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Teaser This comprehensive review details the challenges in assessing the safety of novel ocular long-acting delivery (LAD) technologies, and well as the most common types of toxicity encountered during early toxicity testing of such delivery approaches to the posterior of the eye.

The safety evaluation of long-acting ocular delivery systems

Evan A. Thackaberry[1], Florence Lorget[2], Cindy Farman[3] and Vladimir Bantseev[4]

[1] Ra Pharmaceuticals, 87 Cambridge Park Dr, Cambridge, MA 02140, USA
[2] Sangamo Therapeutics, 501 Canal Blvd, Richmond, CA 94804, USA
[3] Farman Pathology, Reno, NV 89431, USA
[4] Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA

The safety evaluation of ocular long-acting delivery (LAD) technologies is a nascent field. Here, we detail the challenges in assessing the safety of novel LAD technologies, and well as the most common types of toxicity encountered during early toxicity testing. A detailed understanding of the route of administration, pharmacology, and functionality and/or pharmacokinetics (PK) of the LAD, along with all of its component parts, including the active pharmaceutical ingredient and excipients, is crucial for the successful development of next-generation long-acting ocular therapeutics.

Introduction

From a toxicologist's perspective, the route of administration is crucial. Given that most known ocular toxicants come into contact with ocular tissues and structures via topical or systemic administration, the field of ocular toxicology has traditionally been concerned with the study of toxicants delivered by these two routes, either intentionally, as therapeutic agents, or unintentionally, as accidental exposures. However, to date, the focus of sustained delivery efforts has been on intravitreal (IVT), trans-scleral (implant), intracameral, or subconjunctival routes of administration, with the intention of maximizing drug exposure to the target ocular tissue and reducing the frequency of administration. Given that less is known about the ocular toxicity of substances administered by these routes, the safety assessment of sustained-release formulations of therapeutic agents intended to treat diseases of the eye is challenging.

Although data gleaned from ocular irritation tests (Draize or *in vitro* alternatives) and systemic studies with known ocular toxicants can provide a roadmap to assess ocular safety, these new routes of administration provide unique challenges. The challenges include a limited understanding of ocular PK, including tissue distribution following administration, limited knowledge about translatability to humans, localized exposure to high concentrations of therapeutic agents and excipients, physical movement of particles within the eye, and a lack of *in vitro* models designed to mimic these novel routes of administration. Another key difference between

Evan A. Thackaberry is vice president and head of nonclinical development at Ra Pharmaceuticals. He leads the toxicology, drug metabolism and pharmacokinetics, and bioanalytical support for the Ra pipeline, developing novel complement therapeutics across multiple indications. Dr Thackaberry is an active member of the Society of Toxicology (SOT) and a past president of the SOT Ocular Toxicology Specialty Section. He is also an active member of The Association for Research in Vision and Opthalmology (ARVO) and the American College of Toxicology (ACT), and has presented and published extensively in the areas of ocular and formulation toxicology.

Vladimir Bantseev is a senior scientist in the Department of Safety Assessment – Toxicology at Genentech, Inc. As an ocular toxicology subject matter expert, his main focus is on assessing the safety of novel treatments for retinal diseases and he contributes to the development of these molecules from target identification through post-marketing. Dr Bantseev is a Fellow of the American Academy of Optometry, SOT member and an elected board member of the Ocular Toxicology Sub Specialty. He is also an active member of ARVO. He has presented and published extensively in the area of ocular anatomy, physiology, toxicology and ophthalmic drug development.

Corresponding author: Thackaberry, E.A. (ethackaberry@rapharma.com)

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traditional ocular toxicology and the toxicology of sustained-release formulations is the prolonged presence of formulations within the eye. The physical presence of these formulations, polymers, or devices within the eye for an extended period of time can lead to several unique toxicities, including inflammatory or foreign body responses (FRBs), and physical toxicities related to movement of solid particles.

The challenges described above are likely responsible for the limited success achieved within the field of sustained ocular drug delivery to date. Although numerous exciting new technologies are currently in nonclinical, and in a few cases, clinical development, only six true long-acting technologies have demonstrated sufficient safety and efficacy to gain approval for human use: Vitrasert®, Retisert®, Ozurdex®, Iluvien®, Triesence®, and Trivaris®. Of these, all are long-acting steroid formulations except Vitrasert® (a long-acting antiviral). The successful development of so many steroid-containing technologies is telling, because these drugs are capable of reducing or preventing inflammatory reactions to the delivery platforms. Furthermore, two of these formulations (Triesence® and Trivaris®) rely on the inherent insolubility of triamcinolone acetonide, rather than on polymers or excipients, to produce their long-acting release characteristics.

To date, the sustained delivery of nonsteroid molecules in an injectable formulation (small molecule or protein based) has not been achieved, although two long-acting VEGF therapies are in late clinical development. The first is a low-molecular-weight anti-VEGF single chain Fv (brolucizumab), which aims to achieve extended duration of efficacy not by utilizing a novel delivery system, but by providing superior PK and drug loading over other anti-VEGF therapies [1]. The second system is the ranibizumab port delivery system, a refillable drug-device combination [2] that is currently in Phase III clinical trials. If approved, these new therapies will represent the first approved long-acting anti-VEGF options, and could be capable of achieving 4–6 months of efficacy. The success of these therapies could serve as a guide for long-acting delivery paradigms of next-generation retinal therapies.

Here, we focus on the toxicology of ocular LAD technologies. Examples from the literature and the laboratories of the contributing authors are provided when available. Although ocular long-acting delivery research has been a focus of numerous academic and pharmaceutical laboratories for many years, the study of the toxicology of long-acting ocular delivery systems is still a nascent field. Advances in our understanding of the local effects of persistent drug depots within the eye will be crucial for the successful development of next-generation delivery technologies.

