Testing of Intraocular Drugs for Clinical Use

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In recent times, the use of intraocular drugs has become frequent in ophthalmology, in particular in the treatment of retinal diseases. Such treatments are most often off-label use of compounds that have been developed for systemic use, not as primary intraocular agents. The modern history of this off-label use began with drugs for endophthalmitis and then progressed to anticytomegaloviral drugs for human immunodeficiency virus-infection-associated acute retinal necrosis syndrome. More recently, triamcinolone has come into widespread use for patients with macular edema from a variety of causes.

In addition to therapeutic intravitreal agents, intravitreal drugs have become popular as surgical adjuncts to pars plana vitrectomy. Compounds such as ICG, trypan blue, and triamcinolone have been used to assist visualization of the internal limiting membrane, epiretinal membranes, and vitreous cortex and have been widely considered useful adjuncts to vitreoretinal surgery.

One of the problems with the use of intraocular drugs, particularly if not primarily developed for this purpose, is the conflicting publications in the scientific literature about their safety. For example, a search of PubMed for the use of ICG in vitrectomy surgery returned 146 papers published in the area. These included clinical studies in patients, in vivo studies in animals, and in vitro studies of the dye in tissue culture. The conclusions were widely conflicting, with some finding that ICG is safe and others that it is toxic. Some investigators have decided that if the retina is intact (i.e., no macular hole), then the use of ICG is safe, but if the substance comes in contact with the retinal pigment epithelium (RPE), toxicity ensues.

The multiple methods of evaluation of drugs or compounds to be used in the eye lead to much confusion about their toxicity. For example, ICG is widely used to aid in visualizing the internal limiting membrane of the retina so that it can be removed in eyes with macular holes and macular edema, yet there is controversy regarding its potential for toxicity. In tissue culture systems and in some studies in which in vivo evaluation systems were used, the dye has been found to be either safe or toxic at similar concentrations. Some investigators have attributed toxicity to the osmolality of the solution and have suggested that isosmolar preparations of ICG are not toxic. Others have concluded that toxicity may be light dependent, and still others have concluded that the compound is toxic only to the RPE cells and not the retina. We are in the uncomfortable position of having a dye in use as a surgical adjunct whose safety seems to be controversial. Standardized methods of evaluation that are universally acceptable seem to be lacking.

One of the problems in evaluating such drugs and compounds is the lack of a standard agreed-on methodology. Certainly tissue culture is one way to evaluate such drugs, but the results may be misleading. The intraocular milieu is complex and difficult to replicate in a tissue culture system. For example, testing of the toxicity of drugs injected into the vitreous using RPE cell lines may be irrelevant, as most of the drug is in contact with the retinal surface, not the RPE. It is difficult to replicate the clinical situation in vivo in tissue culture, and the relevancy of such results is questionable.

Using in vivo systems or tissue culture cell lines, several groups have made efforts to evaluate the effect of triamcinolone. One problem is that triamcinolone is water insoluble. When injected into the human vitreous cavity, the compound may not even directly contact the retinal surface, because much of it is suspended in the liquid or formed vitreous. The effect of the drug on the retina has been reported by some observers to be beneficial in cases of macular edema due to several different diseases. Such an effect is presumably mediated by the solubilized fraction of drug; the portion of triamcinolone crystals that dissolve in the vitreous fluid. It is difficult to know if, in vivo, triamcinolone crystals are in equilibrium, what the soluble fraction of triamcinolone is in the vitreous, and precisely what concentration of soluble drug is available to act on retinal cells. Do microcrystals in the vitreous get absorbed by intraocular cells and if so, what is the pharmacologic effect of this method of drug delivery?

For example, the RPE is not exposed to triamcinolone crystals in the clinical situation (except perhaps in eyes with macular holes). Therefore, placing such crystals onto RPE monolayers may not be relevant to the clinical situation. What is the free level of drug in tissue culture? Is toxicity due to solid crystals in contact with cells or actually due to the pharmacological effects of dissolved steroid? Most authors have not measured free-drug concentrations at the different time points for which they report data. Furthermore, if toxicity is seen, the mechanism is not clear. The drug may not be interacting molecularly in vivo with its receptors in the same way as in vitro assays. Recent papers published in this journal and others highlight the difficulties with in vitro evaluations of drugs intended for intravitreal injection. Such studies report total (free/soluble and crystalline/insoluble) triamcinolone ace-tonide (TCA) concentration and do not necessarily accurately reflect the true free (soluble)-drug concentration in their cultures, nor do they report the concentration of vehicle to which the cells were exposed. Culture medium is a stable milieu with steady state of the drug, and it would have been helpful to know the free-drug concentration at the different time points for which the various investigators reported data. Furthermore, the mechanism of interaction of free drug within cells is unclear. Presumably, it is not interacting molecularly with the cellular receptors in the same way.

