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Review Article

FACTORS AFFECTING INTRAOCULAR BIOAVAILABILITY OF DRUGS

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ABSTRACT

Ophthalmic drug delivery remains a significant challenge to the clinicians. A number of anatomic and physiological barriers restrict the entry of drug inside the ocular tissues, especially in the posterior segment of eye. The present review discusses various ocular barriers and drug factors which influence the ophthalmic drug delivery. Furthermore, recent advances in ophthalmic drug formulations attempted to overcome these barriers have been explored.

Keywords: Bioavailability, Intraocular, Drugs.

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INTRODUCTION

Bioavailability is the amount of drug reaching systemic circulation in an unchanged form following administration by routes other than intravenous route. Bioavailability by intravenous route is 100% since drug is injected directly into systemic circulation [1,2]. It is an important pharmacokinetic (PK) parameter to predict the action of a drug, since with most drugs, a correlation between plasma concentration and action of drug is observed. However, unlike other tissues, it is difficult to estimate intraocular PK of a drug since serial sampling for the measurement of drug concentration is not possible. Alternative methods, chiefly microdialysis (in animals) and non-invasive PK analysis, are routinely employed to predict intraocular PKs of a drug [3].

As compared to other tissues or organs, ocular drug delivery poses a significant challenge to formulators and scientists. With topical administration of conventional eye drops, less than 5% of drug is said to penetrate the cornea to reach intraocular tissues, while majority is absorbed into systemic circulation [4]. A large amount of drug is subject to "pre-corneal drug loss" (Fig. 1). Targeting a specific tissue of eye with a drug delivery system has been an arduous task, faced with many challenges. Difficulties, in large part, are ascribed to the naturally occurring physiological and anatomical barriers unique to the eye. Normally, these barriers act as protective mechanisms and prevent entry of foreign substances [5], however, these also restrict drug permeation and bioavailability in intraocular tissues.

An attempt has been made in this review to give a brief account of physiological factors and anatomical ocular structures that affect transport of topical and systemic drugs (Fig. 2 and Table 1). In addition, drug formulations and routes of drug administration that influence intraocular bioavailability have been discussed.

ANATOMICAL AND PHYSIOLOGICAL BARRIERS

Cornea

Cornea (~500 μm thick) is considered to be a primary barrier to absorption of topically administered drugs. It displays relative impermeability to both hydrophilic and lipophilic drugs [6]. Corneal epithelium (~50 μm thick) is lipophilic in nature and demonstrates tight intercellular junctions. This accounts for restricted passage of hydrophilic drugs through transcellular pathway and small/large molecules through paracellular pathway.

The stroma (~450 μm thick) is highly hydrated and consists of collagen fibrils in a lamellar arrangement. Hydrated state accounts for restricted penetration of highly lipophilic drugs, whereas collagen lamellae restrict the diffusion of large molecules. Corneal endothelium is moderately lipophilic, demonstrates leaky junctions, and presents a less important barrier [5,7]. On account of such unique structural characteristics, biphasic molecules demonstrate better transcorneal permeation [8].

Tear film

Normal tear film is 4–9 μm thick and has a volume of 7–10 μL which can increase to 30 μL in absence of blinking [7]. On topical administration, the drug has low retention in the pre-corneal region (1–2 min) due to constant flow of tears (1.2–1.5 μl/min) and drainage. Drainage rate is usually much faster than the rate of absorption of drugs [9]. Further, poorly water-soluble drugs bind to mucin present in tear film and demonstrate poor permeation [10].

Induced lacrimation

Normal pH of tear fluid is approximately 7.4. Tear film offers a limited buffering capacity and lacrimation is induced even by mildly irritant solutions. To avoid reflex lacrimation, formulations should have a pH between 7.0 and 7.7. This is, however, difficult to achieve since pH to achieve maximum solubility and stability of drug is often well beyond this range [9]. For example, pilocarpine HCl solution has a pH of 3.5–5.5 and ciprofloxacin HCl solution has a pH of approximately 4.5, whereas timolol maleate solution (0.25%) has a pH of approximately 7.0. Induced lacrimation leads to dilution of topically administered drug and accelerated drug clearance. Rate of clearance increases with an increase in the volume of instilled drug [11]. The average drop volume from commercially available eyedroppers is estimated at 39.0 μL (range: 25.1–56.4 μL) [12]. This volume can be reduced using special droppers. However, these are difficult to manufacture, and often, the problem of non-recognition of application of small volumes is reported by patients [13].

Nasolacrimal drainage and spillage

The volume of solution delivered per drop by conventional droppers largely exceeds the retention capacity of lacrimal sac, that is, ~7 μL. As a result, considerable amount of drug is lost from spillage and drainage through nasolacrimal duct [14]. It has been observed that at least 70% of timolol administered topically reaches systemic circulation within 5 min, through conjunctival and nasal blood vessels [15,16].

