formulations are retained at the site of injection when above this threshold, and rapidly eliminated when the mean particle size distribution falls below. A study in Dr Kompella's lab showed that 200 nm and 2 µm particles were retained at the periocular site of injection; however 20 nm particles were rapidly cleared [7]. More rapid leakage of smaller nanoparticles can be attributed to their rapid backward leakage along the needle track at the site of injection or entry into systemic or lymphatic circulation. Larger particle systems of 2 µm and 200 nm did not leave the site of injection over 60 days of monitoring. Consistent with particle retention in the periocular space, celecoxib-PLGA microparticles sustained retinal drug delivery for at least 2 months, and inhibited retinal vascular leakage, VEGF levels and PGE_2 levels in a diabetic rat model [8].

Although the blood and lymphatic circulations near the site of administration contribute significantly to the clearance of drugs following periocular injections, Dr Kompella stressed that the physiological barriers, including the choroid and RPE underlying sclera, offer significant resistance to solute transport across sclera. For instance, the choroid layer reduces transscleral transport of hydrophilic and lipophilic solutes, including mannitol, fluorescein, atenolol, budesonide and celecoxib in bovine, as well as porcine eyes [9]. The presensce of RPE further reduces the transscleral transport of solutes. Overall, the tissue layers underlying sclera seem to be greater anatomic barriers to solute transport, compared with sclera itself. For the above solutes, it is noteworthy that both bovine and porcine scleras and sclera-choroid layers resulted in similar permeabilities [9].

Based on a PubMed analysis, Dr Kompella noted that there are more toxicity reports for the intravitreous route of administration, compared with all of the periocular routes combined. In addition, there are more pharmacokinetic studies for the vitreous compartment than periocular routes. For both periocular and intravitreal routes, there are more pharmacodynamic studies than pharmacokinetic studies. Interestingly, there are a lot more pharmacodynamic studies in humans employing periocular routes compared with intravitreal administration. Thus, although periocular transscleral drug delivery is more readily assessed in the clinical setting, there is a lack of pharmacokinetic studies in the literature. Systematic assessment of pharmacokinetics and drug distribution mechanisms following periocular administration, including assessment of drug-disposition from various periocular sites, evaluation of barrier properties of the sclera, choroid and RPE, and minimization of the systemic and/or lymphatic loss of the drug and delivery systems will be required for future rational design of delivery systems with enhanced fraction of dose delivered. In addition, there is a need to develop safe and aesthetically acceptable periocular sustained delivery systems to be used in the pharmacotherapy of chronic ocular disorders.

Dr Kompella noted that all discussed novel ocular delivery approaches seem to be feasible at this point; however, each has its own caveats that would need to be addressed. For example, safety is a primary concern in iontophoresis and electroporation methods. Approaches involving photodynamic therapy or drug conjugation using polymers or antibodies that employ intravenous use require repeated injections, rely on the leakiness of vessels for delivery, and the drug release at a molecular level needs to be ensured.

4.4 Injectable *in situ*-forming implant systems for transscleral delivery

Dr Eric J. Dadey (QLT USA, Inc.) discussed the compatibility and utility of the Atrigel® (QLT, Inc.) drug-delivery system for the sustained release of drugs to the eye, which can be administered by the intravitreal, subconjunctival or subtenon routes. The Atrigel delivery system is a polymer-based formulation that can provide sustained drug release for the duration of a few months. The implant system is composed of a solid polymer, liquid carrier and the drug. The product packaging is composed of two syringes that have a luer-lock connection, in which the components are mixed directly prior to administration. The polymer is a biodegradable PLGA backbone with built-in dual hydrophobic and hydrophilic functionalities. There are many features of the system that are controllable on a molecular level (i.e., initial characteristic drug burst from PLGA-containing systems). By varying the molecular weight content, biodegradation of the polymer can be varied to provide drug release control. Further control can be achieved by changing the hydrophilic character of the polymer through reduction of lactide to glycolide ratio (i.e., more lactide units result in longer biodegradation-dependent release). The esterolysis rate of the polymer can be controlled with the same strategy. The system is injected as a liquid, which then solidifies in the body. This delivery system is potentially applicable for small and large molecules, proteins and peptides, and nucleic acids. A number of biocompatible solvents that are used in the Atrigel® have been identified, and the preferred solvent is *N*-methyl-2-pyrrolidone. However, currently approved Atrigel products that contain this solvent are not for ocular use.