Unique aspects of safety assessment of LADs

Species selection and predictivity

The anatomy and physiology of the eye provide many challenges for the development of LAD technologies. The eye itself is a structurally complex organ, and this complexity directly impacts the fate of locally administered drugs. The eye is serviced by two distinct vascular beds, and parts are protected by the blood–retina and blood–aqueous barriers. The exterior of the eye is effective at preventing the penetration of drugs, whereas the interior of the eye can rapidly remove drugs via the aqueous outflow. It is the potential for so many varied routes of administration, along with the structural complexity of the eye, that make assessing the safety of LAD technologies so difficult. Additionally, many of the tissues in the eye, most importantly the retina and the lens, lack regenerative capacity. As a result, lesions of these tissues are generally not reversible. Another major obstacle is the lack of models to study the toxicity and tolerability of LAD technologies. Although *in vitro* assays [3,4] are useful to assess local toxicity to individual cell types and simple tissues (such as the cornea), there are no *in vitro* or *ex vivo* models that address the complex architecture and fluid dynamics of the eye as a whole. Therefore, a true assessment of the toxicity of LAD technologies requires *in vivo* studies.

However, the use of *in vivo* studies is complicated by the anatomical and physiological differences of the eye across nonclinical species, and how these relate to human eyes. The choice of animal model is crucial, and can be dependent on the type of LAD and the toxicities of concern. For instance, the assessment of particle movement, a major liability for IVT suspensions, can be assessed only in the nonhuman primate (NHP), which has a vitreous viscosity similar to that of humans. By contrast, the larger eye of the pig is more useful for the safety assessment of human-sized devices. Although the larger eye of a dog was utilized in the case of fluocinlone acetonide implant (Retisert®), the dog eye is prone to sterile inflammation following surgery [5]. The rabbit is a commonly used lower-order species, whereas rodents are generally not useful when assessing intraocular LADs because of the small size of the eye and differences in ocular anatomy and PK from humans and larger animals. Generally, only low dose/exposure multiples can be achieved even in the large animal models because of the small size of the eye, which limits the volume that can be administered and the manufacturing and safety challenges of using high concentration formulations (e.g., stability, pH, osmolality, and viscosity).

Chronic exposure

The goal of sustained delivery is to provide exposure to a therapeutic molecule over an extended duration, typically weeks to months. To accomplish this, a depot of drug with a slow and continual release is required. The drug can be separated from the target tissues, as with a slow-releasing device or polymer implant, or simply exist for an extended period of time within or near the target tissue, as is the case for the sustained-release triamcinolone formulations. In either case, the chronic presence of the drug and any polymers or excipients associated with it can lead to unique toxicities, such as particle movement or immune responses. In our experience, particle movement to the anterior segment happens within minutes of IVT administration, whereas immune reactions generally take from 2 to 6 weeks to manifest. The safety assessment of these technologies requires time-consuming and expensive chronic toxicity studies.

Complex systems

The complexity of some sustained-delivery technologies further complicates their safety assessment. The goal is to predict the impact on human safety from all stages of clinical use and, as such, the safety of complex systems must be characterized from implantation and/or injection, through drug release and polymer clearance, and upon repeat administration. For instance, polymers undergoing physical degradation within the eye must be assessed at all stages of degradation (not only the first phase of drug release). For nonbiodegradable implants, any accumulation of the polymer must be assessed upon repeat-dose administration, if this is in line

with the intended clinical use. The impact of the drug in these nonclinical models is also a concern. For instance, humanized protein therapeutics (particularly mAbs and Fabs) are often immunogenic in nonclinical models, a response that is only exacerbated by prolonged drug release. It is often difficult to assess the combination of complex formulations plus humanized proteins in nonclinical species [6–9].

These challenges, discussed in detail below, promise a difficult path for the nonclinical toxicologist assessing the safety of sustained ocular drug delivery systems. However, sound study design, appropriate animal model selection, and an understanding of the most likely toxicities observed with each route of exposure and technology type are an excellent starting point in the search for a safe sustained-delivery technology for treating ocular diseases.

Impact of route of administration, PK, and ocular biodistribution

The goal of any LAD technology is to sustain the release of a drug to prolong and target the exposure at the intended site of action, thereby increasing the effective dose while reducing both the dosing frequency and the patient treatment burden. As such, a thorough understanding of the functionality of the LAD and the resulting drug release profile of the therapeutic are crucial. From a safety perspective, the ideal LAD provides high local exposure to drive efficacy while limiting systemic exposure, which is likely to lead to off-target toxicity or on-target exaggerated systemic pharmacology. The most prominent example of this is that of the anti-VEGF therapies, which are well tolerated locally within the eye, but are associated with serious systemic adverse events [10,11]. This guiding principle of limiting systemic exposure is the main reason why topical and transscleral administration are not feasible for most sustained ocular delivery technologies, regardless of the target tissue, unless high systemic exposure can be tolerated without undue safety risks. Although significant advances have been made in the selectivity of modern pharmaceuticals, systemic toxicity remains a significant risk for all classes of ocular therapeutics [12].

The route of administration has a crucial influence on the types of toxicity observed with sustained ocular delivery technologies. This is mainly because of the varied toxicokinetics, but is also influenced by the matrices into which formulations are administered (cellular tissue or acellular vitreous and/or aqueous) and the sensitivity of certain tissues to toxicity. As mentioned earlier, there can be little expectation that toxicities will be similar across different routes of administration, even within a small organ such as the eye. Therefore, the intended clinical route of administration should be used in nonclinical safety studies. When possible, the exact location of intended clinical administration should also be replicated in the nonclinical studies. For example, IVT injections in humans are almost invariably administered into the inferior vitreous; therefore, care should also be taken to inject inferiorly in nonclinical species. Although the attributes of each molecule and LAD technology will dictate the types of toxicitiy observed, Table 1 summarizes the most likely target tissues of each toxicity type depending on the route of exposure, based mainly on the expected biodistribution and associated drug and/or excipient levels.

