Chapter Eleven

Ocular Drug Delivery

INTRODUCTION

The external eye is readily accessible for drug administration. As a consequence of its function as the visual apparatus, mechanisms are strongly developed for the clearance of foreign materials from the cornea to preserve visual acuity. This presents problems in the development of formulations for ophthalmic therapy.

Topical administration is direct, but conventional preparations of ophthalmic drugs, such as ointments, suspensions, or solutions, are relatively inefficient as therapeutic systems. Following administration, a large proportion of the topically applied drug is immediately diluted in the tear film and excess fluid spills over the lid margin and the remainder is rapidly drained into the nasolacrimal duct. A proportion of the drug is not available for immediate therapeutic action since it binds to the surrounding extraorbital tissues. In view of these losses, frequent topical administration is necessary to maintain adequate drug levels. Systemic administration of a drug to treat ocular disease would require a high concentrations of circulating drug in the plasma to achieve therapeutic quantities in the aqueous humor, with the increased risk of side effects.

Three important factors have to be considered when attempting drug delivery to the eye-

1. how the blood-eye barrier (systemic to ocular) or cornea (external to ocular) is crossed to reach the site of action,

2. how to localize the pharmacodynamic action at the eye and minimise drug action on other tissues

3. how to prolong the duration of drug action such that the frequency of drug administration can be reduced.

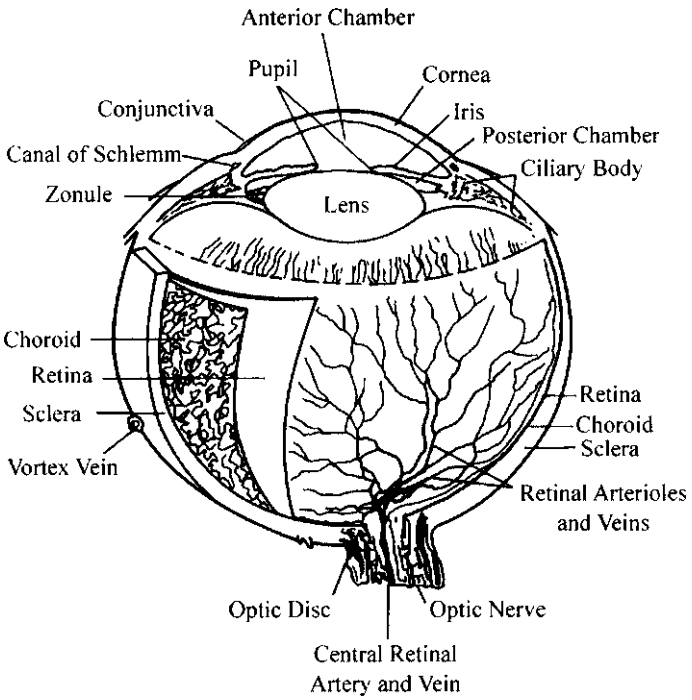


Figure 11.1 Vertical section through the human eye

STRUCTURE OF THE EYE

The outer shape of the eye comprises of two spheres of different radii, one set into the other (Figure 11.1). The anterior sphere, the cornea, is the smaller and more curved of the two and is completely transparent. The posterior sphere or sclera is a white, opaque, fibrous shell which encloses the ocular structures. Both tissues are relatively non-distensible and protect the eye from physical damage. The ring where the two areas join is called the limbus.

The outer tissues of the eye consist of three layers

1. the outermost layers, the sclera and cornea, provide protection for the delicate structures within

2. the middle layer, the uveal tract, has a nutritive function, being mainly vascular and consisting of the choroid, ciliary body and the iris

3. the innermost layer is the retina containing photoreceptors and is concerned with the reception of visual stimuli. The inner eye is divided by the lens that separates the aqueous and vitreous humors. The iris separates the aqueous humor into the anterior and posterior chambers.

The cornea

The cornea is made up of the stroma (up to 90% of its thickness) which is bounded externally by epithelium and the Bowman's membrane and internally by Descemet's membrane and the endothelium (Figure 11.2). The mean thickness of the cornea in man is just over 0.5 mm in the central region and is composed of five to six layers of cells. It becomes 50% thicker towards the periphery as the epithelium increases to eight to ten cell layers. The cells at the base are columnar, but as they are squeezed forward by new cells, they become flatter. These cells can be classified into three groups: basal cells, an intermediate zone of 2–3 layers of polygonal cells (wing shaped) and squamous cells. The permeability of the intact corneal epithelium is low until the outermost layer is damaged, suggesting that tight junctions exist between the cells in this layer. The outer layer of the surface cells possesses microvilli, which presumably help to anchor the precorneal tear film. The cells of the basal layer show extensive interdigitation of plasma membranes and are therefore relatively permeable.

