Expert Opinion

Novel approaches to retinal drug delivery

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Research into treatment modalities affecting vision is rapidly progressing due to the high incidence of diseases such as diabetic macular edema, proliferative vitreoretinopathy, wet and dry age-related macular degeneration and cytomegalovirus retinitis. The unique anatomy and physiology of eye offers many challenges to developing effective retinal drug delivery systems. Historically, drugs have been administered to the eye as liquid drops instilled in the cul-de-sac. However retinal drug delivery is a challenging area. The transport of molecules between the vitreous/retina and systemic circulation is restricted by the blood–retinal barrier, which is made up of retinal pigment epithelium and endothelial cells of the retinal blood vessels. An increase in the understanding of drug absorption mechanisms into the retina from local and systemic administration has led to the development of various drug delivery systems, such as biodegradable and non-biodegradable implants, microspheres, nanoparticles and liposomes, gels and transporter-targeted prodrugs. Such diversity in approaches is an indication that there is still a need for an optimized noninvasive or minimally invasive drug delivery system to the eye. A number of large molecular weight compounds (i.e., oligonucleotides, RNA aptamers, peptides and monoclonal antibodies) have been and continue to be introduced as new therapeutic entities. However, for high molecular weight polar compounds the mechanism of epithelial transport is primarily through the tight junctions in the retinal pigment epithelium, as these agents undergo limited transcellular diffusion. Delivery and administration of these new drugs in a safe and effective manner is still a major challenge facing pharmaceutical scientists. In this review article, the authors discuss various drug delivery strategies, devices and challenges associated with drug delivery to the retina.

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1. Introduction

Drug delivery to the posterior ocular segment poses significant drug delivery challenges due to its unique anatomical and physiological barriers. The majority of any topically administered drug is removed by natural mechanisms, such as blinking, tear turnovers, drug metabolism and binding. The remaining fraction (< 5%) of the drug finally reaches the target site. The poor bioavailability of topically administered agents is also due to the impermeable nature of the human cornea, which is composed of epithelium, substantia propria and endothelium.

Drug delivery to the eye can be broadly discussed under the treatment of anterior and posterior segment diseases. This review article aims to highlight the advancements in the drug delivery arena over the past decade. The treatment of the diseases related to the anterior segment of the eye is relatively less problematic than the posterior segment. The posterior segments of eye require site-specific drug delivery systems to target the vitreous cavity, retinal pigment epithelium (RPE)

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and choroid. Diseases affecting the posterior segment, such as age-related macular degeneration (AMD), diabetic macular edema (DME), cytomegalovirus (CMV), proliferative vitreoretinopathy (PVR) and uveitis, can cause irreparable vision loss due to inadequate drug levels arising from poor delivery.

1.1 Age-related macular degeneration

AMD is a vision-threatening ocular disease affecting the macula [1]. AMD is the major cause of central vision loss, typically in individuals ≥ 65 years of age. The early phase of AMD is characterized by drusen located under the RPE, and pigment alteration. Advanced stages involve dry and wet AMD. The former represents the severe atrophy of photoreceptors and the underlying RPE and choriocapillaris. The latter accompanies choroidal neovascularization (CNV) in invading the subretinal and sub-RPE space. Patients with neovascular AMD have a mean age of 70.5 years, compared with 56.8 years for those with dry AMD; no significant gender difference has been found [2].

The exact mechanism involved in the pathogenesis of neovascularization is poorly understood. Furthermore, the exact cause of primary insult also remains unknown. The natural history of CNV formation involves various pathophysiologic events, such as senile degeneration of the RPE, deposition of drusen, disruption of the Bruch's membrane, CNV formation and cicatrisation of the CNV [3,4]. Higher VEGF levels have been found in patients suffering from wet AMD, which supports VEGF's role in the development of choroidal neovascularization. VEGF is a large polypeptide structurally similar to platelet-derived growth factor (PDGF), it is a potent mitogen for vascular endothelial cells and promotes leukocyte-induced damage to retinal endothelial cells [5]. The early stages of wet AMD can be diagnosed from the presence of large soft drusen and localized detachment of the RPE. Presently, AMD is treated using photodynamic therapy, transpupillary thermotherapy, macular surgery, radiation, retinal translocation, anti-VEGF agents and steroids [6]. An ideal strategy to combat AMD will only result from a thorough understanding of the pathophysiological mechanisms involved in the development of AMD.

1.2 Diabetic macular edema

DME is commonly seen in patients suffering from diabetes mellitus. This disease is characterized by the leaking of fluid from blood vessels and swelling of the retina within the macula [7]. It is associated with the thickening of the basement membrane and a lowering of the pericyte count, which in turn raises the permeability and incompetence of retinal vasculature. A compromise in the blood–retinal barrier (BRB) integrity leads to the leakage of plasma constituents in the surrounding retina, resulting in retinal edema. Various factors linked to the development of DME include the degree of diabetic retinopathy, severe hypertension, hypoalbuminemia, hyperlipidemia and fluid retention. Diabetic macular edema is classified into two types: focal or non-cystoid DME, diffuse or cystoid DME. Focal macular edema is caused by microaneurysms (small aberrations in blood vessels) that can lead to vision loss. Diffuse macular edema is caused by the dilation of retinal capillaries (extremely thin, narrow blood vessels) in the posterior segment [8,9]. Laser treatment is indicated in the treatment of DME (focal laser treatment for focal macular edema and grid laser treatment for diffuse macular edema). Recently, intravitreal or subtenon injections of various corticosteroids have shown promising results in the treatment of diffuse diabetic macular edema [10,11].

1.3 Proliferative vitreoretinopathy

PVR involves the scarring process that may result from failed retinal detachment surgery. As the name suggests, the proliferation of cells takes place in the vitreous and retina [12]. This condition is characterized by the proliferation of cell membranes on the surfaces of the retina and in the vitreous. In 1989, the Silicone Study Group classified PVR into three patterns: diffuse, focal and subretinal [13]. The development of PVR is linked to clinical risk factors, such as aphakia, recurrent retinal detachment, preoperative vitreous hemorrhage, choroidal detachment, the presence of preoperative PVR, silicone oil-based implants and a high vitreous protein level [14-18]. At present, the management of PVR primarily involves surgical treatment. Recurrences of PVR after surgery can be prevented by adjunctive treatment; adjunctive treatments stop the proliferation of cell types and other growth factors involved in PVR. 5-Fluorouracil and low molecular weight heparin in combination have shown promising results in the treatment of patients at risk of developing PVR after vitrectomy and retinal reattachment surgery [19]. Adjunctive treatments achieved via targeted/sustained drug delivery systems can prevent re-proliferation after surgery for established PVR [20].