Conjunctivae

Conjunctival epithelium demonstrates tight intercellular junctions [17], similar to corneal epithelium, which restrict paracellular drug

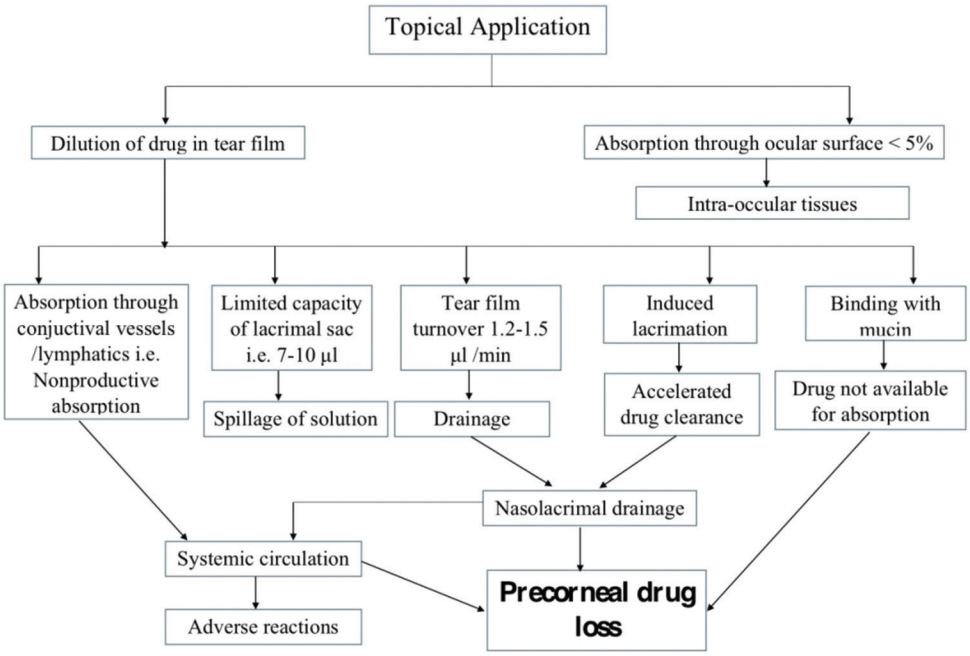

Fig. 1: Factors responsible for "Pre-corneal Drug Loss"

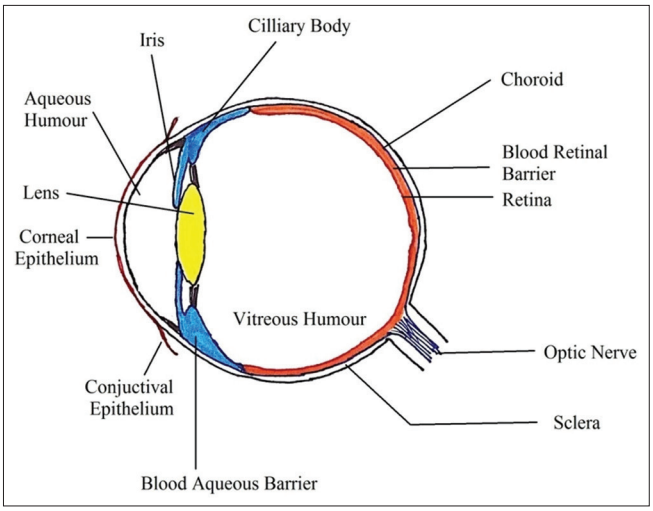

Fig. 2: Anatomy of human eye

transport [10]. Conjunctiva, however, is more permeable to hydrophilic molecules and macromolecules as compared to cornea and offers a 20 times greater surface area. Transconjunctival route is being explored for its potential to deliver large molecules such as protein and peptides. Most drugs in clinical practice, however, are small, lipophilic molecules, which are better absorbed through transcorneal route [18].

Absorption through blood vessels and lymphatics of conjunctivae is often termed as "non-productive absorption" since most of absorbed drug reaches systemic circulation [5,19]. In a study on enucleated rabbit eyes (devoid of circulation) and live rabbit eyes, 30-fold higher drug concentrations were detected in the enucleated eyes as compared to the latter with the use of episcleral implants, suggesting the role of conjunctival and choroidal circulation in drug clearance [20]. Conjunctival circulation is said to play a more important role in drug clearance as compared to choroidal circulation [21,22]. Furthermore, molecular size of drug is inversely related to its clearance through conjunctival or episcleral circulation [23]. Extent of "non-productive absorption" varies between drugs. For example, it has been estimated at 74% for flurbiprofen and up to 70% for timolol [13,15].

Sclera

Sclera consists of irregular arrangement of collagen lamellae interspersed with proteoglycans and glycoproteins [24]. Transscleral route offers several advantages such as (i) large surface area for absorption, (ii) hydrated stroma, which facilitates diffusion of hydrophilic molecules, (iii) absence of metabolic activity, (iv) relatively high permeability to macromolecules, and (v) ease of administration of controlled release dosage forms [23]. Molecular radius of drug is more important than molecular weight for transscleral penetration with globular molecules showing better penetration as compared to linear dextrans [23]. Sclera is permeable to large molecules (up to 150 kDa MW), including proteins such as IgG [25,26], and can act as a drug reservoir [26]. Drugs absorbed through conjunctiva are removed in part by episcleral circulation and uveoscleral outflow. Drugs which escape this elimination can pass transsclerally to reach choroid and even neural retina [3,5]. Furthermore, aging is found to have little effect on transscleral permeability in human eyes [27], which becomes an important consideration for the treatment of conditions associated with aging such as age-related macular degeneration (AMD) or diabetic retinopathy.

Choroid

Choroid is highly vascular, however, the vasculature is leaky. Drugs escaping conjunctival and episcleral circulation can pass transsclerally to reach choroid. Furthermore, systemically administered drugs, due to the leaky vasculature of choroid, can easily reach the choroidal extravascular space. Drug permeation from choroid to retina, however, is restricted by retinal pigment epithelium (RPE) [18] (Fig. 3).

The innermost layer of choroid, choroid Bruch's membrane, can bind to lipophilic molecules, and restrict their permeation, although it allows hydrophilic molecules to pass through. In contrast to sclera, an inverse relation between molecular radius and drug permeation through RPE-choroid has been reported in bovine models. Furthermore, aging is found to reduce solute permeability through Bruch's membrane [23,28].