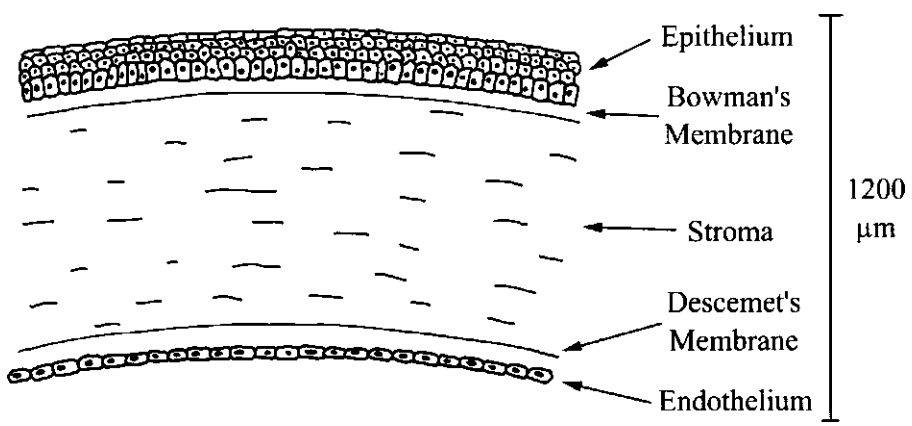


Figure 11.2 The five layers of the cornea

Immediately adjacent to the epithelium is a less ordered region of the stroma or Bowman's membrane. It is not sharply differentiated from the stroma beneath it and could be described as Bowman's layer rather than a membrane.

The stroma substantia propria consists of a set of lamellae, or plates, running parallel with the surface and superimposed on each other like the leaves of a book. Between the lamellae lie the corneal corpuscles, cells that synthesize new collagen essential for the repair and maintenance of this layer.

The stroma can be considered to have a comparatively open structure that normally allows diffusion of solutes having molecular weight below 500,000 Daltons. It can act as a barrier for very lipophilic substances that pass freely through the epithelium, while it is easily penetrated by hydrophilic solutes.

Descemet's membrane is located on the interior surface of the stroma and it is secreted by the endothelium. It is made up of a different type of collagen from that in the stroma. The endothelium consists of a single layer of flattened cells 5 μm high and 20 μm wide. These cells form a regular mosaic, each with close contact with its neighbours. The endothelium is about 200 times more permeable than the epithelium and thus represents a weak barrier. The endothelium is in contact with the aqueous humor of the anterior chamber. The endothelial layer is crossed by a passive flux of water towards the stroma, which has a tendency to swell. An active pump mechanism generates a flux in the opposite direction that controls corneal tumescence.

Although the cornea covers only one-sixth of the total surface area of the eyeball, it is considered the main pathway for the permeation of drugs into the intraocular tissues.

The conjunctiva and sclera

The conjunctiva lines the posterior surface of the eyelids and covers the exterior surface of the cornea. The portion that lines the lids is called the palpebral conjunctiva; the portion covering the white of the eyeball is called the bulbar conjunctiva. The palpebral conjunctiva is vascular and the bulbar conjunctiva is transparent. The area between the lids and the globe is termed the conjunctival sac that is open at the front at the palpebral fissure and only complete when the eyes are closed.

The sclera is composed primarily of collagen and mucopolysaccharide and forms the posterior five sixths of the protective coat of the eye. Its anterior portion is visible and constitutes the white of the eye. Attached to the sclera are the extraocular muscles. Through the sclera pass the nerves and the blood vessels that penetrate into the interior of the eye. At its posterior portion, the site of attachment of the optic nerve, the sclera becomes a thin sieve like structure, the lamina cribrosa, through which the retinal fibres leave the eye to form the optic nerve. The episcleral tissue is a loose connective and elastic tissue that covers the sclera and unites it with the conjunctiva above.

The choroid and retina

The choroid is the middle pigmented vascular coat of the posterior five-sixth of the eyeball. It is continuous with the iris in the front. It lies between the retina and the sclera and prevents the passage of light rays.

The retina is the light sensitive inner lining to the eyeball. It consists of seven nervous layers and one pigmented layer.

The aqueous humor

The aqueous humor is a clear colourless fluid with a chemical composition rather similar to that of blood plasma, but with a lower protein content. Its main function is to keep the globe reasonably firm and it is secreted continuously by the ciliary body into the posterior

chamber. It flows as a gentle stream through the pupil into the anterior chamber, from which it is drained by he canal of Schlemm.