1.4 Uveitis

Uveitis refers to inflammation affecting the uvea, which consists of iris, ciliary body and choroid. Depending on the structures affected by the inflammation, this condition can be classified as iritis or anterior uveitis, iridocyclitis or intermediate uveitis, and choroiditis or posterior uveitis. Posterior uveitis is inflammation affecting the retina and choroid. The most common types of posterior uveitis include toxoplasmosis, pars planitis, CMV retinitis and histoplasmosis. The risk factors for developing posterior uveitis include sarcoidosis, syphilis, Behcet's syndrome, psoriatic arthritis and multiple sclerosis [21-23]. Various corticosteroids [24-26] and immunosuppressive drugs, such as cyclophosphamide, chlorambucil, methotrexate, azathioprine, ciclosporin A, bromocriptine, dapsone and colchicine [26,27] form the mainstay in the treatment of uveitis.

1.5 Cytomegalovirus retinitis

CMV literally means 'very big cell virus', and belongs to the herpes virus family. In humans, the virus is also known as human herpes virus 5. CMV is present in various body

fluids, such as urine, blood, saliva, semen, cervical secretions and breast milk, of an infected person and spreads through sexual contact, blood transfusions, organ transplants, and breastfeeding. HIV-infected patients with T cell or CD4 count < 100 are more prone to this disease [28]. CMV retinitis is characterized by retinal necrosis, granular lesions and retinal edema [29,30]. CMV retinitis leads to blurring of central vision, photopsias (sense flashes of light in retinal irritation), floaters (cellular debris within the vitreous), blind spots (lack of light-detecting photoreceptor cells on the optic disc) and visual-field loss [1]. Traditionally, CMV is treated daily with antiviral drugs such as ganciclovir, foscarnet and cidofovir. Although the preferred mode of delivery is intravenous administration, intraocular implants of ganciclovir appear to provide a better therapeutic profile over intravenous ganciclovir administration, resulting in a dramatic delay in progression of CMV retinitis [31].

As previously mentioned, the topical route is not able to provide adequate drug delivery to the posterior segment, and even systematically administered therapeutic agents demonstrate limited permeability into the retina and vitreous. The two main barriers that prevent the entry of drug from systemic circulation into ocular tissues are the blood–aqueous barrier and BRB [32]. The blood–aqueous barrier may not constitute the primary barrier to drug absorption into the retina and vitreous chamber, but the BRB regulates the transport of agents from the blood into the vitreous and retina [33]. The BRB is composed of the retinal endothelial blood vessels and RPE. For several agents, the permeability behavior across the BRB is comparable to those at the blood–brain barrier [34]. Moreover, the RPE, otherwise known as the outer BRB, contains efflux pumps such as P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP), which further limit the permeation of various xenobiotics and endogenous compounds from the choroid into the vitreous [35,36]. P-gp is also expressed on the retinal endothelial vessels, also known as the inner BRB [37].

Thus, the challenge facing ocular pharmacologists and drug delivery scientists today is not only to develop new therapeutic agents but to find more effective drug delivery strategies to circumvent the ocular barriers without causing significant discomfort or alterations to the protective mechanisms of the inner eye. The challenge is presently being met by various injection methods, systems and controlled release ocular drug delivery systems, some of which are dealt in this review article.

2. Conventional routes for drug delivery to the posterior segment

Generally, four modes of administration can be employed for the delivery of drugs to the posterior segment: topical, systemic, intravitreal and periocular (including subconjunctival, sub-Tenon's, retrobulbar, peribulbar and posterior juxtascleral) [38]. The conventional routes (i.e., topical and systemic) are discussed in this section, whereas intravitreal and various novel periocular routes will be discussed in the following section.

2.1 Topical administration

Topical is the most preferred route of drug administration, primarily for reasons of better patient compliance and cost affordability. This route is most convenient in the treatment of diseases affecting the anterior segment of the eye. Drug absorption following topical administration occurs both by corneal or non-corneal routes [39]. A large fraction absorbed through the cornea penetrates into intraocular tissues, unlike the non-corneal route, which causes significant uptake into systemic circulation via the nasolacrimal duct. Non-corneal routes are considered to be non-productive with respect to drug availability in the anterior chamber of the eye [40,41]. However, a few studies claim significant non-corneal absorption due to the favorable physicochemical properties of the penetrating molecules [42]. Many efforts have been directed towards enhancing the corneal permeability of the drug following topical administration. The application of high concentrations of penetration enhancers to increase bioavailability may cause mucosal irritation and corneal abrasion, leading to toxicological complications [43]. Topical application is associated with many other complications, such as the blurring of vision, extensive precorneal drug loss by high tear fluid turnover, non-productive absorption, drainage through the nasolacrimal duct, impermeability of the corneal epithelium, transient precorneal residence time and metabolism of the drug by anterior segment enzymes. As a result, drug absorption is limited to 5% at best [40,44]. As drug availability in the anterior segment is governed by ocular barriers and the physicochemical properties of drug molecules, research should be directed towards surpassing these barriers by novel routes of administration and/or altering the properties of drug molecules.

2.2 Systemic administration

As previously mentioned, BRB hinders the movement of systemically administered drugs to the posterior eye segment (including the retina, choroid and vitreous). Lalezari *et al.* studied the effectiveness of cidofovir upon systemic administration in the treatment of CMV retinitis with manageable side effects [45]. Barza *et al.* demonstrated that poorly lipid soluble antibiotics such as penicillins, cephalosporins and aminoglycosides achieve intravitreal levels no higher than 10% of serum levels after venous infusion. Such low penetration levels requires frequent administration of high doses, resulting in serious systemic side effects [46,47]. Also, nonspecific absorption of systemically administered agents leads to unwanted side effects and potentially serious toxicities.

3. Novel routes for drug delivery to the posterior segment

The concentration of drug delivered to the posterior segment depends heavily on the mode of administration. Commonly

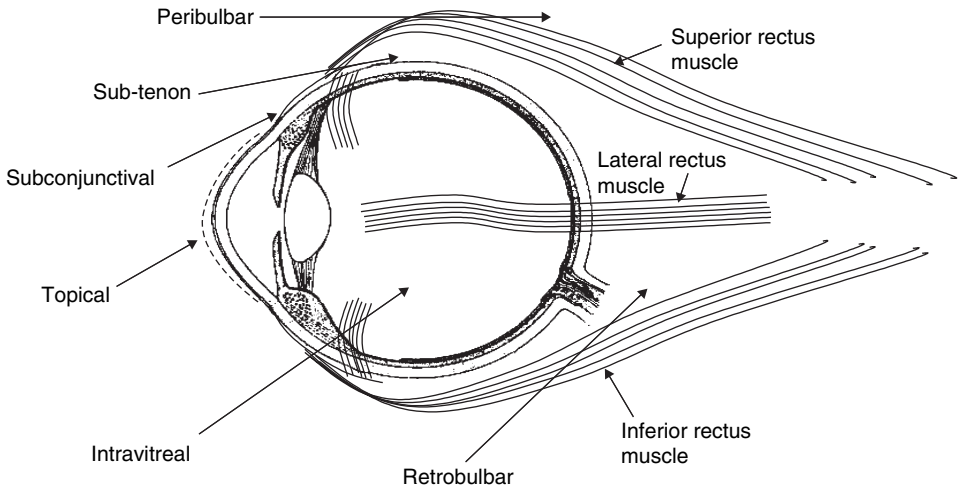

Figure 1. A schematic representation of various ocular routes of administration.
Adapted from *Animal Biology* (3rd edn), Grove AJ, Newell GE (Eds), University Tutorial Press Ltd., London (1950).

employed techniques, such as topical and systemic administration, do not achieve significant levels sometimes – or even the minimum inhibitory concentration – in the posterior segment. But local therapy, for example intravitreal ganciclovir, has shown promising results in the treatment of CMV retinitis in AIDS, and has proven to be an effective alternate to systemic administration [48,49]. In addition, the periocular route of administration may deliver substantial drug levels to the posterior segment relative to topical and systemic routes. **Figure 1** shows the various ocular routes of administration.