Vitreous humor

Vitreous humor consists of solutes, ions, collagen, and hyaluronic acid. Hyaluronan, present in the vitreous, is negatively charged and allows permeation of anionic molecules. Drugs administered intravitreally can be eliminated through anterior and/or posterior pathway.

Anterior pathway involves passage of drug molecules into aqueous humor followed by clearance through aqueous turnover and uveal circulation [5]. Posterior pathway involves drug permeation through blood-retinal barrier and uptake by the transporter systems in RPE. Small, lipophilic drugs, eliminated through both routes, demonstrate shorter intravitreal life as compared to large, hydrophilic drugs eliminated only through anterior pathway [29].

Retina

Inner limiting membrane, separating vitreous humor and retina, restricts entry of not only the drugs but also gene delivery into retina following intravitreal administration [5,30]. Intercellular spaces in retina are approximately 15–20 nm wide and do not demonstrate tight junctions, which allow permeation of small molecules but not of large, cationic molecules [29]. Large molecules also face resistance to permeation by inner and outer plexiform layers. In fact, a "Retinal exclusion limit (REL)," referring to the maximum size of molecule which is able to freely permeate through retina, has been defined. According to Jackson *et al*., REL in human eyes is 76 kDa. REL might be responsible for persistence of hard exudates in retina for months in patients of hypertensive or diabetic retinopathy due to high MW and low diffusion capacity [31].

Blood ocular barriers

a. The blood aqueous barrier is formed by endothelium of iris or ciliary blood vessels and non-pigmented ciliary epithelium. It demonstrates tight junctions, restricting the entry of solutes into aqueous humor [5]. It also restricts passage of systemically administered hydrophilic drugs from plasma into aqueous humor. Inflammation can compromise the integrity of this barrier, increasing the drug permeation from plasma [18,32].
b. The blood-retinal barrier consists of an outer blood-retinal barrier (formed by RPE) and an inner blood-retinal barrier (formed by tight junction endothelium of retinal blood vessels) [3] (Fig. 4). Normally, this barrier restricts the entry of plasma components, water, and toxic substances into retina.

Outer blood-retinal barrier is highly resistant to entry of hydrophilic molecules and shows limited permeability to macromolecules [23]. In a study on bovine RPE, hydrophilic beta-blockers (atenolol and nadolol) demonstrated seven to eight times less permeation as compared to lipophilic beta-blockers (metoprolol, timolol, and betaxolol). Thus, choroid Bruch's membrane and RPE pose significant barriers to permeation of lipophilic and hydrophilic molecules, respectively, making drug delivery to retina an even more arduous task.

Inner blood-retinal barrier is also not easy to overcome for systemically administered drugs. Only 1–2% of plasma concentration of drug can be detected in intraocular tissues. Molecules with MW as low as 3 kDa are not able to penetrate the barrier in cat eyes [33]. Drugs from retina, too, cannot diffuse back into systemic circulation. Because of such formidable barriers, eye drops or systemic therapy are often not capable of achieving therapeutic concentration in retina and intravitreal injections become indispensable [3].

Outer blood-retinal barrier can become compromised in the presence of choroidal neovascularization, which then allows permeation of larger molecules. Bevacizumab, used in cases of classical choroidal neovascularization, is found to reduce retinal thickness and improve visual acuity, probably by reversing the increased permeability [26]. *Staphylococcus aureus* infection is shown to disrupt the outer blood-retinal barrier in *in vitro* models and is capable of inducing endophthalmitis in normal and diabetic mice [34].

On the other hand, systemic illnesses such as diabetes or hypertension and surgical procedures can damage the inner blood-retinal barrier, which then allows permeation of larger molecules [31]. This is supported by experimental evidence that periocular injection of celecoxib in diabetic rats achieves greater retinal and vitreal levels due to disruption of blood-retinal barrier [35]. Pathological conditions can also alter drug availability into inner retinal layers such as Muller and photoreceptor cells [5].

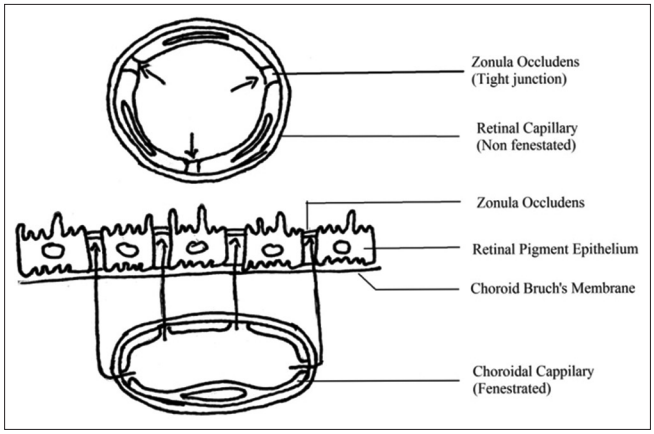

Fig. 3: Structure of choroidal vasculature, choroid Bruch's membrane, and retinal pigment epithelium

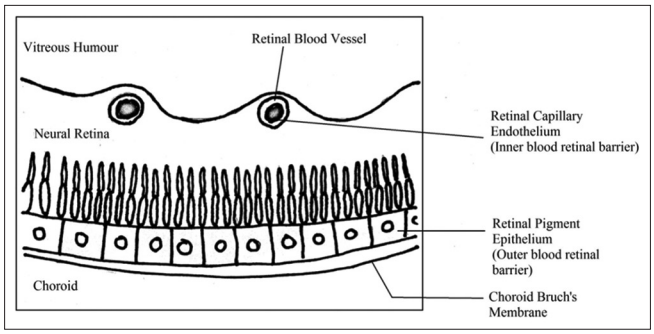

Fig. 4: Inner and outer blood-retinal barrier

Transporter systems in the eye [5,36]

Transporter systems include efflux transporters and influx transporters. Efflux transporters belong to ATP-binding cassette superfamily and include P-glycoprotein, multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP). These transporters are important with regard to bioavailability of anticancer drugs, antifungals, antivirals, steroids, and fluoroquinolones inside ocular tissues (Table 2). Few authors have suggested that only MRP1, MRP5, and BCRP are expressed in freshly excised human corneal epithelial tissue.