The vitreous body is a jelly made up of a form of collagen, vitrosin, and the mucopolysaccharide, hyaluronic acid. Its composition is similar to that of the cornea, but the proportion of water is much greater, about 98 percent or more, compared with about 75 percent for the cornea. The vitreous body serves to keep the underlying retina pressed against the choroid.

The eyelids

The eyelids are movable folds of modified fleshy skin consisting of orbital and palpebral portions positioned in front of the eyeball (Figure 11.3). They have an obvious protective function and play an important role in the maintenance of the tear film and lacrimal drainage. Fibrous tarsal plates provide the framework for the lids.

Between the bulbar and the palpebral conjunctiva there are two loose, redundant portions forming recesses that project back. These recesses are called the upper and lower fornices, or conjunctival sacs. The looseness of the conjunctiva in this region makes movements of lids and eyeball possible.

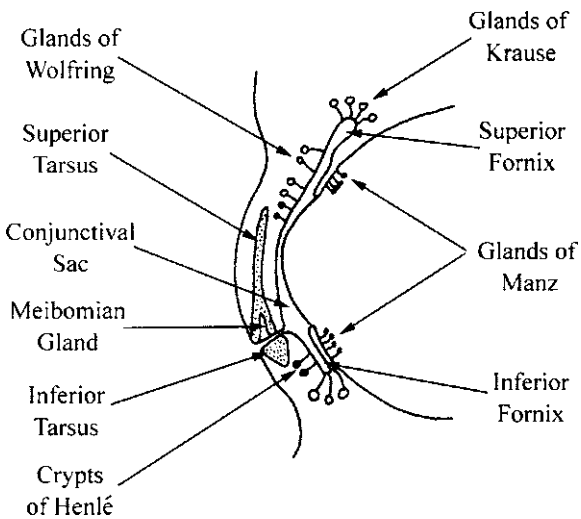


Figure 11.3 Vertical section through the eyelids and conjunctiva

The precorneal tear film

The maintenance of a clear, healthy cornea requires that the anterior surface of its epithelial layer be kept moist. The precorneal tear film is a very thin fluid layer continuously bathing the corneal epithelium, the conjunctiva and walls of the conjunctival cul-desac. Normal secretion of tears by the lacrimal system is necessary for:

1. nutrition of the cornea,
2. protection against bacterial infection,
3. removal of cellular debris and foreign matter
4. formation of a stable, continuous fluid film over the cornea producing a high quality optical surface.
5. lubrication for the movement of the eyelids.

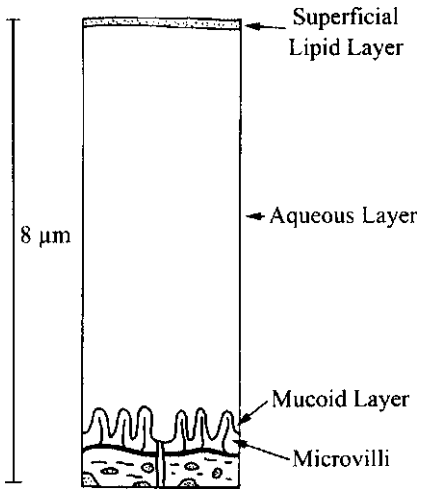
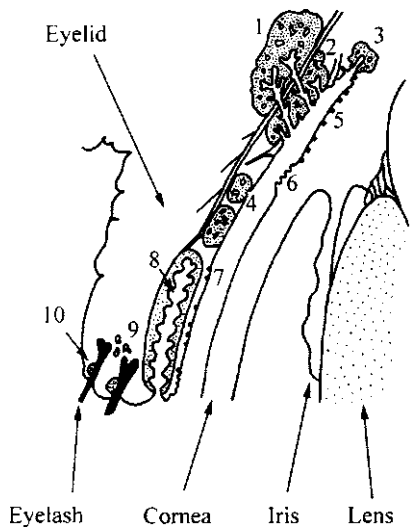


Figure 11.4 The structure of the tear film according to Wolff,

The average tear volume in a human is 7 µl, 1 µl of which is contained in the precorneal tear film, and 3 µl in each of the tear margins. Prior to blinking, the tear volume can increase to about 30 µl. The maximum amount of fluid that can be held in the conjunctival sac is only about 10 µl As the tear layer is so thin, evaporation and lipid contamination of the mucin component of the tear fluids quickly destroys its continuity. This results in dry spots that appear usually within 15 to 30 seconds after a blink, at scattered locations on the corneal surface. The blinking action of the eyelids, which usually occurs before the actual formation of dry spots, is required to re-form the tear film layer. The blink interval should therefore, be shorter than the tear break-up time.