3.1 Intravitreal injection

During the past few years, research into drug delivery to the posterior segment by intravitreal injections has grown. This mode of administration requires direct injection of drug into the posterior segment through the pars plana, evading all the barriers. Studies have been carried out to evaluate the pharmacokinetic parameters of antiviral agents: ganciclovir [50], foscarnet [50] and cidofovir [51]; antibiotics: cefazolin [52], amikacin, moxifloxacin [53], ceftizoxime, ceftriaxone ceftazidime [54], clindamycin [55] and gentamicin [56]; steroids: dexamethasone [55], triamcinolone acetonide [57]; and monoclonal antibodies: rituximab [58], bevacizumab [59]; following intravitreal injections. Vitreal retention times have been shown to become larger with an increase in the molecular weight of the drug. Large molecules (linear molecules > 40 kDa and globular molecules > 70 kDa) tend to have longer retention times due to the tight barrier that surrounds the vitreous humor [60,61]. This route of administration is more suitable for drugs with high molecular weights (> 500 Da) and longer half-lifes. Elimination from the vitreous is primarily governed by a first-order rate process [62]. Although drug delivery through intravitreal injections can achieve increased drug concentrations in the neural retina; adverse effects such as retinal detachment from repeated injections, retinal hemorrhage, endophthalmitis and other retinal toxicities due to high concentrations upon bolus dose administration may result in patient incompliance [63-66]. Ausayakhun *et al.* studied the efficacy and complications of intravitreal ganciclovir (2.0 mg in 0.1 ml per injection) to control CMV retinitis [67]. The results indicated that 60% of the treated eyes remained stable, 13% showed improvement and 26% showed a decrease in visual acuity. Moreover, retinal detachment was observed in 6%, intravitreal hemorrhages in 1% and endophthalmitis in 1% of treated eyes. This study clearly demonstrates the complications of intravitreal injections that should be taken into consideration [67]. Several studies were carried out on similar lines, which suggest that intravitreal injection, although useful, is not an ultimate strategy for posterior segment diseases [68-70]. Advancements in drug delivery system design and surgical techniques have lead to the development of intravitreal implants that can be placed inside the vitreous to deliver constant drug levels over prolonged periods. Unlike intravitreal injections administered 2- or 3-times a week, intravitreal implants can be conveniently replaced every 6 months.

3.2 Periocular injection

The region surrounding the eye is referred to as periocular region. Out of all the existing routes, the periocular route is considered to be the least painful and the most efficient route of drug delivery to the posterior segment of eye. Drug delivered via periocular route is placed in close proximity to sclera, and, as a result, vitreal drug levels can be observed 20 – 30 min after administration. Periocular delivery mainly involves retrobulbar, peribulbar, subtenon and subconjunctival routes.

3.2.1 Retrobulbar injection

Retrobulbar injection involves deposition of drug solution into retrobulbar space within the muscle cone. This route is preferred when the medication needs to be in direct

contact with macular region. Hyndiuk *et al.* concluded that steroids tend to concentrate in the optic nerve after retrobulbar injection [71]. Such injections are usually given with a special 23-gauge sharp 1.5 inch needle with a rounded tip and a 10° bend. The needle is introduced in the quadrant between the inferior and the lateral rectus muscles and directed posteriorly until orbital septum resists its penetration; the needle is directed towards the apex of the orbit and penetrated until it meets the resistance of the intermuscular septum. Following penetration through this structure, the needle reaches the retrobulbar space which can take 2 – 3 ml of solution. Care should be taken to minimize needle movement so as to prevent possible laceration of the blood vessels. Pressure should be applied on the globe to distribute the anesthetic effect and to ensure homeostasis [72].

3.2.2 *Peribulbar injection*

Peribulbar injection has been devised to lower the risk of injury to intraorbital structures associated with retrobulbar administration during cataract surgery. The injection is made in the inferiorlateral quadrant of the orbit using a 26-gauge half-inch disposable needle [73]. Peribulbar injections can be classified as circum-ocular (sub-tenon's, episcleral); peri-ocular (anterior, superficial); peri-conal (posterior, deep) and apical (ultra deep); based on the depth of needle [74]. Ripart *et al.* have reported comparable clinical efficacy using the retrobulbar and peribulbar techniques [75].

To ensure that the needle is in the proper peribulbar space, the eversion of the lower lid when the hub of the needle touches the eyelid skin should be checked. A total of 8 – 10 ml of anesthetic solution (mixture) can be injected in both sites. Although, peribulbar and retrobulbar injections are proven to be useful in analgesia, akinesia, the control of intraocular pressure [76] and postoperative analgesia, complications such as diplopia, orbital haemorrhage, globe perforation, artery occlusion, brainstem anaesthesia, optic nerve trauma and ptosis have been reported [77-81].

3.2.3 *Subtenon injection*

The Tenon's capsule is a fascial sheath of connective tissue sandwiched between the conjunctiva and episcleral plexus. The episcleral or subtenon's space is a void between the Tenon's capsule and sclera [82]. Subtenon injection places the drug in contact with sclera for longer periods due to its avascular nature. The posterior Tenon's capsule has the tendency to degenerate with age, thus helping the diffusion of anesthetic into the retrobulbar cone. A drug solution administered by subtenon's injection has the disadvantage of decreased and difficult molecular penetration through the sclera and choroid. Moreover, rapid removal of the drug by the choroidal circulation can result in a shortened duration of action. It is considered to be the most promising route for targeting posterior segment [82].

3.2.4 *Subconjunctival injection*

The conjunctiva is a membrane that covers sclera. A mucus layer secreted by the goblet cells of conjunctiva hydrates, lubricates, cleanses and serves as a defence against pathogens. The injection of a drug solution beneath the conjunctiva is considered to be a localized and minimally invasive drug delivery technique to the posterior segment of eye. Approximately 500 µl of a drug solution can be injected into the subconjunctival area (bulbar conjunctiva) using a 25/30-gauge, 30 mm long needle. Following a subconjunctival injection, molecules may directly diffuse through the sclera to reach choroid [83]. Ambati *et al.* have demonstrated that significant levels of bioactive proteins can be delivered to the posterior segment of the rabbit eye following a subconjunctival infusion [84]. Prostaglandins have also been found to be more permeable across the sclera than the cornea [85]. High concentrations of dexamethasone has been found in the subretinal fluid of humans following subconjunctival injection, in contrast to oral and peribulbar routes, indicating that subconjunctival administration may deliver greater amounts of drug to the retina [86]. Sustained delivery can be achieved in the posterior segment by using polymeric vehicles [87,88]. Many research articles propound the use of subconjunctival injection for posterior segment delivery because of its numerous advantages over other routes.