Influx transporters belong to solute carrier superfamily and include transporters for amino acids, peptides, vitamins, glucose, lactate, and nucleoside/nucleobases. These are now being explored as targets for prodrugs/drug analogs to enhance intraocular drug delivery. Prodrugs are designed in a way to evade efflux transporters, whereas being recognized as substrates by influx transporters. Common target influx transporters include peptide (PepT1), amino acid (LAT1, LAT2, and B [0,+]), monocarboxylic acid (MCT), and vitamin transporters such as biotin and ascorbic acid (SVCT2). L aspartate acyclovir, targeting B (0,+) transporter on corneal epithelium, demonstrates 4 times higher transcorneal penetration compared to conventional acyclovir. Prodrugs administered through oral and intravenous routes commonly target peptide transporter (PepT).

Furthermore, certain receptors in retina responsible for internalization of nutrients such as folate and transferrin are potential targets for drug delivery to retina.

Drug-related factors

Contact time with anterior surface of the eye is the primary determinant of absorption and intraocular bioavailability of a drug following topical administration. Solution drainage shortens

the contact period with cornea and conjunctiva, thus restricting intraocular drug delivery [37]. Fluid dynamics in the pre-corneal region is altered by various factors, for example, pH, irritant nature, vehicles, tonicity, etc., and must be considered while formulating a compound [13].

Viscosity of solution

Although normal saline can be used as a vehicle in ophthalmic formulations, slightly viscous solutions are usually favorable to patients. Increase in the viscosity also helps to retain the drug for a longer period in the "pre-corneal" region, thus, increasing the contact time [13]. This results in decrease in rate of drainage in unanesthetized rabbit eye [38]. Acceptable viscosity level is considered as 20–30 centipoises [3]. Viscosity of aqueous solutions can be enhanced by addition of polymers such as polyvinyl alcohol, polyvinyl pyrrolidone, methylcellulose, and polyacrylic acid [9], for example, ketorolac 0.45% solution available commercially contains carboxymethylcellulose as viscosity enhancer. However, from a manufacturer's perspective, viscous solutions are difficult to filter and sterilize. Further, increase in viscosity beyond a certain range does not improve the intraocular delivery of drug. On the other hand, it may even lead to interference with visual field or blockage of puncta and canaliculi.

Optimum lipophilicity/hydrophilicity

Optimum lipophilicity of a drug for corneal absorption is indicated by an "n octanol water partition coefficient" between 10 and 100 [9]. Further, increase in lipophilicity usually restricts the diffusion of drug molecules since these bind to lipid membranes of cornea. A parabolic relation between lipophilicity of molecule and its permeability across rabbit cornea has been described by Schoenwald and Ward. A lipid prodrug is often used to achieve optimum lipophilicity in case of poorly soluble drugs such as 5-FU [36]. On the other hand, hydrophilicity of poorly water-soluble drugs can be improved using cyclodextrin-based solutions, for example, cyclodextrin-based solutions of pilocarpine, prostaglandins, acetazolamide, and cyclosporine [39]. However, an appropriate concentration of cyclodextrin (<15%) is required for optimum delivery of active ingredient in case of aqueous solutions [40]. A number of processes are used to achieve an optimum lipophilicity/hydrophilicity of drug molecule, which includes use of ester prodrugs, phosphate ester prodrugs, oxime prodrugs, and oxazolidine prodrugs (Table 3).

Table 1. Anatomical and Physiological barriers to intra-ocular drug delivery

Anatomical barriers	Characteristics
Cornea	Epithelium – Major barrier to passage of hydrophilic drugs; tight intercellular junctions restrict paracellular diffusion. Stroma – Barrier to passage of highly lipophilic drugs
Tear film	Drugs bind with mucin, Dilution of topical drugs Induced lacrimation and tear film turnover accelerate drug clearance
Conjunctiva	More permeable than cornea to hydrophilic drugs and macromolecules; Greater surface area Epithelium – tight intercellular junctions
Sclera	Hydrated stroma- Hydrophilic drugs are better absorbed More permeable to macromolecules Molecular radius is an important parameter to determine permeation
Choroid	Receives less blood flow, hence, less drug permeation from systemic circulation Choroid Bruch's membrane restricts permeation of lipophilic drugs
Vitreous humour	Hyaluronan- More permeable to anionic drugs (due to negative charge) Large, hydrophilic drugs are retained more in vitreous humor
Retina	Permeable to small, lipophilic or hydrophilic molecules Inner limiting membrane restricts entry of drugs from vitreous into retina.
Blood ocular barriers	Blood aqueous barrier- tight junctions restrict entry of solutes into aqueous humour and entry of hydrophilic drugs from plasma into aqueous humour Outer blood retinal barrier- Major barrier to hydrophilic drugs Inner blood retinal barrier- Major barrier which restricts entry of systemic drugs into retina
Physiological barriers	
Lacrimal sac	Low retention capacity i.e., 7-10 uL. Spillage of topical solutions Nasolacrimal drainage
Conjunctival circulation	"Non productive absorption" ; most of absorbed drug reaches systemic circulation Drug clearance is inversely related to molecular size of drug
Episcleral circulation and uveoscleral outflow	Remove a portion of drug bypassing conjunctiva
Choroidal circulation	Plays a role in drug clearance through "non productive absorption"
Transporter system in eye	Efflux transporters reduce bioavailability of anticancer, antifungal and antiviral drugs, steroids and fluroquinolones