Following topical administration of most small-molecule therapeutic agents, only a small percentage of the dose (1–10%) reaches the intraocular environment, with a large percentage of the remaining dose being absorbed systemically, either via the vessels of the conjunctiva, or by gastrointestinal absorption via the nasolacrimal duct [13]. The intraocular exposure of large molecules following topical administration is even lower, although their systemic exposure is also low because of poor vascular and gastrointestinal absorption [14]. Although unlikely to be used for sustained-release formulations, topical administration serves a good starting point in this discussion, because its primary target tissue is the cornea, with extraocular tissues the second most likely target. The corneal epithelium is uniquely sensitive to toxic insult, because of the rapid turnover of these cells and their crucial barrier function, and this tissue is a common target of both topically and systemically administered drugs [15,16]. Eye drops [topical antibiotics and intraocular pressure (IOP)-lowering therapeutics] are the most common topical delivery system, although, for the purposes of this discussion, transscleral administration (non-implant) produces a similar exposure profile, although more focused on the sclera than on the cornea. Examples of corneal toxicity following topical exposure abound in the literature; many of these result from accidental exposures to caustic or acidic chemicals. Several studies have also demonstrated the toxicity of the preservative benzalkonium chloride (BAC) [17,18]. Abdelkander and Mansour [19] studied *in situ* thermal gels as a topical delivery system, and identified corneal damage with several versions. The assessment of corneal toxicity by topically administered formulations can also be done using *in vitro* models, such as corneal endothelial cells, human corneal epithelial cells and corneal organoculture [20].

Similar to topical, subtenon injection (episcleral space) is also likely of limited use in sustained delivery for diseases of the intraocular structures. Corneal exposure is still likely to be high, but in this case, exposure will be higher in tenon's capsule and the sclera. Subtenon administration is not common, but has been reported for steroids and chemotherapeutics [21]. Clinically, a case of systemic steroid toxicity following subtenon injection has been published [22].

Subconjunctival injection or implantation offers a lower systemic C_{max} for small-molecule drugs compared with topical or subtenon administration, mainly because of the decrease in nasolacrimal absorption. However, the systemic C_{max} is still higher than IVT injections with poor penetration of most therapeutic molecules into the globe of the eye, which limits its usefulness for sustained delivery [14,23,24]. Exposure is likely to be highest in the conjunctiva immediately surrounding the injection site, along with the sclera. Subconjunctival poly(lactic-co-glycolic acid) (PLGA) implants have been investigated and appear to be well tolerated in rabbits [25]. This is perhaps not surprising, given that this route is most akin to a subcutaneous (SC) administration, and numerous SC PLGA depots have been approved.

Intracameral administration deposits drug and/or LADs in the aqueous chamber. The highest concentrations of drug are seen in the adjacent cornea and lens, along with the iris and trabecular meshwork of the outflow [23,26]. As with most routes of administration, systemic exposure is likely to depend highly on the rate of release from the LAD technology, because released drug is likely to be quickly cleared by the outflow. This clearance mechanism is an advantage for intracameral dosing when targeting the trabecular meshwork as a therapy for glaucoma. Given the potential for interference with the outflow mechanisms, including clogging of the trabecular meshwork, intracameral administration of

TABLE 1

Target tissues and toxicities of concern based on route of administration of sustained ocular delivery technologies.

Route of administration	Target tissues	Exposure	Toxicities of concern
Topical (eye drops)	Cornea and conjunctiva	High	Exaggerated pharmacology or off-target toxicity; irritation; cytotoxicity of excipients; penetration by drug-loaded particles
	Anterior ocular tissues	Low	Exaggerated pharmacology or off-target toxicity
	Extraocular organs (particularly liver)	High	Exaggerated pharmacology or off-target toxicity
Trans-scleral (topical)	Sclera	High	Exaggerated pharmacology or off-target toxicity; irritation; cytotoxicity of excipients
	Internal ocular tissues	Low	Exaggerated pharmacology or off-target toxicity
	Extraocular organs (particularly liver)	High	Exaggerated pharmacology or off-target toxicity
Subconjunctival injection or implant	Conjunctiva, sclera, and tenon's capsule	High	Exaggerated pharmacology or off-target toxicity; irritation; immune response; cytotoxicity of excipients
	Internal ocular tissues	Low	Exaggerated pharmacology or off-target toxicity
	Extraocular organs	High	Exaggerated pharmacology or off-target toxicity
Subtenon injection	Sclera and tenon's capsule	High	Exaggerated pharmacology or off-target toxicity; irritation; cytotoxicity of excipients
	Cornea	Moderate	Exaggerated pharmacology or off-target toxicity; irritation; cytotoxicity of excipients
	Internal ocular tissues	Low	Exaggerated pharmacology or off-target toxicity
	Extraocular organs (particularly liver)	High	Exaggerated pharmacology or off-target toxicity
Intracameral injection or implant	Cornea and lens	High	Exaggerated pharmacology or off-target toxicity; cytotoxicity of excipients; immune response; penetration of corneal epithelia; damage to lens (chemical, immune, or physical) leading to cataract formation
	Anterior outflow	High	Exaggerated pharmacology or off-target ; blockage of outflow by particles
	Posterior ocular tissues	Low	Exaggerated pharmacology or off-target toxicity; immune response
	Extraocular organs	Low–moderate	Exaggerated pharmacology or off-target toxicity
Trans-scleral implant	Sclera and conjunctiva	High (implant); low–moderate (device)	Exaggerated pharmacology or off-target toxicity; irritation; immune response; cytotoxicity of excipients
	Vitreous and retina	High	Exaggerated pharmacology or off-target toxicity; immune response; cytotoxicity of excipients; physical damage from device/implant contact
	Lens	High	Exaggerated pharmacology or off-target toxicity; damage to lens (chemical, immune, or physical) leading to cataract formation
IVT injection or implant	Vitreous and retina	High	Exaggerated pharmacology or off-target toxicity; immune response; cytotoxicity of excipients; physical damage from implant or particle movement; blockage of visual axis
	Lens	High	Exaggerated pharmacology or off-target toxicity; damage to lens (chemical, immune, or physical) leading to cataract formation
	Anterior ocular tissues	Moderate	Exaggerated pharmacology or off-target toxicity; immune response
	Anterior outflow	Moderate	Exaggerated pharmacology or off-target toxicity; immune response; blockage out outflow by particles

particles should generally be avoided. Overall, the anterior chamber is sensitive to changes in pH, osmolarity, or the presence of cytotoxic drugs or excipients.