4. Novel drug delivery systems

Intravitreal and periocular routes of drug administration generally achieve high drug levels in the posterior segment, in contrast to oral, systemic or topical routes. The rapid clearance of agents from the site of action is still an issue. Although this problem can be addressed by frequent drug administration, such a regimen results in poor patient compliance. The sustained delivery of therapeutic agents in the vitreous has been attempted by several groups. The most favored are polymeric systems such as micelles, gels, nanoparticles, microcapsules and even implants. These system acts as a depot for long-term controlled drug delivery.

4.1 Liposomes

Liposomes are membrane-like vesicular system consisting of one or more concentric lipid bilayers separated by water or an aqueous buffer compartment with a diameter of 1 nm and 10 µm. These systems, categorized as small unilamellar vesicles (10 – 100 nm), large unilamellar vesicles (0.1 – 10.0 µm) and large multilamellar vesicles (more than one lipid bilayer) depending on their size and the number of lipid bilayers, have found applications in various ophthalmic formulations. These systems are among the earliest drug delivery systems used in the eye. The nature of the hydrophilic and hydrophobic parts in liposomes plays an important role in various drug delivery strategies. A lipid-soluble drug can be incorporated into the membrane portion of the vesicle, and water-soluble compounds can be incorporated in the central aqueous compartment [89].

Various drugs, such as antibiotics [90,91], antivirals [92,93] and ciclosporin [94,95], have been encapsulated in liposomes for intravitreal delivery. In the case of CMV retinitis, as ganciclovir levels fall below the minimum inhibitory concentration CMV

starts replicating. Therefore, the management of CMV retinitis requires frequent intravitreal injection of ganciclovir. To overcome the undesirable side effects of frequent intravitreal injection, various sustained release devices have been developed. Liposomal delivery to the posterior segment may improve drug efficacy/distribution, sustain the release and reduce the toxicity [96]. In fact, the first injectable intravitreal release system studied was ganciclovir liposomes [93,97-99]. These vesicles act as sustained release drug depot held in the aqueous core, and have been shown to provide therapeutic intravitreal drug levels for a prolonged period of time compared with conventional intravitreal injections [100-102]. For example, the clearance rate for ganciclovir from intravitreally injected liposomes has been shown to be 22-times slower than a control; no evidence of retinal toxicity was observed in one study after clinical or light microscopy examination of the treated eyes [101]. Encapsulating the lipophilic prodrug ganciclovir in liposomes has also been carried out [103], and liposomal delivery has also enabled the sustained release and stabilisation of antisense oligonucleotides in the ocular fluids [104]. Targeted drug delivery to retinal tissues has even been achieved by encapsulating agents in heat-sensitive liposomes; these vesicles were administered into the marginal ear vein and a moderate argon laser pulse applied to deliver locally a bolus dose to the retinal vasculature [105,106].

Liposomes are potentially useful as ocular drug delivery systems; however, a relatively short shelf life and the difficulties of sterilization have limited their commercial success.

4.2 Micro- and nanoparticles

As with liposomes, sustained intravitreal therapeutic drug concentrations can be achieved with controlled release from micro- and nanoparticles. These particulate delivery systems can improve patient compliance and reduce side effects. They are designed as drug carriers where the active ingredient (drug or biologically active material) is dissolved, entrapped or encapsulated, and/or the active ingredient is absorbed, adsorbed or attached. Particles with a size > 1 μm are usually termed microparticles or microspheres, whereas those < 1 μm are generally termed as nanoparticles. Nanoparticles can also be referred to as nanospheres and nanocapsules: the former consists of a matrix-like structure, where active compounds can be firmly adsorbed onto the surface, entrapped or dissolved in the matrix; the later has a polymeric shell and an inner core.

Various polymers have been used in the development of micro- and nanoparticles for intraocular delivery and are discussed in several reviews [107-109]. The most common ones are polylactide (PLA) and poly(lactide-co-glycolide) (PLGA). These are FDA-approved polymers that degrade *in vivo* via the Kreb's cycle, resulting in biocompatible by-products (lactic and glycolic acids). These polymers can be fabricated or formulated into devices such that they can provide controlled drug release from a few days to years. Intravitreal delivery of PLGA- and PLA-based microparticles has been shown to cause no apparent cytotoxicity on the retina, as shown by electrophysiological and histological examinations [110,111].

Initial experiments have used fluorescein-loaded biodegradable microparticles that were injected intravitreally in rabbits. These studies helped determine the clearance of the dye from PLGA- compared with PLA-based microspheres [111]. Later studies with doxorubicin (adriamycin) [112], retinoic acid- [113] and 5-fluorouracil-loaded [114,115] microspheres were investigated for the treatment of PVR. Rabbit eyes inoculated with human cytomegalovirus were used to test the antiviral effect of ganciclovir released from PLGA microspheres [116]. PLGA microspheres containing ciclosporin A are effective in the treatment of severe chronic posterior uveitis in patients unable to tolerate systemic or periocular therapy [117].

Recent work from the laboratory of the present authors has shown that suspending ganciclovir-loaded PLGA microparticles in a PLGA–PEG–PLGA thermosensitive gel minimizes the initial burst release [118], and may provide a formulation suitable for intravitreal injection that offers minimal obstruction to vision [119]. In this study, a conscious rabbit microdialysis model with permanently implanted probes was selected as the method for investigating the vitreous ganciclovir levels following intravitreal administration of the formulation.

In vivo studies using a drug entrapped in nanoparticles for intravitreal delivery are relatively limited. Merodio *et al.* have studied ocular disposition and tolerance of ganciclovir loaded bovine serum albumin nanoparticles in rats following intravitreal administration. Even after 2 weeks, a significant proportion of nanoparticles remained in the vitreous [120]. No inflammatory responses were observed in the histological evaluation of the retina, mainly at the photoreceptor layer, and adjacent tissues. Another set of experiments by de Kozak *et al.* using the PEG-conjugated nanoparticles loaded with tamoxifen were evaluated in experimental autoimmune uveoretinitis rat models [121]. PLA nanoparticles loaded with rhodamine 6G and nile red, when injected in the vitreous, have demonstrated transretinal movement, with a preferential localization in RPE cells. This study has also revealed that a single intravitreal injection of nanoparticles can continue to deliver drug to RPE for ~ 4 months [122].