Table 2: Efflux transporter systems in the eye and its clinical relevance

Efflux transporter system	Tissue of presence	Clinical relevance
P-glycoprotein (Class I and III)	Cornea, conjunctiva, RPE, and retinal blood vessels Negligible expression in human corneal epithelium	Efflux of lipophilic drugs, for example, lipophilic beta-blockers
Multidrug resistance protein1 (MRP1)	RPE and conjunctival epithelial cells	Efflux of anionic drugs or neutral drugs conjugated with acidic ligands; Responsible for the development of resistance against nucleoside analogs in cancer chemotherapy
Multidrug resistance protein (MRP) 2 and 5	Corneal epithelium	
Breast cancer resistance protein	Corneal epithelium	Anticancer drugs, for example, mitoxantrone, topoisomerase inhibitors, and methotrexate are substrates

Table 3: Prodrugs used to improve lipophilicity/hydrophilicity of drugs for ocular use [36]

Process	Characteristics	Examples of drugs
Esterification (ester prodrugs)	Improve lipophilicity, hydrophilicity and *in vivo* lability; Improved corneal penetration Parent drug is released by esterases present in corneal epithelium, iris-ciliary body, retina, and optic nerves.	Adrenergic agonists or antagonists, carbonic anhydrase inhibitors, cholinomimetic drugs, PGs, and steroids. Ganciclovir mono-valerate – better corneal penetration compared to parent drug. Ester prodrugs of PGF2α analogs, for example, latanoprost, travoprost, and unoprostone exhibit high selectivity for PG F receptor with reduction in adverse reactions Dipivefrin – ester prodrug of epinephrine is more lipophilic than parent drug and exhibits better penetration than parent drug with a higher level in aqueous humor; 20-fold reduction in dose as compared to parent drug
Phosphate esters	Increase aqueous solubility of poorly water-soluble drugs; excellent chemical stability.	Cannabinoid analogs, for example, arachidonylethanolamide and *R*-methanandamide Vidrarabin
Carbamate prodrugs	High *in vivo* stability (not easily degraded to release parent drug)	Limited use
Oxime prodrugs	Activation by hydrolysis in iris-ciliary body (oxime hydrolase) followed by ketone reductase	Beta-blockers, for example, alprenolol, betaxolol, timolol, etc., long-lasting intraocular pressure (IOP) reduction
Oxazolidine prodrugs	Increased lipophilicity of beta amino alcohols, Limited aqueous stability	Phenylephrine oxazolidine shows 10 times improved corneal penetration as compared to parent drug with a 10–15-fold reduction in dose and systemic adverse reactions.
Prodrugs derived from sulfonamide functional groups	More reactive and hydrolyze easily	Sulfonamide derivatives of acetazolamide, methazolamide

Drug concentration

An increase in drug concentration in ophthalmic solutions can improve the corneal penetration in accordance with the Fick's law of diffusion. A plateau effect, however, exists between drug concentration and clinical effect, which may be attributed to reflex lacrimation and resultant drug loss with the use of hypertonic solutions. In a study by Drance and Nash, maximum reduction in IOP was observed with pilocarpine 4% solution as compared to 1%, 2%, and 8% solutions [8].

Particle size, shape, molecular weight, and dissolution rate

Drug dissolution determines the amount of drug in solution actually available for diffusion across the ocular surface. Smaller particle size offers a greater surface area and increases surface specific dissolution rate and permeation across cornea. This property becomes important in case of sparingly soluble drugs in suspensions such as steroids. With regard to the particle shape, sharp angle particles are more irritant and induce reflex lacrimation, which, in turn, enhances drug clearance.

Transcorneal permeability of hydrophilic substances is primarily governed by molecular weight. Drugs with MW <500 Da demonstrate better corneal penetration through passive diffusion. Since most drugs in ophthalmic practice demonstrate small molecular weight, these show good permeation whereas drugs such as bacitracin (MW 1411), colistimethate or colistin sulfate (MW 1250), and polymyxin (MW 1200) can penetrate cornea only in diseased conditions [8].

Surface charge of molecules

Mucin layer on anterior surface of cornea and pores in the corneal epithelium possesses negative electrical charge and repel anionic drugs while positively charged molecules demonstrate better penetration [9]. Corneal penetration by PnG liposomes is demonstrated to be in order of positive> negative> neutral. Enhanced permeation will also lead to a better response *in vivo*. Thus, cationic liposomes of tropicamide and acetazolamide produce greater degree of mydriasis and IOP reduction, respectively. Furthermore, delivery of certain drugs to posterior segment can be increased by the use of positively charged emulsions, for example, cyclosporine [7]. To improve the delivery of anionic drugs, chitosan is being explored for use in nanoparticles and matrix [41].

A positive surface charge, thus, seems a desirable property for an ophthalmic drug, however, it does not always ensure greater bioavailability in all ocular tissues. Positively charged molecules get bound to negatively charged proteoglycan matrix of sclera and demonstrate poor penetration through transscleral route [5]. Choroid Bruch's membrane is also less permeable to positively charged molecules [23]. To add to this, hyaluronan present in vitreous humor is negatively charged, which can form immobile complex with positively charged molecules, thus restricting their movement and drug delivery to retina [5]. This disadvantage is observed during polymeric and liposomal gene delivery to retina [42] and can be overcome to a great extent by PEGylation. In contrast, negatively charged albumin nanoparticles diffuse more freely in vitreous as compared to their cationic counterpart [5]. Hence, structures in posterior segment appear more favorable to the diffusion of anionic molecules.