IVT injection of small molecules or proteins leads to high vitreal and retinal levels, followed by delayed release of the therapeutic agent. Although approximately 100% of the IVT drug injected is eventually eliminated via the systemic circulation, the C_{max} and steady-state plasma levels are kept low by the slow release from the vitreal reservoir [27]. Trans-scleral implants, which release primarily into the vitreous, would deliver the therapeutic agent similar to an IVT infusion. Both IVT injections and trans-scleral implants could provide high local exposure to the retina and choroid, the target of therapies for age-related macular degeneration (AMD) and neuroprotection, while minimizing systemic exposure (C_{max} and SS_{plasma}). Despite the benefits of IVT injection of therapeutics, there are relatively few published data on the toxicity of formulations administered by this route, such as to the retina and lens. In addition, immune responses and particle movement following IVT administration are of significant concern, affecting these tissues as well as more distant tissues, such as the choroid and

ciliary body, as well as impacting aqueous outflow. Implants, either trans-scleral (Retisert® and Vitrasert®) or IVT (Ozurdex® and Illuvein®), have been demonstrated to be clinically safe and are approved in the USA and/or Europe, although as mentioned earlier, most of these products contain an extended-release steroid, which might mitigate any immune response to the polymers involved. Published reports of other nonsteroid-containing formulations are limited, and those that have been reported have generally not been well tolerated. Injection of nanoparticles (NPs) into the vitreous has been shown to cause retinal damage and inflammation [28], polymer hydrogels have been shown to cause retinal and lens toxicity [29,30], as have silica particles [31]. In general, large implants produce fewer toxicities, whereas smaller particle-based technologies tend to have tissues with immune responses and particle movement.

Although the basic physical distribution of therapeutics after administration by different routes has been studied using a few model compounds, drug release into the surrounding tissue and ocular distribution characteristics will depend not only on route of administration, but also on the physical and biochemical characteristics of the molecule, and the LAD technologies utilized. For example, anti-VEGF therapeutic proteins, such as ranibizumab or bevacizumab, are eliminated mainly via the aqueous outflow of the eye following IVT administration, whereas lipophilic small molecules, such as diclofenac are mainly eliminated by diffusion across the retina into the choroidal circulation when administered by the same route [32–34]. LAD technologies affect drug exposure mainly by altering drug release at the site of administration. Lower release rates tend to reduce both local concentrations and systemic exposure C_{max}, whereas the 'burst' effect seen with some polymer LAD technologies can provide a useful local loading dose, but are also expected to produce a higher C_{max} immediately following administration. Regardless of the route of administration, drug, and LAD technology utilized, drug molecule PK and distribution should be fully characterized in parallel with the toxicological assessment of the drug delivery system to fully understand and predict efficacy and safety.

In addition to assessing the local and systemic exposure to the therapeutic molecule, it is often important to assess the toxicokinetics of solvents, polymers, excipients, or degradation products of the formulation or drug carrier, which can be present at very high local concentrations within the eye.

Although the eye is generally considered metabolically inactive, almost all ocular tissues contain some levels of Phase I and Phase II metabolic enzymes [35,36]. These enzymes have a key role in maintaining the normal homeostasis of the ocular tissues. Oxidoreductases, including the prevalent cytochrome P450, hydrolytic enzymes such as esterases, and peptidases and conjugating enzymes such as *N*-acetyl transferase and glutathione-*S*-transferase, are paramount enzymatic systems in the eye. Tissue distribution and level of expression of these various enzymes cater to the various functions of the ocular tissues [37].

For metabolically labile drugs, careful considerations should be given to transporter and enzymatic systems when considering ocular drug delivery strategies to optimize tissue uptake, distribution and residence time, while minimizing toxicity. Prodrug strategies that leverage both the transporter and enzymatic systems appear to be promising for the drug delivery of small molecules.

Inflammation, immunogenicity, and immune surveillance in the eye

The eye is often described as an immunologically privileged organ, and is generally able to suppress immune responses within the globe via anterior chamber-associated immune deviation (ACAID) [38–42]. However, when the globe of the eye is breached, inflammation and immune-mediated reactions can, and do, occur within the eye. These reactions can be directed against the drug itself or components of the LAD systems, such as implants, polymers, or excipients.

Animals in nonclinical studies injected intravitreally with therapeutic proteins, such as ranibizumab and aflibercept, produce significant ocular inflammation [43,44]. This inflammatory reaction is not observed in humans, and, therefore, is considered to be related to the administration of humanized proteins in animals. However, the translation between animal models and humans of inflammatory reactions against other LAD components, such as NPs and microparticles, microspheres, hydrogels, polymers, and other excipients is unknown. Some of these components can elicit a foreign body reaction. Importantly, a FBR might be generated against both durable and biodegradable devices, and particulates [45,46]. Although the mechanisms involved in the formation of a FBR in the eye has not been studied in depth, it might follow a similar sequence of events as has been described for biomaterials for other nonocular routes of administration [47–51]. Importantly, although FBRs may be clinically acceptable in nonocular tissues, they are not tolerable in the eye because of the risk of compromising the integrity of ocular structures (e.g., fibrous material in the vitreous potentially leading to retinal tears), as discussed below.