The precorneal tear film was first thought to be a three layered structure consisting of a superficial oily layer, a middle aqueous layer, and an adsorbed mucus layer (Figure 11.4), secreted by several glands (Figure 11.5)[1]. Recent research suggests that the mucus fraction extends through the tear film[2]



Main lacrimal glands
1. Orbital lobe
2. Palpebral lobe

Accessory lacrimal glands
3. Glands of Krause
4. Glands of Wolfring

Mucin secretors
5. Goblet cells
6. Glands of Manz
7. Crypts of Henle

Oil secretors
8. Meiomian gland
9. Glands of Moll
10. Glands of Zeis

Figure 11.5 The glands secreting the components of the precorneal tear film

The superficial oily layer is approximately 0.1 μm thick and consists of wax and cholesterol esters secreted by the Meibomian glands, the glands of Zeis at the palpebral margin of each eyelid, and the glands of Moll situated at the root of each lash. This layer reduces the evaporation from the underlying aqueous phase by a factor of 10 to 20, preventing the cornea from drying.

The aqueous layer lies below the oily layer and is the largest component of the tear film (6–10 μm thick), consisting of watery lacrimal secretions provided by the numerous accessory lacrimal glands of Kraus and Wolfring, most of which are situated in the upper conjunctival fornix.

The mucoid layer is secreted by conjunctival goblet cells, the crypts of Henlé, which are situated on the conjunctival surface of the upper and lower tarsus, and the glands of Manz positioned in a circular ring on the limbal conjunctiva. Mucin is involved in adhesion of the aqueous phase to the underlying cornea, and thus keeps the cornea wettable.

Physical properties of tears

The normal pH of tears varies between 7.0 and 7.4. The pH of the tear film is influenced by any dissolved substances, particularly by the bicarbonate-carbon dioxide buffer system. When the eyelids are open, the pH of the precorneal tear film increases through loss of carbon dioxide. Solutions instilled into the lower fornix with pH below 6.6 and above 9.0 are associated with irritation, reflex tears and rapid blinking.

The surface tension of tear fluid at 33°C, the surface temperature of the eye, has been measured as between 44 and 50 $mN.m^{-1}$ [3]. The instillation of a solution containing drugs or adjuvants that lower the surface tension may disrupt the outermost lipid layer of the tear film. The lubricant and protective effect of the oily film disappears and dry spots may be formed due to tear film evaporation. The dry spots cause irritation and reflex blinking is elicited to eliminate the sensation of a foreign body in the eye. In many cases, the sensation may be delayed for 30 minutes to 1 hour following the application, dependent on the concentration and nature of the instillate.

The evaporation process influences the tonicity of human tears when the eye is open. The osmolarity after prolonged eye closure or during sleep is 293 to 288 mOsmol. After the eye is opened, the osmolarity progressively rises at a rate of 1.43 $mOsm.Kg^{-1}.h^{-1}$ to 302 to 318 $mOsm.Kg^{-1}$. Due to their molecular weight and low concentrations, proteins contribute only slightly to the total osmotic pressure, but they do influence tear viscosity. The viscosity of human tears ranges from 1.3 to 5.9 mPa.s with a mean value of 2.9 mPa.s.

Lacrimal drainage system

An efficient drainage system exists to remove excess lacrimal fluid and cell debris from the precorneal area of the eye (Figure 11.6). Tears initially drain through the lacrimal puncta, which are small circular openings of the lacrimal canalculi, situated on the medial aspect of both the upper and lower lid margins. They then pass through the mucous membrane lined lacrimal passages. The superior and inferior canalculi (approximately 8 mm in length and 1 mm in diameter) unite in the region of the medial canthus to form the common canalculus. This opens into the lacrimal sac about 3 mm below its apex. At its lower end, it is continuous with the nasolacrimal duct that passes downwards to open into the inferior meatus of the nose with a valvular mechanism at its opening. The tears finally pass into the nasopharynx.