A comparative study between microparticles (2 μm) and nanoparticles (200 nm and 50 nm) encapsulated with sodium fluorescein revealed that particles ≤ 200 nm could be phagocytosed by the retina. Even after 1 month, larger particles were only observed in the vitreous and trabecular meshwork. Indirect ophthalmoscopy has shown that 2 μm particles were deposited on the retina, causing dense vitreous opacities, but mild opacities were observed with nanoparticles [123].

4.3 Implants

Although injectable drug delivery systems such as liposomes, micro- and nanoparticles are easy to administer, once injected it would be difficult to retract those particles, in the instance of complications such as toxic responses. Thus, it could be advantageous to use implants instead for

controlling the rate and duration of drug release. Ocular implants can be removed as needed by surgical intervention. Such implants may be divided into two broad categories depending on the polymer in the matrix:

- Nonbiodegradable implants [31,124] are not metabolized to any significant extent and are not eroded *in vivo*.
- Biodegradable implants [125,126] eventually degrade *in vivo* into soluble components by enzymatic and/or non-enzymatic processes.

A nonbiodegradable implant containing ganciclovir was first introduced in 1996 for CMV retinitis (Vitrasert®; Chiron Vision). This reservoir type of device contains a ganciclovir tablet coated with polyvinyl alcohol and ethylene vinyl acetate. It is surgically inserted in the posterior segment of the eye, through the pars plana, where it delivers ganciclovir locally over a period of 5 – 8 months in a well-controlled (zero-order) manner. The implantation of Vitrasert, being large, requires the creation of 4 – 5 mm sclerotomy at the pars plana. Once the device is depleted of drug, it needs to be replaced with a new device. Side effects occurs in ~ 13 out of 110 eyes with this device, and are associated with complications in the posterior segment, such as vitreous hemorrhage, rhegmatogenous retinal detachment, endophthalmitis and cystoid macular edema with epiretinal membrane [127]. Nevertheless, several other implants have been designed with drugs such as fluocinolone [128], dexamethasone [129] and ciclosporin [130] for the treatment of severe uvetis. Retisert® (Bausch and Lomb), another nonbiodegradable implant containing fluocinolone, was approved by FDA in 2005 for the treatment of chronic noninfectious uvetis [131]. Similarly, implants incorporating both triamcinolone and 5-fluorouracil are proven to be effective in PVR [132]. The limitations of intravitreal implants has led to the development of intrascleral implants that are less invasive and are made of polyvinyl alcohol and ethylene vinyl acetate containing betamethasone [133].

Implants made of biodegradable polymers can be further classified as hydrophilic or hydrophobic. Hydrophilic polymers have been less explored *in vivo* for retinal drug delivery relative to the hydrophobic ones. Hydrophobic biodegradable polymers have been shown to be effective for the preparation of sustained delivery devices for various drug types, such as antivirals [125,134-137], antifungals [138], antimetabolites [139-142], immunosuppressive agents [143] and steroids [142,144,145]. However, the most widely used hydrophobic polymeric implants are made of PLGA and PLA. These polymers degrade by bulk erosion and suffer from initial and final burst releases. To overcome final burst release, a device with a polymeric blend of 80 (PLA-70,000): 20 (PLA-5000) has been developed. This implant has demonstrated sustained ganciclovir release for a period of 24 weeks, with drug levels maintained well above the minimum therapeutic levels [135]. The use of a sustained release 5-fluorouridine-containing PLGA rod in the treatment of experimental PVR has been shown to reduce retinal detachment from 89 to 11%, with no apparent toxicity [141]. Similar experiments by Hashizoe *et al.* have revealed that retinal detachment was lowered from 100 to 64% by using a scleral plug [140]. More recently, the co-delivery of multiple drugs has also been investigated from a single implant for the treatment of PVR [142]. PLGA-based implants consist of three cylindrical segments containing 5-fluorouridine, triamcinolone and human recombinant tissue plasminogen activator. Several studies have been performed to assess the toxicity and biocompatibility of these implants using slit lamp examination, electroretinography and light microscopy. Results revealed no ocular inflammatory reactions with slit lamp biomicroscopy [137] and no change on electroretinograms [137-139,145] even after complete drug release. Histological evidence also demonstrates the biocompatibility of these implants: no abnormalities in rabbit retinal tissue adjacent to the implanted site and the posterior pole have been observed [138,139,145].

5. Novel targeting strategies to posterior eye segment

In spite of progress made in retinal drug delivery, the field remains challenging due to drug hydrophilicity, the presence of efflux proteins and drug ionization. Due to the polar and hydrophilic properties of a drug molecule (such as ganciclovir, cidofovir or foscarnet), its entry into retinal cells could be restricted, which is often prerequisite in the treatment of a disease such as CMV retinitis. In addition, efflux proteins can limit transepithelial permeation of a large number of lipophilic compounds. Earlier work from the laboratory of the present authors used the conventional lipophilic prodrug design, which increases permeability but suffers from poor aqueous solubility [146]. Recently, transporter-targeted prodrug design has met with considerable success in imparting sufficient permeation of several molecules across biological membranes. This strategy can also be employed to enhance intracellular drug concentrations in the retina following systemic, intravitreal and transcleral administration (**Figure 2A** and **B**).

Other strategies that have been employed in the area of drug delivery include iontophoresis and phonophoresis. Drug delivery to the eye using these novel strategies has also been attempted. The following subsections will discuss their application.

5.1 Transporter-mediated drug delivery

Various nutrient transporters are expressed on both the apical and basal sides of epithelial cells. Studies have also been done to prove the presence of numerous transporters on the neural retina [147-151]. Initially it was thought that membrane transporters were responsible for transferring native compounds across cell membranes, but later studies have shown their role in drug transport across various tissues. Transporter-mediated drug delivery involves targeting drug molecules to these transporters, in order to enhance their permeation across membranes. By chemical modification or coupling to a ligand (promoeity) that is a known substrate for the transporter, the parent drug can be transported. This prodrug (i.e., drug with