Results in studies evaluating triamcinolone in vitro have caused concern that damage to the cultured cells could be the result of mechanical contact of TCA crystals with the cell monolayer, a condition that would be very different from that in the in vivo retina. Gravity would cause the crystals to settle.

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on the surface of cells in culture, thereby exacerbating potential mechanical damage, whereas in vivo, any crystallized drug would tend to settle in the inferior vitreous and would not produce any significant mechanical injury to most of the neural retina. Because of the presence of the more condensed vitreous cortex, which serves as a cushion to the drug’s mechanical effect, crystallized drug in a living eye could cause far less physical damage. The reports lack a description as to what happened to the size of TCA crystals during the incubation period. Were the crystals small enough to be phagocytosed by cells?

Evaluation of liposomes, crystalline drugs, and insoluble drugs that function as long-acting delivery systems is particularly difficult.8–12 Such drug delivery systems are placed into the vitreous cavity in the patients’ eyes, and the delivery of therapeutic drug to the tissue of interest can occur in several ways. First, there is a dissolution of equilibrium, at which point the drug is released from its solid form; or, if it is entrapped in a liposome, there is a rate at which the drug crosses the lipid membrane of the liposome (which may or may not be a linear function of time, potentially resulting in complex pharmacokinetics).13 Both processes result in free, solubilized drug in an aqueous environment, which would then be subjected to the forces that act on such a soluble drug. These include several exit pathways from the vitreous cavity including the anterior chamber angle, the uveoscleral pathway, and the path across the retina along with the vitreous water, which is pumped by the RPE into thechoriocapillaris. In addition to this soluble mechanism, small particles of solid drugs come into contact with the retina and other intraocular cells, which, once they reach a certain size, may be engulfed by the cells. In areas of exposed RPE, this may be an important source of uptake.

Free-drug molecules interact with cells in several different ways, depending on the chemical composition of the compound, including receptor-mediated signaling effects such as the opening of ion channels or the initiation of intracellular second-messenger signaling cascades; receptor- or transporter-mediated endocytosis; and, in the case of lipophilic compounds (e.g., steroids), diffusion across the cell membrane bilayer. Another important but often overlooked source of drug carrier and delivery particle clearance is the potential phagocytosis by different cell types. The eye contains several highly active phagocytic cell populations as part of its normal physiology, which can putatively uptake micro- and nanosized particles, depending on where these particles are located in the eye. RPE cells phagocytose photoreceptor outer segments daily in a circadian cycle. In the neural retina, both microglial cells and Müller glial cells are phagocytic.14

Another way to prolong the duration of action of intravitreally injected drugs is to design drugs that have prolonged intracellular durations of action. One of the first such drugs used was cidofovir (HPMPC).15,16 Studies of intravitreal injection of this nucleoside phosphonate anti-CMV drug have shown that the vitreous half-life is short, approximately half a day. The duration of anti-CMV action in eyes with CMV retinitis is therefore expected to be on the order of a week or so. When cidofovir is absorbed into cells, the phosphorylated drug is strongly polar, and this polarity prevents exit of the drug through the cell membrane. Thus, the intracellular half-life is quite long. Cidofovir has been shown to control CMV retinitis for 6 weeks after a single injection. This strategy can be used in other ways. Liposomes can be designed that incorporate a drug into the wall of the liposomes if the drug is lipid derivatized. These liposomes may be engulfed by retinal cells and may also fuse with the cell membrane.17

There is evidence to show that such drugs have longer durations of action in the retina than predicted by vitreous elimination curves. Other classes of drugs may have prolonged intracellular duration and actions. Compounds such as ribozymes and small interfering RNA enter cells and have enzymatic and other properties that interfere with the production of messenger RNA.18,19 Such compounds are under development for ophthalmic uses and have been tested as inhibitors of the scarring in eyes with recurrent retinal detachment. They are also under clinical development as inhibitors of VEGF production in eyes with choroidal neovascularization. The theoretical potential of a prolonged duration of action by these drugs after entering cells has not yet been realized in clinical trials but remains an exciting possibility.