The drainage of tears is an active process involving the lacrimal pump that is dependent on the integrity of the orbicularis muscle of the eyelids. Closure of the eyelids draws the lacrimal fluid from the puncta and canalculi into the sac by a suction effect. Opening the lids forces the lacrimal fluid from the sac into the nasolacrimal duct and then

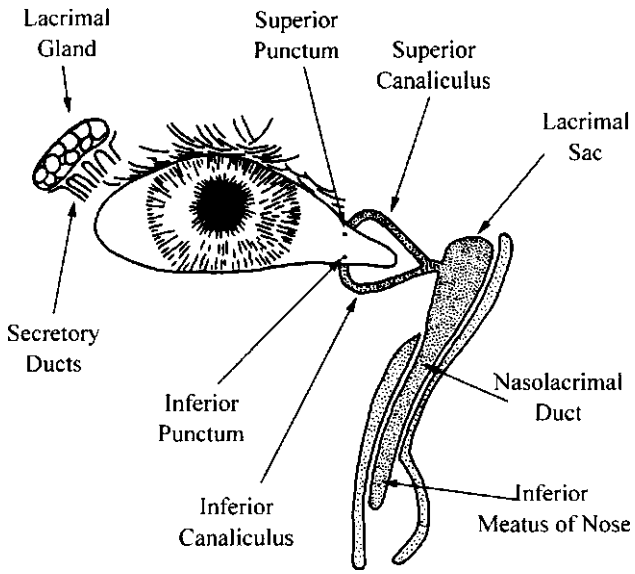


Figure 11.6 Lacrimal drainage system

into the nose through the lower end of the sac. The valvular mechanism opens during this movement.

Blood-eye barriers

Several barriers prevent material entering the ocular circulation. The vessels of the iris have thick walls that prevent leakage of materials into the aqueous humor. The epithelium in the ciliary processes is a unique membrane that prevents the passage of most molecules, including antibiotics and proteins. However, molecules may enter the posterior chamber of the eye during the active secretion process that forms aqueous humor. Topically applied fluorescein is seen to leak across the choroidal circulation, without passing into the retinal pigment layer. Injury or inflammation can damage the blood-eye barriers, since the capillary endothelial and epithelial cells separate, resulting in destruction of the intercellular barrier and leakage of material.

The external eye is readily accessible for drug administration; however, as a consequence of its function as the visual apparatus, mechanisms are strongly developed for the clearance of foreign materials from the cornea to preserve visual acuity. This presents problems in the development of formulations for ophthalmic therapy.

Systemic administration of a drug to treat ocular disease would require a high concentrations of circulating drug in the plasma to achieve therapeutic quantities in the aqueous humour, with the increased risk of side effects. Topical administration is more direct, but conventional preparations of ophthalmic drugs, such as ointments, suspensions, or solutions, are relatively inefficient as therapeutic systems. A large proportion of the topically applied drug is immediately diluted in the tear film and excess fluid spills over the lid margin and the remainder is rapidly drained into the nasolacrimal duct. A proportion of the drug is not available for therapeutic action since it binds to the surrounding extraorbital tissues. In view of these losses frequent topical administration is necessary to maintain adequate drug levels. This results in transient periods of over and under-dosing (Figure 11.7).

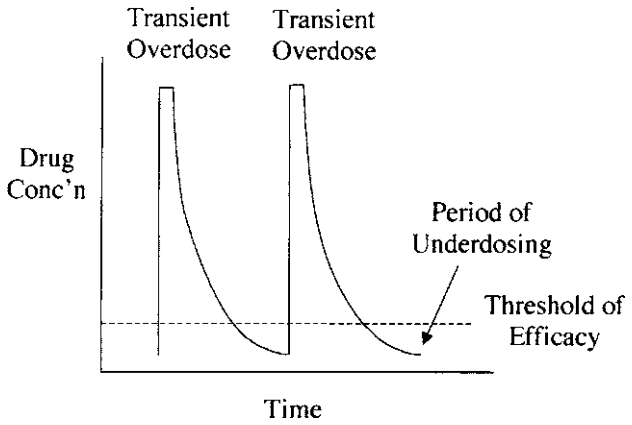


Figure 11.7 Sawtooth pattern of therapy following administration of ophthalmic drugs as eye drops

Three factors have to be considered when drug delivery to the eye is attempted. Firstly, how to cross the blood-eye barrier (systemic to ocular) or cornea (external to ocular) to reach the site of action; secondly, how to localise the pharmacodynamic action at the eye and minimise drug action on other tissues and finally, how to prolong the duration of drug action such that the frequency of drug administration can be reduced.

FACTORS AFFECTING DRUG PERMEATION

The sequential barriers of epithelium, stroma and, to a lesser extent, the endothelium pose difficulties for drug absorption through the cornea (Figure 11.2). The epithelial and endothelial cells are rich in lipids and are mostly permeable to fat soluble substances. The stroma is acellular with a high water content, making it permeable to water soluble substances. In order for drugs to be well absorbed, they need to have a mixed hydrophilic/hydrophobic nature with an intermediate partition coefficient in the range of 10 to 100.