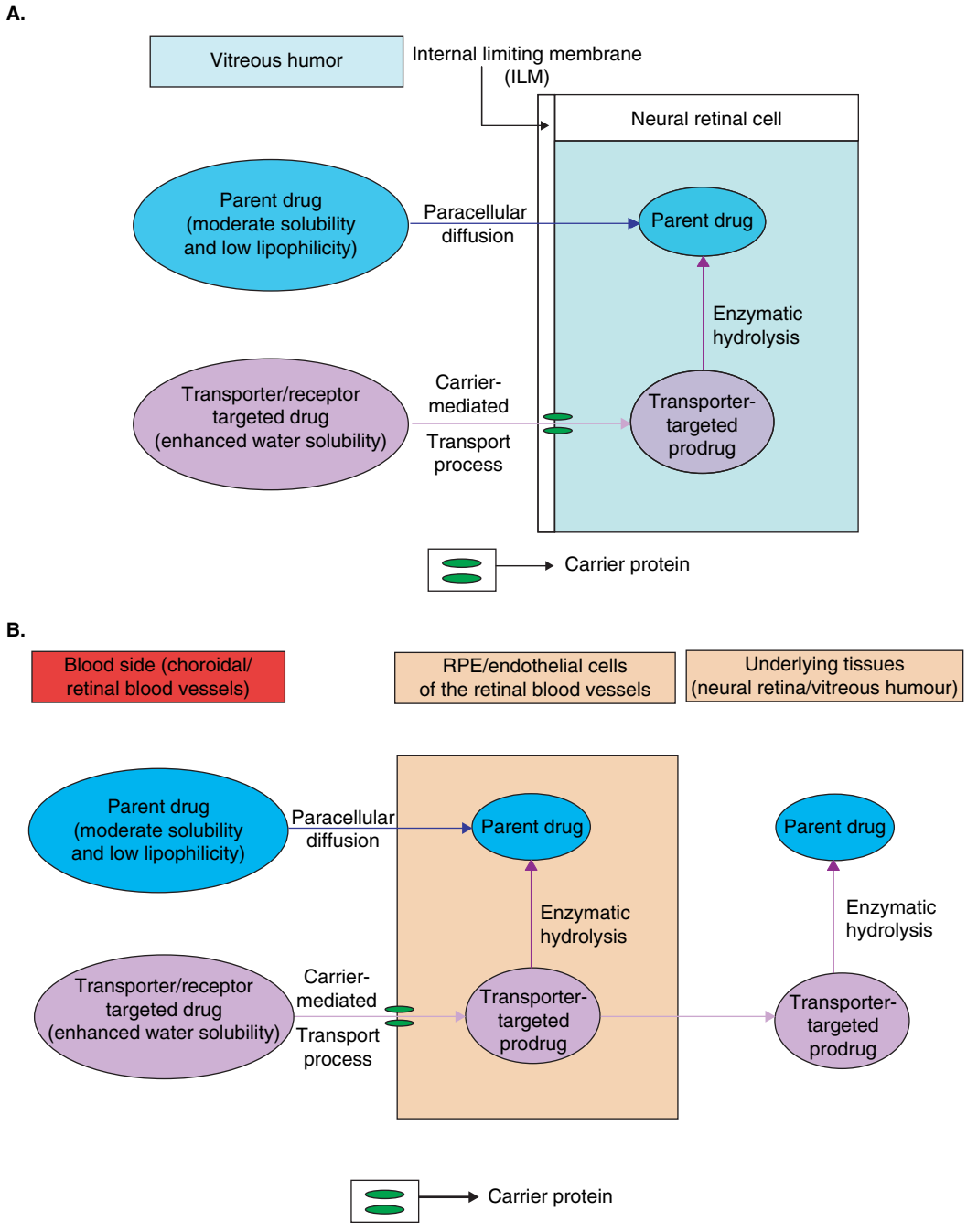

Figure 2. A schematic representation of retinal drug levels following (A) intravitreal administration of parent drug and transporter targeted prodrug (B) systemic administration of a parent drug and a transporter-targeted prodrug.
RPE: Retinal pigmented epithelium.

a promoeity) is translocated across the cell membrane if the promoeity or prodrug is recognized by the transporter. By proper selection of the promoeity, the desired solubility and lipophilicity characteristics can also be achieved. Moreover, the prodrug, which is subsequently enzymatically cleaved, releases the parent drug and promoeity (leaving group), which, being a nutrient, causes no toxicity concerns. Various transporters that are generally targeted for the purpose of drug delivery include peptides, amino acids, monocarboxylic acids, folates, nucleosides and nucleobases, organic anions and organic cations [152]. A few notable transporters are discussed in the following sections relative to retinal drug delivery.

5.1.1 *Peptide transporters*

Peptide transporters translocate dipeptides, tripeptides and peptidomimetics across the various epithelia [153].

These membrane translocators are proton coupled and are classified into PepT1, PepT2 and peptide/histidine transporters (PHT 1 and PHT 2). These transporters translocate a wide variety of substrates. Therefore, many drugs with diverse chemical structures and pharmacological activities can be delivered by these transporters into various cells. Cephalosporins such as cefadroxil [154], ACE inhibitors, β-lactam antibiotics, renin inhibitors, and a few other compounds without a peptide bond such as 5-aminolevulinic acid [155], are substrates of peptide transporters. The high oral bioavailability of these drugs is attributed to the active transport by PepTs on the brush border membranes. Many nonsubstrate compounds can be delivered via PepTs through prodrug derivatization (i.e., by esterification with dipeptides and tripeptides), such as benzyl alcohol, acyclovir [156], ganciclovir [157] and zidovudine, with increased oral bioavailability. There is ample evidence to suggest that peptide transporters are expressed on the rabbit corneal and retinal epithelia, and also on both the blood–aqueous and blood–retinal barriers [158-160]. The delivery of acyclovir and ganciclovir across the corneal and retinal barriers has been improved by a targeted prodrug design. The permeability of dipeptide prodrugs of nucleoside analogs, valine–valine–acyclovir, valine–valine–ganciclovir and tyrosine–valine–ganciclovir, was found to be significantly higher compared with their parent counterparts and prototypes, valine–acyclovir and valine–ganciclovir. Because of their high drug affinities and capacities, peptide transporters are also used to circumvent drug efflux proteins. In summary, peptide transporters are very good targets for drug delivery to the ocular tissues.

5.1.2 *Amino acid transporters*

Amino acid transporters are a relatively large class of transmembrane proteins classified on the basis of their substrate specificity, charge and sodium, potassium and chloride ion co-transport. These are mainly classified as cationic, anionic and neutral amino acid transporters and are sub-classified into sodium-dependent and -independent transporters. System L, a large amino acid transporter; system Y^+, a cationic amino acid transporter; and system $b^{0,+}$, a cationic and neutral amino acid transporter are sodium-independent transporters. System X^-, an anionic transporter; system A, B^0, $B^{0,+}$ and ASC (alanine–serine–cystein), β-amino acid transporters; are sodium dependent. Drugs such as gabapentin, L-DOPA and α-methyldopa are some of the substrates of amino acid transporters. As amino acid transporters are very specific in recognizing substrates, peptidyl prodrugs of L-DOPA and α-methyldopa were not recognized by these transporters, although these compounds have been shown to be recognized by the peptide transporters. Various amino acid transporters, such as $B^{0,+}$, LAT1 and ASC, have been found on different ocular tissues. Prodrugs of nucleoside analogs acyclovir and ganciclovir were targeted to these transporters expressed on the corneal epithelium and RPE to achieve higher ocular bioavailability [161,162].