With these drugs, there may be tissue culture methods to determine the duration of action in cells. However, final results of such pharmacokinetic studies would require a pharmacodynamic study in living animal eyes. Implantable delivery systems such as those in the ganciclovir implant result in soluble drug in the vitreous. If this results in a steady state concentration of the drug, tissue culture systems may be appropriate for the study of their effects. With drugs that are minimally soluble in water and therefore act as their own delivery systems, such as crystals (trimacinolone) or minimally soluble drugs, it may be extremely complex to model the dynamics of drug distribution without using a living animal eye. Other bioerodible systems in which drug is released as a polymer and eroded and absorbed, such as the Posurdex system produced by Allergan (Irvine, CA), are designed to release the drug at a constant rate, achieving so-called linear release. If this linear release rate is appropriate, a steady state intraocular concentration is maintained for prolonged periods. Maintaining the steady state level depends on the equilibration of the drug release rate with exit pathways.

The delivery systems just described or prolonged-release drugs probably cannot be adequately assessed in tissue culture as it is extremely difficult to model the exit pathways and steady state concentrations of drugs. Tissue culture models and studies are appropriate and clinically relevant for investigating specific hypotheses related to molecular and cellular mechanisms of action or toxicity under the highly controlled conditions that these models offer, which may be difficult or impossible to study in vivo due to confounding and uncontrollable variables and conditions. However, tissue culture systems cannot realistically be expected to replicate the physiological or pathophysiological complexity of the intact eye or even a part of the eye (e.g., the RPE–retinal interface) in the absence of clearly defined hypotheses designed to take advantage of the isolated properties these systems offer.

In complex organs such as the eye, it may be reasonable to approach drug testing for local use in a three-step manner. The initial step may be evaluation of a drug or compound in tissue culture. For this, one could use a neural cell line derived from the retina and the RPE, as well as possibly a ciliary body cell line or corneal cell line. One must understand, however, that placement of a drug in the eye will result in different concentrations over time than one may see after placing it in tissue culture medium. As such, tissue culture assays impose limits on the conditions and specific variables that can be investigated and used to define the range of clinically relevant parameters. After a screening process, which may be more efficient in tissue culture, an investigator might move on to look at the compound in animal eyes.

Obviously, the primate model is closest to human, but primates are expensive and there may be ethical issues regarding their use early in an investigation. Many investigators have used two lower species: the rodent such as the mouse or rat and the rabbit because of its large eye. The pig is another species frequently used in studies, but for in vivo experiments, these animals are large and difficult to handle. The presence of
ocular pigment is also important to consider. Many drugs bind to melanin, and thus using an appropriately pigmented animal instead of an albino species may result in drug distribution and pharmacokinetic properties more similar to that found in humans. Crystalline drugs such as triamcinolone or crystalline ganciclovir or cidofovir in a living eye would slowly release from the crystal drug into the vitreous fluid, depending on solubility, particle size, and vitreous fluid rate turnover. The free drug in the vitreous can be measured from a vitreous sample obtained through a fine needle tap at any time point. Multiple vitreous taps would allow the investigator to study the free-drug dynamics in the vitreous. The final phase of testing in clinical trials for intraocular drug toxicity and pharmacokinetics is under way.

It is important to understand the complex issues involved in intraocular drug delivery. Currently, there are several approved drugs for intraocular use, such as ranibizumab (Lucentis; Genentech, South San Francisco, CA) and pegaptanib (Macugen; Pfizer, New York, NY). Pharmaceutical companies are involved in development of others, including preservative free triamcinolone, plasmin enzyme to induce vitreous detachment, small interfering RNA to reduce vascular endothelial growth factor (VEGF) production, and others. Perhaps most important, there are numerous off-label uses of drugs and substances injected in the eye, but the scientific literature remains confusing with regard to intraocular properties and toxicity. Until investigators are able to agree on and use rigorous standardized methods and conditions to evaluate such drugs in the relevant systems, we will continue to be faced with confusion surrounding the use of drugs such as commercial triamcinolone. ICG, antibiotics, antivirals, tissue plasminogen activator, and others.

References