The stroma can be considered as a single compartment with the aqueous humor since the endothelium separating them is only a weak barrier and hence is not rate limiting. Both the epithelium and the aqueous tissues can act as drug reservoirs. The hydrophilic stroma serves as a depot for water-soluble compounds such as catecholamines and their metabolites, whereas the epithelium is the main depot for lipophilic molecules such as chloramphenicol.

Ionization and pH

The majority of drugs are ionizable, but for a drug to pass the lipid barriers of the epithelium and endothelium, it needs to be hydrophobic, which generally means unionized. The ionized form prefers the trans-stromal route since this is primarily hydrophilic. After an ophthalmic formulation is instilled into the eye, it is mixed with the tears present in the conjunctival sac and with the precorneal tear film. The pH of the mixture is mainly determined by the pH of the instilled solution as the tear film is of small volume with low buffering capacity. Since the pH can so easily be influenced by the delivery vehicle, it can be exploited to optimize absorption. For example, pilocarpine, a weak base, is administered in a vehicle of low pH, in which it is ionized. In this form it can be absorbed before the tear film secretion returns the local pH to physiological levels. Adjustment of the vehicle buffer capacity allows some measure of control over the duration of the pH disturbance.

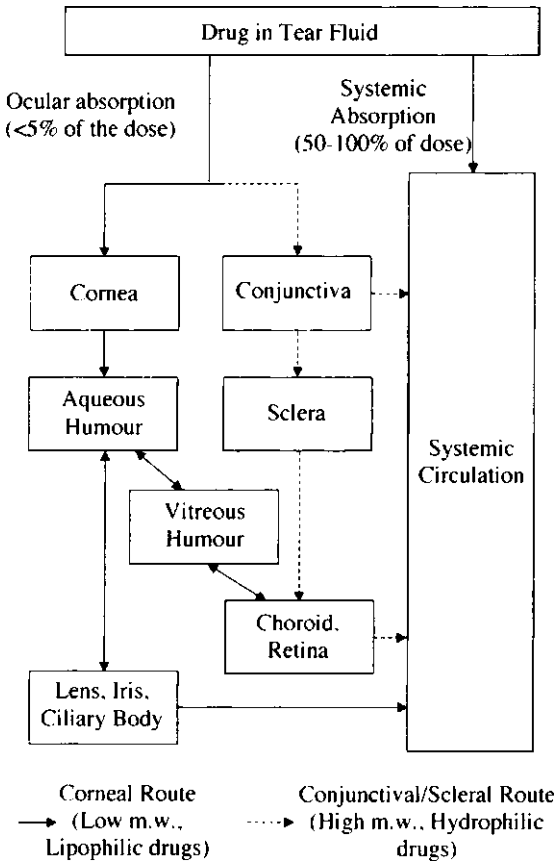


Figure 11.8 Transcorneal penetration into the aqueous humour. The losses are significant and it usual for less than 5% of the instilled dose to penetrate into the intraocular tissues following a 25 ul drop.

Protein binding

Estimates of the total protein content of tears range from 0.6 to 2% w/v, the major fractions consisting of albumin, globulin and lysozyme. Drugs bound to the protein in the tear fluid may not permeate the cornea due to the additional bulk of the protein molecule; also binding of drugs to protein in conjunctival tissues competes for the drug available for corneal absorption (Figure 11.8). Binding increases in certain disease states, particularly inflammatory conditions, due to higher secretion of proteins in tissue exudate.

If two drugs have to be applied to the same eye, an interval of five to ten minutes should elapse between administration of each drug. If the second drug is applied too soon after the first, it may displace the first drug, which will then be rapidly cleared, thereby reducing its effect.

Pigmentation and drug effects

There have been only a limited number of studies carried out in this area, but these suggest that the intraocular distribution of drugs vary with levels of eye pigmentation. For example, a ten-fold increase in pilocarpine deposition in the iris-ciliary body of the pigmented rabbit eye is found when compared with albinos, although the pilocarpine concentration in the aqueous humor was indistinguishable between the two[4]. Pilocarpine is metabolised by tissue