5.1.3 *Monocarboxylic acid transporters*

Monocarboxylic acid transporters (MCTs), expressed in various tissues, transport monocarboxylic acids such as lactic acid, pyruvic acid, and those generated from glucose metabolism. Transport across cell membranes has been found to be proton dependent. There are at least nine subtypes of MCTs (MCT1 – 9) that have been isolated from various mammalian tissues [163-165]. It has also been discovered that MCTs are expressed on the retina. RPE expresses MCT1 on the apical and MCT3 on the basolateral side. Both transporters work in conjunction to transport lactic acid from the neuronal retina into the blood [166]. MCT1 is involved in the transport of monocarboxylic acid drugs, such as valproic and salysilic acids across inner blood–retinal barrier [167]. Other drugs such as β-lactam antibiotics are substrates of MCTs [168]. The prodrug carbenicillin, carindacillin, was found to be transported by MCTs across the intestinal brush border membrane [169]. MCTs could be excellent targets for drug delivery to the retina.

5.1.4 *Folic acid transport systems*

Folic acid transport systems are classified under reduced folate carriers (RFCs) and folate receptors (FRs). FRs are subdivided into three types: FRα, FRβ and FRγ. These receptors express a higher affinity for folic acid and its derivatives than their reduced forms. Unlike the receptors, RFCs exhibit a higher affinity for the reduced form of folic acid. There is evidence to suggest that the polarized expression of FRα and RFC-1 on the RPE [170]. Both of these systems work in conjunction to transport folic acid vectorially from the choroidal blood to the retina. Such transport systems are also expressed in the rabbit retina [171]. Delivering drugs to the retina using the folate transport system could be an effective strategy to increase the ocular bioavailability of drugs impermeable across RPE.

5.2 Improving ocular bioavailability with prodrug derivatization

Targeting prodrugs to the transporters expressed on the retina – or, more precisely, on the RPE – and the endothelial cells of the retinal blood vessels may provide an opportunity to increase the retinal and vitreal drug concentrations. The following subsections discuss how common routes of administration may be employed to target transporters on the retina.

5.2.1 *Systemic administration*

The administration of drugs via systemic routes may achieve 1 – 2% of plasma levels in the vitreous humor. Thus, if such a drug is targeted via a transporter expressed on the BRB, it could significantly enhance the concentration in the retina; prodrugs may be subsequently cleaved in the retina to generate high drug levels (**Figure 3A**). Nonspecific uptake of the prodrug by various tissues expressing a particular transporter for which the prodrug is designed is the major pitfall of this strategy.

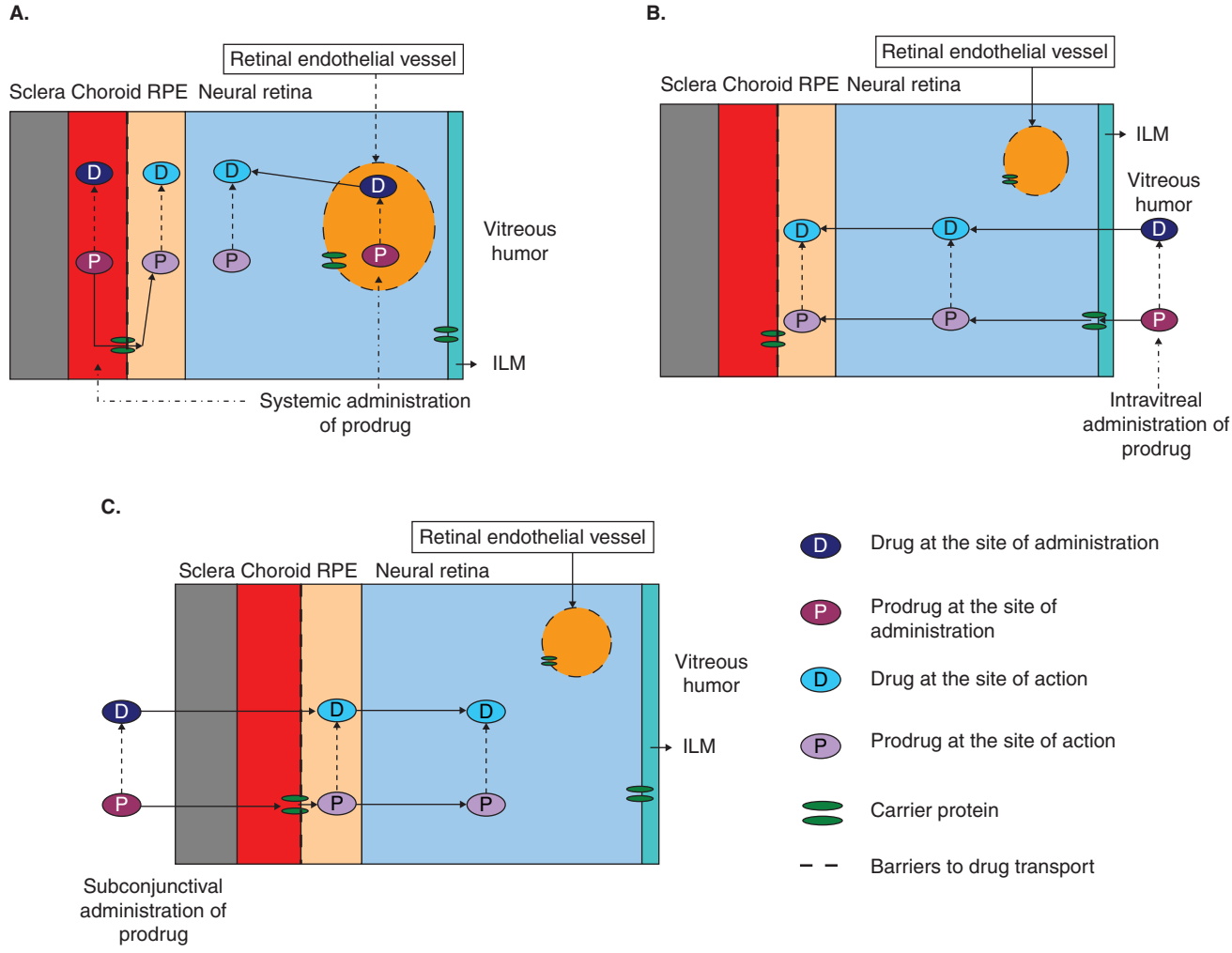

Figure 3. Strategies involved in enhancing drug delivery to the posterior segment by transporter-mediated drug delivery.
A. Transporter-targeted drug delivery following systemic administration; **B.** Transporter-targeted drug delivery following intravitreal administration; and **C.** Transporter-targeted drug delivery following *trans*-scleral/subconjunctival administration.
D: Drug; ILM: Internal limiting membrane; P: Prodrug; RPE: Retinal pigmented epithelium.

5.2.2 *Intravitreal administration*

A direct intravitreal injection of the prodrugs into the vitreous may allow recognition by the specific transporter, thereby resulting in more efficient uptake by the neural retina and RPE cells (**Figure 3B**). This strategy overcomes the limitation of systemic administration of the non-site-specific transporter-mediated uptake of prodrugs.

5.2.3 *Subconjunctival administration*

Subconjunctival administration generally achieves a higher concentration of drug in the vitreous relative to systemic delivery. Administering prodrugs that are targeted to specific transporters expressed on the basolateral side of RPE via the subconjunctival route may enhance vitreous levels. These prodrugs may diffuse through the sclera into the choroidal circulation, where they interact with the transporters on the RPE. This strategy is depicted in **Figure 3C**. Subsequently, the prodrugs may be converted back to the parent drug in the retinal tissue, eliciting the required therapeutic activity.