The performance of Atrigel was evaluated and optimized by *in vivo* subcutaneous implantation. Understanding of *in vitro/in vivo* correlation for currently used drugs within this delivery system requires further refinement. From a safety perspective, there is little reaction to the subcutaneous implant; however, vasodilation seems to be associated with the needle-injection trauma. Several examples of commercial formulations were reviewed, including leuprolide acetate peptide. Data from leuprolide acetate suggested that the preclinical release profiles are predictive of clinical experience with no required changes in formulation. Ocular tolerability examples with vehicle alone, and one ocular drug delivery example with octreotide were presented without any safety findings. A good correlation between the intraocular, periocular and subcutaneous release profiles of octreotide release was apparent from the implants, suggesting that subcutaneous measurements may be translatable into the ocular setting. It is noteworthy that the intravitreal injection data presented showed 50-times the tissue concentrations compared with subtenon administration. Atrigel sustained-release drug-delivery system seems to be tolerated following intra- and peri-ocular injections. Macrophage activation was reported initially; however, after implantation the injection site-specific effect self-resolved. Dr Dadey noted that from a systemic point of view PLGA polymer safety is not an issue due to elimination of biodegradation by-products in the Krebs cycle. Overall, PLGA polymers can be used in a variety of hydrophilic and hydrophobic solvents, with the preferred carrier as *N*-methyl-2-pyrrolidone, having six FDA-approved products. Application of the Atrigel platform technology will require an NDA, as it is not considered a device by the government regulators.

4.5 Vitreous as a drug reservoir

Dr Clive G. Wilson (Strathclyde University) discussed the vitreal characteristics across species and their changes with age. The deceptively simple vitreous humor historically has not been scrutinized because, in itself, it is not a target for pharmacotherapy. However, it has some important implications for drug delivery. The vitreous functions as a shock absorber for the retina, maintains a reservoir of nutrients for the lens, prevents invasion by extraocular cells or proteins, and helps maintain IOP. It is a transparent gel that is 98% water, with a refractive index of 1.33, and a viscosity value of 2- to 4-times that of water. The vitreous compartment is not well-mixed nor homogeneous, its viscosity is dependent on the resident concentration of Na^+-hyaluronate and varies in different spatial regions. The vitreous viscosity is lower in the aged eye compared with young eyes. A key element is the presence of connecting collagen fibrils. These are highly species dependent, and they tend to collapse with age. These fibrils can be visualized using dark-field illumination imaging techniques.

Multiple notable differences between vitreous content across species and with age were described, considering their potential implications on intravitreal dosage form performance. For example, human vitreal protein content is higher compared with that of rabbits. Vitreous in aged eyes tends to shrink compared with younger eyes, displaying features of vacuolation, central liquefaction and an increase in protein content. Lacunae develop in the periphery of the aged vitreous compartment in addition to thickened central fibres. Hence, a remaining question for intravitreal drug delivery is the optimal dosage form design to fit into this heterogeneous space. One of the key messages from Dr Wilson was that most clinicians discount the importance of vitreal structure and status from a uniform intravitreal drug distribution viewpoint. The assumption that drugs applied intravitreally quickly diffuse in a uniform fashion needs to be revisited in a more critical manner.

Some key questions were raised for future discussion that must be better understood for successful intravitreal drug delivery: how does drug distribution vary if dosage forms are injected into one of several subcompartments of the vitreous? What are the consequences on disposition of intravitreal injections relating to the complexity of this system? In older patient, ageing vitreous can accentuate distribution effects, particularly for high molecular weight drug candidates and particulate delivery systems. Current animal models and computer simulations grossly ignore these considerations and therefore pose a risk of developing inadequate dosage forms for the clinic.

4.6 Intravitreal implants

Dr Paul Ashton (pSivida, Ltd) provided an overview of several generations of sustained drug delivery to the vitreous and retina using the intravitreal implant systems developed by pSivida. The Vitrasert® implant, which received FDA approval in 1996, was the first device that provided sustained intravitreal pharmacotherapy > 6 – 8 months, and required surgical insertion through a 6 mm incision. Retisert™ was the next generation of this ocular implant device, which received FDA approval in 2005. Improvements in Retisert include a longer sustained-release period of ~ 3 years, and the requirement of a smaller surgical incision of 3.5 mm. The third-generation delivery system, Medidur™, provides comparable sustained-release features to that of Retisert, but can be injected with a 25-gauge needle without the necessity of an incision. Medidur is currently entering Phase III clinical trials and has been granted fast-track review by FDA.