Toxic responses to LAD formulations

For all of the toxic effects described herein, route of administration is crucial to predicting the risk of possible specific ocular and extraocular tissue liabilities. Table 1 summarizes the most likely target tissues of each toxicity type depending on the route of exposure. Similar to the field of sustained ocular delivery in general, our understanding of the toxicities associated with these formulations and devices is still developing. Many reviews and published studies of IVT LAD methodologies exist [52–59]; however, the safety of these LAD technologies is generally not the main focus of this work. The only way to truly evaluate the effects of a potential ocular LAD substance is *in vivo* testing via the clinical route of administration in a relevant species. Reactions seen following routes of administration other than ocular, such as by the SC route, do not necessarily translate to what will occur in the posterior compartment of the eye [55]. Here, we describe the five major types of toxicities observed with LAD–drug combinations (Box 1); local chemical-mediated, local immune-mediated, local physical-mediated, ocular phototoxicity, and systemic (extraocular) toxicities.

BOX 1

The five major types of LAD-induced toxicities

- Local chemical-mediated toxicity.
- Local immune-mediated toxicity.
- Local physical toxicity.
- Ocular phototoxicity.
- Systemic (extraocular) toxicity.

Local chemical-mediated toxicities

When one thinks about a typical toxic reaction to a drug, one is typically envisioning a chemical toxicity, either off-target, such as acetaminophen liver toxicity, or exaggerated pharmacology, such as the increased risk of bleeding with warfarin. These types of toxicity are common with LAD technologies, and they are often rendered more complicated by the complex nature of the LAD systems. Key to understanding a chemical toxicity of a sustained-release ocular formulation is to understand what component(s) of the formulation is/are causing the toxicity. Direct ocular toxicity caused by the drug, such as that described for doxycycline [60] adalimumab [61], or topotecan [62], limit the usefulness of these drugs for IVT administration, but can be mitigated by lowering the dose (or rate of release from sustained-release formulations), or choosing a different therapeutic compound. An in-depth assessment of the biology of the target will often provide insight into potential exaggerated pharmacology, such as cell cycle inhibition in rapidly dividing corneal epithelial cells. Off-target toxicology is more difficult to define, but can be assessed via simple cytotoxicity assays, or by screening for off-target receptor binding (e.g., kinase inhibitors) using cell-based assays. The high drug load, or prolonged local exposure, produced by some sustained delivery technologies could uncover new or exacerbate previously known toxicities of approved products. For example, although steroid-induced increased intraocular pressure is observed with oral and inhaled steroids at a very low incidence, this adverse effect has become more common with the development of ocular-targeted steroid administration, in particular the long-acting IVT triamcinolone products [63–65].

Chemical toxicity produced by excipients within a sustained-release formulation, such as solvents, polymers, surfactants, or their degradation products, are often more difficult to remedy, because the effects might be mediated by the very physiochemical properties of the excipient required for a key function within the drug delivery system. For instance, certain solvents might have direct toxic effects on the retina and lens (Fig. 1a,b), and preservatives, such as BAK, have been shown to be toxic to the cornea [18,66,67]. The retina can be sensitive to high local levels of common excipients, such as arginine or DMSO [68,69]; therefore, caution should be used when testing formulation components with no previous intraocular use. When novel formulations are being used to deliver previously approved therapeutics, excipients are the most likely cause of the toxicity observed, especially if overall exposure is not significantly increased compared with previous repeat dose studies. As published recently, testing of the novel individual components of the drug delivery system is required to determine which excipient is responsible for the toxicity [70].

Metabolites or degradation products of the excipients used can also be a potential cause of chemical toxicity within the eye. These compounds might be intended byproducts, such as polymer degradation products, or unintended metabolites resulting from enzymatic metabolism. Given that the presence and concentration of these compounds will be temporally distinct from the original excipients, the timing of the toxicity observed is often a guide to the responsible molecular species. For example, a toxicity seen only after 3 weeks following intracameral injection, which corresponds to the maximal production of polymer degradation product, might suggest that these products are responsible for the observed toxicity. Once again, the importance of assessing the nonclinical safety of the sustained-release technology over its entire life cycle is highlighted here. If a toxic degradation product or metabolite is only produced (or only reaches toxic levels) in the final days before a formulation is cleared from the eye, then nonclinical studies must be long enough to assess this clearance to identify the toxicity.

Local immune-mediated toxicities

As described above, the immune privilege of the eye does not prevent it from mounting a strong immune response to intraocularly administered therapeutic depots. The administration of large

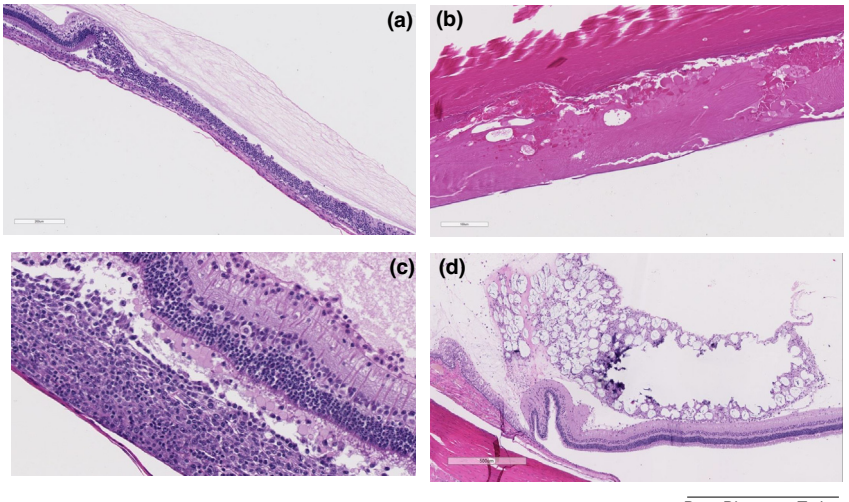

FIGURE 1

Histopathology findings with long acting delivery systems. **(a)** Injection of a solvent into the eye has resulted in almost complete necrosis of the retina because of contact with the solvent. **(b)** Degeneration of lens fibers because of contact of injected solvent with the posterior aspect of the lens. **(c)** Marked antidrug antibody (ADA)-related subretinal inflammation in response to intravitreal (IVT) administration of a bispecific antibody in rabbit. (d) Foreign body inflammatory response to IVT injection of a polymer. Clear spaces, many containing blue-staining material, represent the presence of the polymer, often within multinucleated giant cells or individual macrophages. Fibrosis associated with the inflammation has adhered to the retina and caused traction detachment.