5.3 Evasion of cellular efflux by prodrug derivatization

Schlosshauer and colleagues observed that efflux pumps P-gp and MRP are expressed on the blood side of the bovine RPE [34]. These researchers have studied the transport of P-gp and MRP substrates. In their study, Rhodamine 123, a P-gp substrate, was found to be effluxed from the neural retina into the blood in a manner similar to the blood–brain barrier. In a similar study, Duvvuri *et al.* looked at the ocular disposition of the drug quinidine in the presence of P-gp inhibitors such as verapamil. From these studies, they concluded that there is expression of P-gp on the RPE [172]. The cellular efflux of many drugs such as anticancer and antiviral agents can be overcome by targeting nutrient influx transporters.

Transporter-targeted prodrug derivatization can achieve evasion of efflux pumps, resulting in improved ocular bioavailability. Covalent linkage of a ligand (promoeity) may result in loss of recognition by efflux pumps, and, at the same time, may cause translocation of the adduct through influx transporters.

5.4 Iontophoresis

It is a noninvasive technique for driving molecules through tissue by electromotive repulsive forces using small electrical charges applied by an iontophoretic chamber charged with an active agent [173-175]. Iontophoresis depends on the physicochemical parameters such as the pH of the tissue [176], drug concentration [177], temperature [178], properties of the drug molecules [173]; and electrical parameters such as current [179], voltage [180] and impedance [181]. This technique suffers from certain side effects, including pain (due to excessive current density), burns (due to electrolyte changes within the tissues) and tissue damage [182]. OcuPhor™, an ocular drug delivery system containing diclofenac, has been designed by Iomed, Inc. Promising results have been obtained, with high concentrations of drug achieved in the posterior segment. This technology can serve as a suitable noninvasive alternative for the treatment of ocular diseases such as AMD and diabetic retinopathy [183]. Iontophoresis-enhanced permeation has been investigated successfully with several drugs such as lidocaine, gentamicin [184], amikacin [185], tobramycin [186], cefazolin [187], ticarcillin [187] insulin and nafarelin [188]. However more concerted efforts are required to render this technique as a clinically useful alternative for posterior segment delivery [189].

5.5 Phonophoresis

In 1963, Griffin and Touchstone demonstrated that by using ultrasonic sound of 1 watt/cm^2 for 5 min, cortisol can be significantly transported through swine skeletal muscle [190]. Zderic *et al.* investigated the application of ultrasound (1-s bursts of 20 kHz) on the *in vitro* corneal permeability of various glaucoma drugs with different lipophilicities. The permeability of rabbit cornea increased by 2.6-times for atenolol, 2.8-times for carteolol, 1.9-times for timolol and 4.4-times for betaxolol, after 60 min of ultrasound exposure [191]. The exact mechanism by which ultrasound enhances the delivery of drugs is still not understood, but certain studies propound that minor structural alterations in the cornea may cause an increase in drug absorption [192]. Ultrasound technology can further be conveniently modified for the administration of drugs to the posterior segment.

6. Conclusion

With numerous diseases affecting the posterior segment of the eye, delivering drugs to the retinal tissue is a clinically significant problem that challenges ocular pharmacologists and drug delivery scientists. Disease such as AMD, DME and PVR warrant the discovery of newer therapeutic macromolecules. New strategies need to be developed to deliver these molecules without causing patient discomfort and alterations to the protective ocular barriers. For instance, the treatment of the wet form of AMD requires a wide variety of molecules, from small steroids to monoclonal antibodies. Each molecule behaves differently with respect to its delivery. When given intravitreally, molecules generally exhibit very short half-lifes, which necessitates their frequent administration. Although subconjunctival and other periocular routes provide relatively lower bioavailability in the retinal tissues than intravitreal injections, they overcome the major limitations of patient incompliance, vitreal hemorrhage and retinal detachment.

Sustained release drug delivery systems such as liposomes, micro- and nanoparticles and implants have proven to be advantageous over simple intravitreal injection of a drug solution. These systems can deliver precise amounts of drug, offer prolonged and controlled release, and prevent the degradation of drug in the vitreous. Intravitreal implants have been developed as an alternative to frequent intravitreal injections. However, the risks involved with intravitreal administrations are not entirely eliminated. Moreover, repeated surgery is required for the implantation and removal of such devices. Particulate systems may provide an alternative to implants, wherein small delivery systems can be injected into the vitreous without the need for surgical interventions. *In situ* gelling systems can also offer a sustained release depot, which does not require surgery.

The past decade has witnessed significant advances in the field of transporter-targeted drug delivery. Valacyclovir and valganciclovir are two well-known drugs that use transporter-mediated drug delivery. Numerous transporters have been identified that can be exploited to enhance the bioavailability in the retina of drugs administered systemically, intravitreally and subconjunctivally. Prodrug derivatization could also improve lipophilicity and water solubility, which is useful in achieving enhanced permeation. Bypassing efflux pumps may further enhance the drug bioavailability via prodrug derivatization.

7. Expert opinion

Diseases affecting posterior segment of the eye are increasing at an alarming rate. To meet the demand for highly effective treatment, it is not only imperative to develop new therapeutic targets, but also to find more effective drug delivery strategies which can effectively deliver drugs in therapeutic concentrations. Membrane transporters and receptors have found wide application in the arena of drug delivery. There are numerous examples of transporters and receptors that have been identified, cloned and shown to be expressed in various tissues. These carriers may not only be used to target specific cells or tissues, but can facilitate the permeation of drug molecules. This strategy is particularly suitable for molecules that poorly permeate the biological barriers or ionize under physiological conditions.

Several transporters have been identified and characterized on the retina and the BRB. These transporters could be used for efficient drug delivery via various modes of administration, such as intravitreal, intravenous or transcleral/subconjunctival. The ocular bioavailability of poorly bioavailable compounds with high potency can be enhanced using a transporter-targeted prodrug approach. It has also been noticed that, apart from these influx transporters, numerous efflux pumps may prevent drug entry into target sites such as the retina. For similar reasons, the compounds that are substrates for efflux pumps may have poor ocular bioavailability. Thus, translocation by influx transporters can bypass the efflux pumps and may enhance the ocular bioavailability of such compounds. Moreover, derivatization of the parent molecule into a prodrug could significantly increase solubility and enable the administration of higher doses.

In the future, an amalgamation of different delivery systems and strategies may open up a new dimension in providing better therapies. In addition, nanotechnology seems to have a lot of potential in drug delivery. Nanoparticles can deliver therapeutic molecules to the targeted locations efficiently and may also be useful in sustaining their release. Receptor-targeted nanoparticles may play a crucial role in delivering drugs and antibodies to the posterior eye segment. Nanoparticles may further provide stability to the drug molecules in various blood and ocular fluids.

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