The basic premise of delivery systems has not changed over several generations of products. Release from a device is modified by optimizing the accessible surface area and using a selective-permeability polymer coat/cover. The drug is also coated, with a laminated series of polymers with varying degrees of permeability, with some being impermeable. Therefore, the engineering allows for a biphasic release achievement if desired. The mechanism of release after insertion into the vitreous depends on water passing through outer surface pores on a permeable membrane cover into the implant core, where it dissolves the resident drug, creating a permanent, high concentration gradient for the drug in the insert. This configuration results in linear release.

Vitrasert was first used for sustained delivery to treat CMV with the antiviral agent ganciclovir. Vitrasert is a non-erodible implant having a main side effect of retinal detachment, with 10% prevalence. However, it should be noted that many patients with CMV get retinal detachment without proper pharmacotherapy. Retisert, the second-generation device, was first developed in the treatment of uveitis – an autoimmune disease that causes severe inflammation of the eye and is a leading cause of blindness in the US. Fluocinolone acetonide is the corticosteroid used with Retisert. A clinical trial over 3 years was conducted in patients with one eye receiving the implant. The systemic side effects typically associated with steroid treatment were eliminated. However, ocular side effects, such as elevated IOP and the need to have filter surgery, were increased. Therefore, when using this implant, a two-stage process of implantation followed by filtration surgery is generally anticipated. The same drug was also effective in treating DME, for which the presented data suggested that implanted patients showed advantageous clinical end point readouts and did not need surgical intervening. There was a standard of care comparison with an available implant that came out consistently inferior to the Retisert device. For this indication, the primary side effect was also IOP, with the subsequent need for surgery. There were also cases of cataract formation.

Medidur is entering Phase III trials for the target indication DME. The duration of drug release is controlled by the physical length of the implant, and can vary from weeks or months to a year. As the third-generation device, Medidur offers the advantage of being erodible or non-erodible. Because this is a substantially smaller device than its predecessors that can be injected with a 25-gauge needle, and does not require surgery, it has the potential to deliver a wide range of drugs. However, the agents need to be potent and target appropriate biomarkers of disease, due to the Medidur's small size and limited load.

5. Species differences and development of AG13958

Dr Kay Rittenhouse (Pfizer, Inc.) discussed current challenges and opportunities in pharmacokinetics and pharmacodynamics for ocular drug delivery, efficacy and safety. Dr Rittenhouse reviewed ocular comparative physiology of several model species. There are many physiological differences between species that make translational ocular drug development challenging. For example, anterior chambers of human and rabbit eye are similar in comparison, but these two species differ greatly in posterior chamber constitution. Rabbit vitreous is significantly less viscous compared with human vitreous. Another challenge to ocular drug delivery is the inability to obtain human pharmacokinetic data. Noteworthy results included the pharamcokinetic parameters of Macugen in primates. There, the intravitreal $t_{½}$ was found to be 94 h, and the plasma $t_{½}$ was 102 h, providing more credence to the frequent observation that pharmacokinetic parameter of $t_{½}$ is mirrored for intravitreal–plasma correlations.

The distribution of some drug candidates in the back of the eye following subtenon's and intravitreal space implantation were discussed. AG13958 – a drug candidate for wet AMD undergoing clinical trials by Pfizer – displays low water solubility and has been delivered through the subtenon's capsule and intravitreal routes of administration. During preclinical evaluations of AG13958, the rabbit model was used for pharmacokinetic studies following subtenon's capsule injection. It is known that this specific periocular space is looser in the rabbit model than in monkeys. In light of these differences, rabbit choroidal concentrations were 1500 versus 50 ng/ml at 16 weeks following intravitreal and subtenon route of administration, respectively. One key conclusion offered by Dr Rittenhouse was that despite the larger dosing spaces/volume offered by the subtenon route, significantly lower levels of drug reach the site of action due to permeability barriers, requiring further study.

AG13958 has demonstrated efficacy in several preclinical pharmacology models. The candidate provided complete inhibition of VEGF2 over at least 32 h in a 8-day non-neonatal rat model of neovascularization. In addition, it was determined that AG13958 was equally effective in the neonatal rat model, as well as in laser-induced CNV rodent

models. AG13958 is in a clinical study Phase I/II program, and is using intravitreal injection as the route of administration. Outcomes of that trial will be available shortly, which should provide further clarity about translational efficacy models and their correlation to humans.