amounts of protein or polymers within any ocular tissue, including the vitreous and aqueous humor, quickly overcomes any native immune suppression, and can produce strong inflammatory responses (Fig. 1c,d). These responses are not well characterized to date, but can include antidrug antibody (ADA)-mediated responses, mixed cell inflammatory responses, FBRs, and possibly others. In contrast to extraocular tissues (such as the SC space) in which mild immune responses to local depots are well tolerated, significant immune responses in the eye are frequently damaging and sight-threatening because of the delicate and nonregenerative nature of the intraocular tissues. Based on the experience of the authors, immune responses are the most frequently encountered toxicities with sustained ocular delivery systems, particularly with suspension or implant technologies, and they can range in severity from minimal to severe, potentially compromising the conduct of the study and/or preventing further development of a LAD system. Polymers that have been shown to the 'biocompatible' or 'safe' for use in humans via other routes of administration might not be tolerated within the eye, because well-tolerated immune responses produced in other tissues in the body might be severe within the context of the eye, particularly if intraocular pressure is elevated as a result, or fibrosis is produced within the globe, which could result in retinal detachment.

ADA-related inflammation is characterized by mixed cell inflammatory reaction, often progressing to thickening of the retinal nerve fiber layer, perivascular sheathing, swelling around the optic nerve, and degraded fundus view following repeated administration or extended exposure to a biologic therapeutic [6,7,9]. ADAs are generally detected in the plasma, but the relationship between inflammation and ADA-positive titers is generally not direct, possibly because of the presence of locally produced ADAs within the eye [71]. Importantly, ADA-type immune responses to humanized proteins, such as ranibizumab and aflibercept [8,9,43,44], have been shown not to translate to humans. This makes sense in the context of the long industry experience with systemically administered humanized antibodies, for which ADA responses have also been shown to lack translatability to humans [72]. However, such reactions still present a challenge to the development of biologic therapeutics, because these immune reactions can often progress to the point to preventing continued dosing on chronic toxicology studies. The rabbit is generally more sensitive to the development of these ADA-driven inflammatory reactions, limiting the utility of this species for repeat-dose toxicology studies of IVT biologics [7].

Non-ADA-related general mixed cell inflammation is common with both particle and/or implant and suspension sustained delivery ocular formulations [73–76]. Outside of the globe, following topical, subconjunctival, or subtenon administration, such responses might be well tolerated if they are not severe and rapidly resolve, whereas similar responses following intracameral or IVT administration are less likely to be tolerated. It is generally not possible to differentiate ADA-related and non-ADA-related inflammation histologically; therefore, assessment of ADA titers in the serum or vitreous are crucial in defining the mechanism of the inflammation. Tolerability will depend on the severity and time course of the response, and the half-life of formulation. Formulations causing a significant response over the course of weeks or months are unlikely to be tolerated clinically.

FBRs are common with particle or implant technologies. When these are present in extraocular locations (subconjunctival, extrascleral, etc.), they might be tolerated and, in fact, would be seen as a normal response to a medical device. However, FBR within the globe of the eye are of greater concern, particularly following IVT or intracameral administration. In these cases, tolerability is determined by the time course and severity of the reactions. Minimal reactions that are rapidly cleared from the eye (generally because the sustained-release technology is rapidly cleared form the eye) might be well tolerated. However, severe reactions, or reactions allowed to build over time might irreversibly damage ocular structures, either by fibrotic attachment, or as a result of cellular invasion or extracellular matrix remodeling. Some LAD substances are combined with corticosteroid drugs, which might inhibit the foreign body inflammatory reactions but might not do so completely. This has at least been shown to be the case in extraocular locations, including the demonstration that a FBR to the LAD polymer eventually occurs once the steroid is gone [51]. Complicating matters further, there is some evidence in the literature that polymer type, surface chemistry, size, and shape might make a difference in the nature of immune reactions to foreign materials [46,77,78]. As mentioned earlier, data from other routes of administration (ocular or extraocular) is not predictive of tolerability if given by IVT and intracameral routes, necessitating the assessment of any ocular LAD technology via its intended route of clinical administration.

FBRs have been shown to occur in rabbit, NHP, and human eyes in response to ocular LAD technologies, intraocular lenses, and perfluorocarbon vitreous replacements [6,28,45,46,59,79–82]. Although the translatability of the nonclinical findings to the clinic are unknown, any tolerability findings are a significant concern because of their progressive nature and their potential to threaten the sight of the patient upon involvement of the retina. In our experience, FBRs are common responses to polymer-based IVT depots in rabbits and NHPs [46]. These reactions and their sequelae to the retina and other ocular structures generally indicate poor tolerability and, when observed, preclude further development of the LAD system. Examples of the consequences of such reactions are provided in Fig. 1d, including retinal detachment and/or tearing, and penetration of foreign body-associated microparticles through the retina.

Local physical toxicities

Non-immune local physical toxicities can be broadly categorized into three distinct groups; visual disturbances, impact or penetration of ocular tissues, and blockage of the aqueous outflow. All three can be severe, and they are all difficult to assess nonclinically, particularly when occurring within the globe of the eye. This is because particle movement within the eye is dependent on numerous poorly understood factors, among which eye size and vitreous viscosity, which vary widely by species, are crucial. In general, the NHP is the most relevant species to assess these physical toxicities, because it has a vitreous viscosity most similar to humans [83–85]. However, the NHP is not perfect as a model, because the human vitreous liquefies as it ages [86]. A typical NHP on a pivotal safety study is likely to be a juvenile (2–4-years old), with vitreous characteristics similar to young adult human. Rabbit, dog, and pig have very viscous vitreous. These species might be

useful when assessing the fit of a device or depot with the eye (using scaled devices or implants), but not for movement within the vitreous.