Melanin binding

Basic and lipophilic drugs have affinity for melanin, normally present in uvea and RPE. Melanin binding significantly reduces the availability of drug for intraocular action and warrants use of larger doses. Drug binding to melanin in iris-ciliary body can affect the drug response in anterior segment, while that in choroid and RPE affects the penetration of drug in posterior segment and retina. Pitkänen *et al*. suggested that lipophilic beta-blockers are expected to have significant binding to melanin in human choroid-RPE [43] while sclera, devoid of melanin, does not pose this problem [5].

Metabolizing enzymes [23,44]

Phase I and II metabolizing enzymes of CYP450 family and lysosomal enzymes are present in the eye. These enzymes are detected in high concentration in iris-ciliary body, choroid, and RPE and to a lesser extent in cornea, lens, aqueous humor, and vitreous humor. Metabolic activity is also detected in the blood ocular barrier which lowers intraocular drug concentration. This is an important consideration during the development of ophthalmic drugs, especially prodrugs.

Route of administration

Topical delivery

Topical administration is usually employed for diseases affecting ocular surface or anterior segment. The route is suitable for the administration

of moderately lipid soluble, small molecules, while it's largely unsuitable for macromolecules. It is not an optimum route for the treatment of diseases affecting posterior segment since most drugs are not able to reach the posterior segment following topical administration [26]. Very few drugs show considerable penetration to posterior segment following topical administration in animal models, for example, dexamethasone-cyclodextrin eye drops (reaches posterior segment tissues), dexamethasone-gamma cyclodextrin microparticulate suspension (reaches retina), nepafenac (reaches retina, has a potential use in choroid and retinal neovascularization), memantine HCL (exhibits high melanin binding), dorzolamide (reaches posterior segment tissues including retina), and brimonidine (displays pigment binding and considerable levels in retina; potential role in neuroprotection) [45-50]. In a study involving 10 participants, 1% voriconazole eye drops was found to achieve therapeutic concentration against sensitive *Candida* species in vitreous humor following hourly administration, indicating the need for more frequent administration [51]. Furthermore, topical betaxolol 0.25%, when administered for 28 days or more, was detected in retina and optic nerve head in enucleated eyes (n=7) of patients of glaucoma [52]. Micellar formulations (~10–20 nm), applied topically, have been explored for retinal delivery of dexamethasone and voclosporin and demonstrate encouraging results [5].

Subconjunctival delivery

Subconjunctival space is expandable up to 500 µL and can act as a drug reservoir [26]. This route is traditionally employed for drug delivery to uvea, however, a considerable amount of drug is lost through "non-productive" absorption. At present, interest in this route is increasing for drug delivery to posterior segment using controlled release formulation [18]. Importance of this route was highlighted in a retrospective survey of 13,886 cataract surgeries, in which prophylactic subconjunctival injection at the end of cataract surgery was found to be associated with less incidence of endophthalmitis as compared to pre-operative topical antibiotics [53].

Systemic therapy

Oral route is commonly employed to treat chronic retinal diseases due to advantages such as patient compliance and non-invasiveness. However, bioavailability in target tissue with oral administration remains limited and use of higher doses can result in systemic toxicity. For example, oral acetazolamide used in the treatment of glaucoma often produces systemic side effects and necessitates drug withdrawal. The route is considered useful for delivery of analgesics, antibiotics, antivirals, and anticancer drugs [5]. Macromolecules, however, cannot be delivered through this route due to limited absorption and first-pass metabolism [26].

Following systemic administration, drugs can permeate through choroidal vasculature to reach the extravascular space in choroid. However, choroid receives only a small fraction of total blood flow, which limits the amount of drug passing to choroid. Further, permeation of drug from choroid to retina is restricted by RPE [18], which makes the drug delivery to retina a challenge. Experimental evidence suggests that gold nanoparticles up to 20 nm when injected IV into mice are able to penetrate the blood-retinal barrier to get distributed in retina [5].

With regard to distribution in other ocular tissues, few drugs such as micafungin (retina-choroid), marbofloxacin, and liposomal amphotericin B (AMB) (aqueous and vitreous humor in inflamed eyes) are shown to distribute in various tissues of rabbit eye following IV administration [54-56]. AMB shows an inflammation dependent sequential distribution from aqueous to vitreous humor [56].

Another important consideration with regard to systemic administration of drugs is occurrence of adverse drug reactions (ADRs), which can be particularly troublesome in geriatric patients who are more prone to development of posterior segment pathologies. Occurrence of ADRs limits the use of high doses and drugs with low safety margins [5,15].

Intrastromal delivery

Here, the drug is injected into corneal stroma to overcome low permeation through corneal epithelium. Cornea can also act as a reservoir for macromolecules, which can prolong the intraocular half-life, for example, intrastromal injection of IgG and albumin [26].

Intracameral delivery

It involves injection of drug directly into anterior chamber of eye. The route is commonly employed to deliver antibiotics following cataract surgery and bevacizumab to reduce corneal and iris neovascularization. This route, however, does not achieve significant drug concentration in the posterior segment. Need of repeated injections (due to rapid turnover of aqueous humor) with a subsequent increase in the risk of infection as well as accidental injection of particulates, which can block the trabecular meshwork, are some of the important disadvantages of this route [26].