6. Conclusion

In summary, the 2nd Ophthalmic Drug Development and Delivery Summit provided critical updates on clinical studies, new drug targets, animal models, and intravitreal and periocular drug-delivery systems along with the remaining challenges in developing therapeutic approaches for AMD, diabetic retinopathy and glaucoma. The symposium format and organization was an efficient platform for fostering dialog between attendees on a range of scientific topics and challenges. The Summit remains a growing avenue for building collaborative partnerships in ophthalmic drug development.

7. Expert opinion

Drug development and delivery for the posterior segment is undergoing a major developmental thrust and resource allocation from pharmaceutical companies and biotechnology firms alike. The successful launch of Macugen and Lucentis, and the demonstrated efficacy of corticosteroids and Avastin® (Genentech) are paving the way for the development of several new drug candidates for treating diseases of the posterior segment. Preclinical studies are particularly promising with receptor tyrosine kinase inhibitors. With all of the new drugs under development, there is a need for better delivery approaches to enhance patient comfort and compliance. Fortunately, the ophthalmic field is among the most innovative in novel drug delivery system development and commercialization. Intravitreal implants such as Retisert can sustain drug delivery for as long as 3 years. Similar implants are under development for transscleral delivery. To eliminate the surgical placement required with such implants, injectable biodegradable particulate systems and *in situ* forming biodegradable implants are under development. Although the intravitreal route offers high retinal drug bioavailability, drug delivery from the periocular routes is limited. With the identification of vascular, lymphatic and tissue barriers to transscleral drug delivery, it is anticipated that future drug-delivery systems will overcome some of these limitations to deliver a greater dose fraction to the retina and other tissues of the posterior segment. The success of any new drug or delivery system depends on a clear understanding of preclinical models and carefully designed clinical trials with respect to end points. With new drug and delivery system discoveries, and ongoing discussions on better approaches to clinical trial design for ophthalmic drug products, it is anticipated that more products will enter the posterior segment market in the near future.

Bibliography

1. DUVVURI S, JANORIA KG, MITRA AK: Development of a novel formulation containing poly(d,l-lactide-co-glycolide) microspheres dispersed in PLGA-PEG-PLGA gel for sustained delivery of ganciclovir. *J. Control. Release* (2005) 108(2-3):282-293.
2. MAJUMDAR S, KANSARA V, MITRA AK: Vitreal pharmacokinetics of dipeptide monoester prodrugs of ganciclovir. *J. Ocul. Pharmacol. Ther.* (2006) 22(4):231-241.
3. GILGER BC, SALMON JH, WILKIE DA *et al.*: A novel bioerodible deep scleral lamellar cyclosporine implant for uveitis. *Invest. Ophthalmol. Vis. Sci.* (2006) 47(6):2596-605.
4. KIM H, ROBINSON MR, LIZAK MJ *et al.*: Controlled drug release from an ocular implant: an evaluation using dynamic three-dimensional magnetic resonance imaging. *Invest. Ophthalmol. Vis. Sci.* (2004) 45(8):2722-2731.
5. RAGHAVA S, HAMMOND M, KOMPELLA UB: Periocular routes for retinal drug delivery. *Expert Opin. Drug Deliv.* (2004) 1(1):99-114.
6. AYALASOMAYAJULA SP, KOMPELLA UB: Retinal delivery of celecoxib is several-fold higher following subconjunctival administration compared to systemic administration. *Pharm. Res.* (2004) 21(10):1797-804.
7. AMRITE AC, KOMPELLA UB: Size-dependent disposition of nanoparticles and microparticles following subconjunctival administration. *J. Pharm. Pharmacol.* (2005) 57(12):1555-1563.
8. AMRITE AC, AYALASOMAYAJULA SP, CHERUVU NP, KOMPELLA UB: Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. *Invest. Ophthalmol. Vis. Sci.* (2006) 47(3):1149-1160.
9. CHERUVU NP, KOMPELLA UB: Bovine and porcine transscleral solute transport: influence of lipophilicity and the Choroid-Bruch's layer. *Invest. Ophthalmol. Vis. Sci.* (2006) 47(10):4513-4522.

Affiliation

Michelle Marra[1], Hovhannes J Gukasyan[1], Swita Raghava[2] & Uday B Kompella[†2]
[†]Author for correspondence
[1]Research Formulations, Science & Technology, Pfizer Global R&D, La Jolla Laboratories, Pfizer, Inc., San Diego CA 92121, USA
[2]College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198-5840, USA