Visual disturbance might not be seen as a classic 'toxic' response, but it is crucial to assess nonclinically, because patients will generally not tolerate significant visual disturbances caused by drug particles from a suspension drug product formulation, which can manifest as floaters, haziness, or blockage of the visual axis for an extended period of time. These types of issues are generally only a concern for IVT, transscleral (implant), or intracameral technologies. Visual disturbances can generally be assessed by a veterinary ophthalmologist via slit lamp and indirect exams. Particles, depots, and devices within the visual axis will likely affect vision nonclinically, and these disturbances would likely predict similar clinical findings. For some technologies, such as devices or large depots, the size of the eye makes a difference in this regard. In these cases, a scaled device or depot should be used to assess the impact on vision.

Maintaining the integrity of the 3D architecture of the eye as well as of the different tissues constituting it is paramount to preserving its function. Therefore, careful consideration should be given to any material that can alter its physical integrity, such as large implants and particulate systems. Large implants can move over time, thus exercising mechanical forces upon the tissues where they were inserted and/or damaging tissues because of unwanted blunt contact. Therefore, it is important to evaluate such risk using scaled implants. NPs and/or microparticulate systems present the risk of tissue penetration. Sequelae of retinal penetration include inflammation, necrosis, and degeneration with often irreversible loss of function (Fig. 2). Penetration and retention are also possible with large implants, such as an IVT polymer implant being embedded in the lens or contacting the cornea [87].

The final physical toxicity is blockage of the aqueous outflow, which is perhaps the most damaging and difficult to model nonclinically. The consequences of such effects in humans are well understood based on the example of closed angle glaucoma. When fluid is not able to exit the eye via the aqueous outflow, or it is sufficiently restricted, IOP rises. Acutely, this can be a sight-threatening emergency, with surgery to relieve the pressure via an aqueous shunt required. Chronic high IOP will damage the cells within the optic nerve, eventually destroying sight. This toxicity is only seen with intracameral and IVT technologies. Blockage following intracameral injection of a particle suspension (NPs or microparticles) makes sense, because the particles would quickly be swept into the iridocorneal drainage apparatus via the aqueous flow. This can occur with particles as small as 1.5 μm in diameter [88,89]. Particle movement from the back of the eye following IVT dosing into the anterior chamber can also occur with particles as large as 150 μm (Fig. 3). Clinically, such movement has been documented with the steroid suspension Triesence®, although without associated increased IOP [90,91]. Indeed, the presence of an artificial lens (pseudophakia) or the absence of the lens (aphakia) is likely to impact this movement significantly. Adding to the complexity, it is also possible for foreign particles within the outflow to elicit an inflammatory response [6], which can further block aqueous flow and increase the risk of increased IOP. Intracameral movement can be assessed in most nonclinical species, because the viscosity of the aqueous is similar. However, as a general rule, the administration of long-acting microparticulate or NP formulations in the anterior of the eye is likely to be poorly tolerated for several reasons, including visual disturbances and outflow blockage. Assessing such liabilities following IVT injection requires the use of NHPs, because they have a vitreous viscosity most similar to that of humans. As is the case with the assessment of other toxicities, it is important to assess movement of particles within the eye over the course of the full life cycle of a formulation, from injection to complete clearance. This is because physiochemical changes during degradation and clearance might increase the likelihood of particle movement.

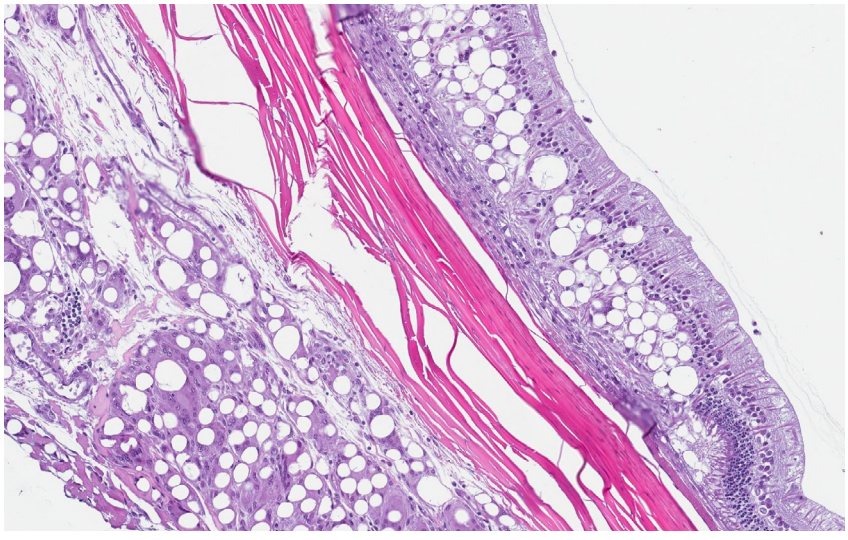

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FIGURE 2

Vacuolization of the retina and periocular tissues in a rabbit administered poly(lactic-co-glycolic acid) (PLGA) microspheres. This vacuolization represents penetration of the microspheres through the retina and sclera and associated inflammatory responses.

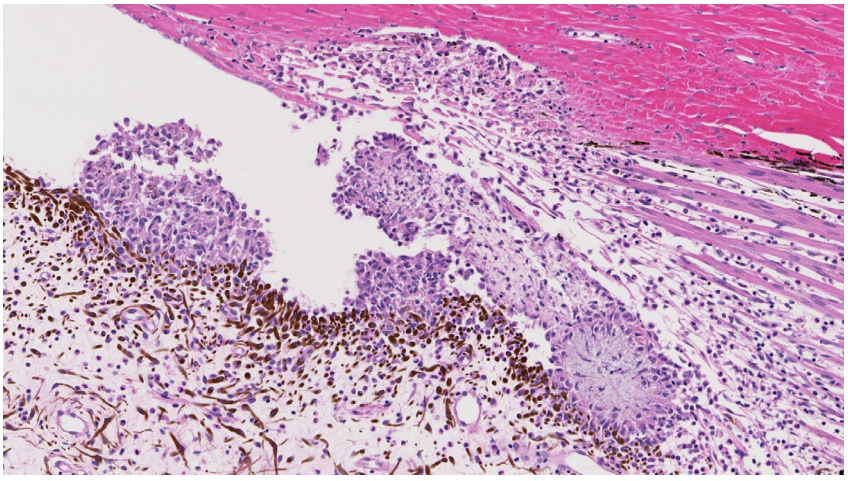

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FIGURE 3

Inflammation of the iridocorneal drainage angle of a monkey administered a hydrogel intravitreally. The hydrogel (faint blue material) has migrated into the drainage angle, inciting an inflammatory response and leading to obstruction of the angle.