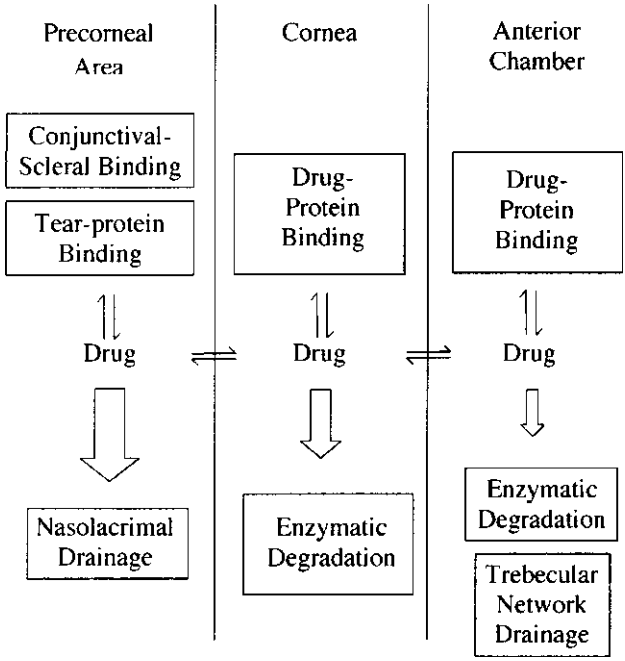


Figure 11.9 Losses during disposition into the eye. The precorneal losses are significant; binding and clearance processes further reduce the concentration of applied drug to the interior tissues

esterases and esterase activity is highest in the iris and ciliary body, followed by the cornea and aqueous[5]. In the pigmented eyes, the pharmacological effect was reduced owing to a higher amount of esterase activity in the cornea and iris-ciliary body.

Drug distribution in the eye

The amount of drug reaching the anterior chamber of the eye is determined by the net result of two competing processes,

1. the rate of drug loss from the precorneal area.
2. the rate of drug uptake by the cornea

Once the drug penetrates the cornea and enters the aqueous humor, it is distributed to all the internal tissues of the eye. The anterior chamber is in contact with the cornea, iris, ciliary body, lens and vitreous humor. The drug is rapidly distributed to these tissues and concentrations mirror those of the aqueous humor. Deeper into the intraocular tissues, the concentration is reduced by aqueous humor turnover and non-specific binding as illustrated in Figure 11.9.

Drug penetration through the sclera and conjunctiva

The sclera could constitute an important penetration route for some drugs, particularly those with low corneal permeation. Drugs may diffuse across the sclera by three possible pathways:-

1. through perivascular spaces

2. through the aqueous media of gel-like mucopolysaccharides
3. across the scleral collagen fibrils.

An *in vitro* study using isolated corneal and scleral membranes of the rabbit has shown that scleral permeability was significantly higher than the respective corneal permeability for many hydrophilic compounds[6]. The ratio of permeability coefficient of sclera to that of cornea was reported 1.2–5.7 for some blockers and 4.6 for inulin. The permeability coefficients were in the order propranolol > penbutolol > timolol > nadolol for the cornea and penbutolol > propranolol > timolol > nadolol for the sclera. It was suggested that the mechanism for scleral permeation was diffusion across the intercellular aqueous media, as in the case of the structurally similar corneal stroma. This mechanism alone however, cannot explain the substantially higher permeability of penbutolol and propranolol compared with the other compounds of similar molecular weight. A partitioning mechanism may account for these observations. The conjunctival/scleral route has also been reported to be the predominant pathway for the delivery of p-aminoclonidine to the ciliary body[7].

FACTORS INFLUENCING DRUG RETENTION

Proper placement of the eyedrops

Accurate and proper placement of an eye drop may considerably improve the efficacy of drug delivery, as the capacity of the conjunctival sac is dependent on the position of the head and technique of instillation. A drop is placed in the inferior cul-de-sac by gently pulling the lower lid away from the globe and creating a pouch to receive the drop. After gently lifting the lid to touch the globe, a small amount liquid is entrapped in the inferior conjunctival sac, where it may be retained up to twice as long as when it is simply dropped over the superior sclera. Drainage from the cul-de-sac may be further reduced by punctual occlusion or simple eyelid closure, which prolongs the contact time of the drug with the external eye. This serves two purposes; first it maximises the contact of drug with the periocular tissues increasing absorption through the cornea and secondly, the systemic absorption is reduced (Figure 11.10).

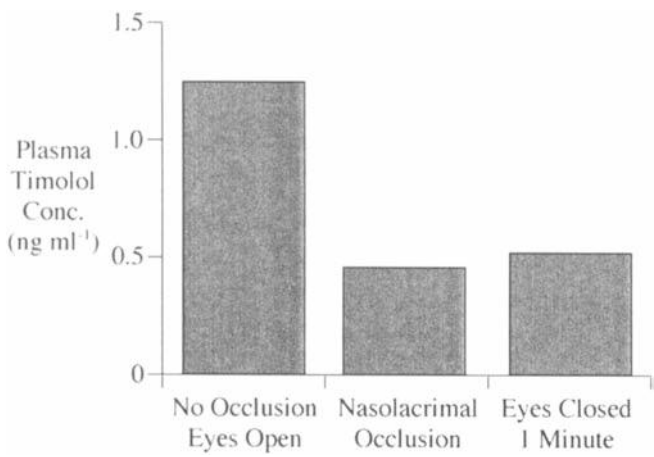


Figure 11.10 Effect of eyelid closure or nasolacrimal occlusion on systemic absorption of timolol