Intrascleral delivery

Sclera is more permeable to macromolecules [57] and can also act as a drug reservoir. Intrascleral hollow microneedles have been used to deliver microparticles, nanoparticles, and solutions along with enzymes such as hyaluronidase and collagenase to facilitate scleral penetration [26].

Intravitreal injection

The route delivers drug directly into vitreous. Diffusion of drug inside vitreous, however, is often not uniform. While small molecules distribute rapidly, large molecules display limited distribution. Distribution is also affected by molecular weight and surface charge of molecules, pathologies affecting vitreous or age-related vitreous liquefaction [5,29]. Implants can be used to avoid frequent injections or use of systemic drugs and these also allow administration of smaller dose, for example, ganciclovir implants and fluocinolone acetonide implant. Implants are usually placed at pars plana and can be either biodegradable or non-biodegradable. Non-biodegradable implants offer a prolonged half-life, however, these require surgical removal [15]. A miniature implant containing fluocinolone acetonide, which can be inserted using a 25-gauge needle in an outpatient setting, has been approved by the US FDA in 2014 for the treatment of diabetic macular edema [58].

Periocular administration

Periocular routes, that is, subconjunctival, subtenon, retrobulbar, and peribulbar administration, are used to deliver drugs to posterior segment. Posterior subtenon route is shown to deliver highest concentration in vitreous with minimum systemic concentration as compared to other periocular routes in live rabbits [22]. Drugs administered through periocular routes reach posterior segment through transscleral pathway, choroidal circulation, or through anterior pathway. Particle size is an important determinant of kinetics following periocular administration, with particles >200 nm in size being retained at site of injection for over 2 months. Fibrin sealants can also be used to deliver drugs for a sustained period through these routes. These have been used for delivery of drugs such as tobramycin for keratitis, topotecan, and carboplatin for retinoblastoma and insulin for diabetic retinopathy in animal models [5].

Drug formulations

Traditional ophthalmic vehicles

Traditional ophthalmic formulations such as solutions, ointments, and suspensions comprise more than 90% of commercially available ophthalmic formulations [4,59]. Ophthalmic solutions such as eye drops and lotions are preferred since these are safe, convenient, easy to store, and sterilize [14]. However, less than 5% of drug administered

using these formulations is able to penetrate cornea. Addition of mucoadhesives and viscosity-enhancing polymers enhances the contact period and increases the intraocular drug availability with these formulations [9]. Eye ointments are semisolid preparations which prolong the contact period and enhance intraocular drug delivery. However, these also interfere with vision [60] and, as a result, are commonly employed at bed time [14]. Suspensions are used to dispense sparingly soluble drugs and to obtain slow dissolution rate. Ensuring a proper particle size (<10 um) [8] and narrow size range improve intraocular bioavailability with suspensions [13].

Mucoadhesive polymers

These adhere to conjunctival mucin and enhance retention by increasing thickness of pre-corneal tear film and tear reservoir [11]. Examples of such polymers include hydroxypropylcellulose, polyacrylic acid, polyethylene glycol, dextrans, hyaluronic acid, polygalacturonic acid, and xyloglucan. Anionic and cationic polymers demonstrate better mucoadhesion as compared to non-ionic polymer. Polycarbophil-cysteine conjugate is shown to increase transcorneal permeation in *in vitro* rabbit model by alteration of tight junctions without damaging corneal tissues [3,61].

Ocular inserts

Ocular inserts are solid devices placed in the conjunctival sac, which release the drug at a slow rate. These have several advantages such as prolonged and sustained drug delivery, improved patient compliance, and increased intraocular bioavailability, for example, pilocarpine ocusert. Since drug is retained for a longer period in pre-corneal region and less amount is drained into nasal mucosa, systemic ADRs are less [13]. Other ocular inserts include medicated contact lenses and collagen shields. Ocular inserts have not gained popularity particularly among older patients, because of difficulty in application and occasional expulsion during sleep [15,62]. Another dosage form similar to contact lens is mini-disk (4–5 mm in diameter) which ensures extended release of drugs and is currently being explored. Soluble ophthalmic drug inserts have also been devised. These oval wafer-shaped formulations soften once getting moist by tear fluid and adhere to the ocular surface. These have been explored for delivery of neomycin, kanamycin, atropine, pilocarpine, dexamethasone, sulfapyridine, and tetracaine [40].

In situ gelling systems (Hydrogels/aqueous gels)

These solutions change to a gel-like consistency on change in environmental conditions. These are commonly employed for diseases such as glaucoma and dry eye syndrome and are found to interfere less with vision [14]. A number of *in situ* gelling systems are available.

pH-dependent *in situ* gelling systems contain polymers such as cellulose acetate phthalate and polyacrylic acid derivatives such as carbopols, methacrylate, and polycarbophil [9,63,64]. These polymers change to gel-like consistency when exposed to pH of tear fluid (7.5), for example, cellulose acetophthalate (pH 4.5) coagulates when it is exposed to tear fluid [13]. Various drugs are formulated with pH dependent *in situ* gelling system such as azithromycin 1% topical solution containing polycarbophil [3]. Ionic strength-dependent gelling system contains gellan gum, which changes its consistency when exposed to increased ionic strength in tear fluid such as that observed during reflex lacrimation [9], for example, timolol maleate *in situ* gel forming system [3]. Temperature-dependent gelling system consists of polymers which change to gel-like consistency when exposed to body temperature, for example, poloxamer 407. However, poloxamer 407 is commonly combined with poloxamer 188 or other mucoadhesive polymers, since when used alone, it does not offer any distinct advantage [65], for example, poloxamer 407 copolymer containing pilocarpine nitrate solution [66].