Ocular phototoxicity

For systemically administered drugs, evidence of melanin binding often leads to assessment of phototoxic potential, particularly for compounds that absorb within the ultraviolet/visible (UV/Vis) spectra or are known to be photolabile. However, for drugs administered directly to the eye, melanin binding is not necessary to produce locally high concentrations within ocular tissues. Therefore, phototoxicity is a potential concern for all small molecules administered directly to the eye that absorb in the UV/Vis spectra, regardless of whether they bind melanin.

Assessing the potential for ocular phototoxicity nonclinically can be a challenge. The first step is to assess the absorption spectra. For molecules that absorb within the natural light range (290–700 nm), the molar extinction coefficient (MEC, an assessment of the degree of absorption) should be calculated at the peak absorbance wavelength [92]. Only molecules with an MEC $>$ 1000 are considered potentially phototoxic. For those molecules that absorb in the UV/Vis spectra and have an MEC $>$ 1000, numerous *in vitro* assays are available to assess phototoxic potential, the most widely accepted of which is the 3T3 neutral red uptake assay. However, this assay has a high false positive rate [93], and follow-up *in vivo* studies are generally warranted in these cases. When assessing phototoxic potential *in vivo*, it is ideal to use the intended clinical route of exposure. However, there are no known models of ocular phototoxicity [94], and *in vivo* nonrodent ocular phototoxicity models have not been validated [95]. Given the small size of the eye and anatomical differences between rodent and human eyes, ocular administration in rodent phototoxicity models is not recommended. Instead, the standard oral/intravenous rat phototoxicity model in the Long-Evans rat is recommended. Although not specific for ocular phototoxicity, this is the only validated model to assess phototoxicity *in vivo*, and current data suggest that the mechanism of phototoxicity is likely to be similar regardless of the target tissue (skin or eye). In this case, care should be taken to meet or exceed the anticipated ocular exposure (via the intended clinical route) in the skin or eyes of rats in the standard phototoxicity model.

When assessing phototoxic risk for ocular LADs, the toxicokinetics and biodistribution should be considered along with the physiochemical properties of the molecule. The cornea and lens of humans and most nonrodent toxicology species (rabbit, minipig, dog, and monkey) filter out most UVA/B light before it reaches the vitreous [96]. As a result, phototoxic reactions to intravitreally or trans-sclerally administered molecules absorbing in this range are unlikely in the posterior segment of the eye. However, molecules that are cleared via the aqueous outflow or distribute to the lens or cornea might still cause phototoxic reactions in these tissues. Molecules administered via subconjunctival or intracameral injection are afforded less protection from UV irradiation.

Finally, solvents, polymers, and excipients used in ocular LAD technologies should also be assessed for phototoxicity liability. The screening paradigm for these compounds should be similar to that mentioned earlier for small-molecule therapeutics.

Extraocular systemic toxicities

A major goal of local ocular administration is to increase local exposure to therapeutic molecules while limiting systemic exposure. However, systemic exposure is observed with all ocular routes of administration, although the ratio of local to systemic exposure varies by route. The kinetics of the exposure is also crucial when assessing systemic toxicity. Eventually, the majority of the drug dose administered locally to the eye will reach the plasma. However, the combination of the route of administration and sustained-release profile impact the C_{max}, SS_{plasma}, and $AUC_{0\text{-}\infty}$.

Topical and subtenon administration will provide the highest systemic exposures for small-molecule drugs in terms of C_{max} and SS_{plasma}, and are most likely to be associated with systemic toxicity. For example, eye drops containing beta-adrenergic antagonists have been shown to cause cardiovascular adverse effects because of high systemic absorption following topical administration [97].

Systemic toxicities have also been reported for alpha-adrenergic agonists, steroids, mydriatics, and antibiotics [22,98–100], most commonly in children because of their small body weight. Although not a common route of administration, systemic steroid toxicity following subtenon administration has also been reported [22]. Intracameral or subconjunctival administration is expected to provide a more intermediate level of systemic exposure to release drugs, although the relative exposure will be highly dependent on the release rate from the LAD technology.

Systemic exposure following IVT administration (IVT injection or trans-scleral implant) is the lowest of all current methods. However, although the risk of C_{max}-driven toxicities following IVT administration is low, AUC-driven toxicities are still a potential concern. For example, although not seen nonclinically, IVT administration of anti-VEGF therapies has been shown to lead to an increased risk of thromboembolic events over time. It is hypothesized that this effect results from chronic inhibition of systemic VEGF [11]. In contrast to the systemic toxicity observed with steroids administered via other ocular routes of administration, IVT injection of triamcinolone (Triesence® or Travaris®) or fluocinlone (Retisert®) has not been associated with systemic toxicity to date [63,101], likely because of the low systemic exposure produced.

Concluding remarks

Ocular toxicology studies with novel therapeutics and LAD technologies present a significant challenge to nonclinical toxicologists because of the unique toxicities observed and the intended chronic clinical use. However, with these challenges come opportunities to utilize cutting-edge technologies, such as advanced imaging and electrophysiology, to assess ocular tolerability and function in a non-invasive manner. A robust understanding of the strengths and limitations of nonclinical models and the numerous ocular safety endpoints is an excellent starting point towards the development of sight-saving therapeutics. Thoughtful study design and careful assessment of safety endpoints by experienced professional are key to early detection of ocular toxicology in these models.

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