Colloidal dosage forms

Colloidal dosage forms consist of small particulate systems (100–400 nm), suspended in an aqueous solution and administered as eye drops [9]. These include liposomes, nanoparticles, and micro or nanoemulsions. These offer advantages such as sustained release, improved permeation through blood ocular barriers, ability to bypass efflux transporters, and better stability profile as compared to proteins or peptides [5].

Nanoparticles entrap, dissolve, encapsulate, or adsorb drug molecules. Nanocapsules are usually more effective than nanospheres, probably due to greater diffusion of unionized drug from the oily core of nanocapsules compared to that from the hydrophilic core of nanospheres. Major limitations of nanoparticles include concerns about stability, control of particle size, and drug release rate [9]. Furthermore, concerns have been raised regarding aggregation, toxicity, and clearance of certain nanoparticles [67].

Liposomes (80–100 μm diameter) consist of concentric lipid bilayer separated by water partitions. These can be used to incorporate lipophilic drugs (in the lipid bilayer) or hydrophilic drugs (in the aqueous compartment) and demonstrate positive, negative, or neutral surface charge [9,68]. Liposomes have a tendency to accumulate in conjunctival folds after drainage has subsided and, thus, deliver drug through transconjunctival and transscleral routes [69]. However, physical instability, difficulty in sterilization, limited drug loading, and interference with vision restrict their use.

Niosomes are surfactant vesicles with a lipid bilayer structure similar to liposomes. Surfactant in niosomes helps the penetration. As a result, niosomes deliver a greater amount of hydrophilic drugs as compared to liposomes. Furthermore, these demonstrate improved chemical stability and low cost of production as compared to liposomes, are biodegradable and non-immunogenic [9].

Microemulsion (ME) can be used to enhance solubility of lipophilic and hydrophilic drugs. These emulsions consist of oil phase, aqueous phase, and surfactant (s) and can be oil in water ME, water in oil ME, or bicontinuous ME [9,70,71]. Advantages of this delivery system have been demonstrated in studies on rabbits. In one such study, pilocarpine submicron system was found to exert a greater and prolonged ocular hypotensive action as compared to pilocarpine HCL 2% eye drops, although the effect was slow to develop [72]. In another study, cationic emulsion of latanoprost demonstrated better safety profile as compared to benzalkonium chloride containing solutions of 0.005% latanoprost [73]. MEs are now increasing recognized as promising formulation for ocular drug delivery [70].

Drug-coated microneedles, approximately 500–750 μm length, have been tested in animal models for delivery of drugs to anterior and posterior segments through intracorneal and intrascleral routes, respectively. Drug molecules dissolve rapidly following insertion of microneedles, after which microneedles can be removed, for example, pilocarpine-coated microneedles. Use of microneedles reduces the risk associated with intraocular injections and can deliver greater amount of drugs to the interior of eye. For example, microneedles inserted into live rabbit cornea deliver 60 times greater concentration of fluorescein in anterior segment as compared to topical application [5,74]. Hollow microneedles have also been used to deliver soluble molecules, nanoparticles, and microparticles. In a preliminary toxicity study on rabbit eye, implantable microneedle made up of biodegradable polymer and methotrexate induced no inflammatory changes [75].

Collagen shields and collasomes

Collagen shields, prepared from porcine sclera, are stored in a dry state and hydrated before introduction to the eye. Although these deliver a higher concentration of drug to cornea and aqueous humor, these are not designed to fit individual patient's eyeballs and often cause interference with vision apart from accidental expulsion. Collasomes prepared by suspending small fragments of collagen in 1% methylcellulose are devised to overcome these limitations [40].

Methods to improve drug permeation

Iontophoresis uses an electrical current of 1–2 mA to transport ionized drug across cornea. Positively charged molecules move toward anode and negatively charged molecules move toward cathode [9]. This method has been tested in animal models for delivery of ciprofloxacin, gentamycin, and antisense oligonucleotides with promising results. Drugs for posterior segment diseases, for example, dexamethasone phosphate, methylprednisolone, carboplatin, and methotrexate have also been delivered successfully using this approach [5]. Ocular iontophoresis offers a significant potential for non-invasive ocular drug delivery for the treatment of posterior segment pathologies [76]. Iontophoresis using a microneedle-based system has also been tried to deliver nanoparticles to the posterior segment of the eye through suprachoroidal space [77].

Ultrasound-mediated drug delivery

Beta-blockers, when administered along with ultrasound (20 kHz for 1 h), show significant improvement in corneal penetration [5].

Ocular penetration enhancers

Surface active agents can enhance the ocular penetration of drugs by lysis of superficial cells of cornea in a dose dependent manner. Examples include benzalkonium chloride, polyoxyethylene glycol lauryl ether, polyoxyethylene glycol sterayl ether, polyoxyethylene glycol oleyl ether, digitonin, and sodium salt. However, these chemicals can lead to transient irritation and the risk of irreversible damage to cornea cannot be ruled out [13]. This restricts the use of high concentration and ionic surfactants [9].

CONCLUSION

Ophthalmic drug delivery remains a significant challenge due to the presence of a number of physiological and anatomical barriers. Knowledge of properties of different barriers can help clinicians select an appropriate drug and/or formulation for ocular pathology. Knowledge of intraocular PK in human eyes remains limited and dependency on animal models is retained. Major advances in ophthalmic formulations, primarily focusing on enhancing contact period with ocular surface and enhancing solubility of drugs, have been made in recent past and offer considerable promise in ocular drug delivery.

AUTHORS' CONTRIBUTIONS

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CONFLICTS OF INTEREST

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