Influence of instilled volume

When an ophthalmic formulation is instilled into the eye, it is mixed with the precorneal tear film. The osmotic pressure of the mixture depends upon the osmolarity of the tears and of the ophthalmic formulation. If the osmotic pressure obtained is within defined limits, no discomfort is experienced, but if the osmotic pressure is outside these limits the patient experiences irritation eliciting reflex tears and blinking. The original osmolarity of the precorneal tear film is regained two minutes after the non-isotonic solution is administered, mainly due to a rapid flow of water across the cornea. The instillation of a hypotonic drug solution creates an osmotic gradient between the tear film and the surrounding tissues. This induces a flow of water from the eye surface to the cornea, hence the drug concentration on the eye surface is temporarily increased.

The rapid rate of tear turnover and efficient drainage in the eye profoundly affects the bioavailability of topically applied ophthalmic drugs. Increasing the tear volume suddenly, such as following the instillation of an eyedrop, produces rapid reflex blinking which quickly re-establishes the normal tear pool. Typically, the volume of an ophthalmic solution will be 30–50 µl, which is instilled into the lower fornix. The reflex blink can cause as much as 20 µl to spill over the lid margin onto the skin. Most of the remainder is pumped into the nasolacrimal duct until the total tear volume returns to its normal value of 7 µl. As the precorneal volume of fluid (lacrimal and instilled) becomes smaller, the turnover rate of lacrimal fluid will have a greater influence on the residual drug concentration. Therefore, a larger instilled volume will maximize the penetration of ophthalmic drugs, but a larger proportion will be wasted through drainage.

Significant drug absorption can occur through the mucous membrane of the nasolacrimal duct, with as much as 80% of the instilled volume draining into the gastrointestinal tract from where it can be absorbed into the systemic circulation. Severe systemic side-effects can arise which can lead to toxicity problems, particularly in geriatric and paediatric patients. To minimise loss to other absorptive pathways, the volume of the eyedrop should be sufficiently small that the tear film is not significantly perturbed. Volumes of 5–10 µl have been found to minimize side effects due to systemic absorption via the drainage apparatus, and special eyedrop tips which are capable of delivering this volume have been developed[8 9]. The extent of drug loss due to administration of volumes too large for the eye to retain has been shown in a number of cases. A 10 µl drop of 10% phenylephrine is as potent as the normal 30 µl drop of drug of the same concentration[10]. Ten and 20 µl drops of tropicamide produce more pupillary dilation compared to 40 and 80 µl drops; moreover, local irritation was seen with the larger drop size[11]. Unfortunately, although smaller drop sizes may be retained for even longer periods in the conjunctival sac and reduce systemic side effects still further, volumes less than 8 µl require high drug concentrations with consequent problems in formulation.

Preservatives

The vast majority of ophthalmic formulations contain preservatives, most commonly benzalkonium chloride. At high concentrations, preservatives can cause irritation and damage to the ocular surface. In strengths greater than 0.01%, benzalkonium chloride can damage the corneal epithelium by desquamation. Disruption of the corneal barrier increases the non-selective absorption of several compounds of differing water solubility and molecular weight. There is currently considerable interest in developing preservative free formulations to overcome these problems.

Effect of systemically administered drugs

Tear film dynamics can be affected by systemically administered pharmacological agents and locally applied adjuvants. Timolol applied topically reduces tear flow whereas pilocarpine stimulates tear flow. The adjuvant benzalkonium chloride disrupts the tear film whereas methylcellulose increases the stability of the tear film. Certain drugs can influence blink rate as well as tear secretion; for example, general anaesthetics may completely inhibit lid movements.

Drugs that are used to treat common conditions such as hypertension and allergic conditions can also influence tear film dynamics. If a patient presents with a complaint involving the tear film, it is important to rule out whether this is a side effect of other medication. Antihypertensives administered systemically (e.g. reserpine, diazoxide) stimulate tear flow whilst antihistamines reduce tear flow.

ROUTES OF DRUG ADMINISTRATION

There are three main routes commonly used for administering drugs to the eye:

1. Topical—drops or ointment
2. Systemic—oral or injection
3. Intra-ocular injection

Topical administration

Drops

The most common form of topical administration is the eye drop. It is apparently easy to use, relatively inexpensive and does not impair vision. The major problems with these types of formulation are their inability to sustain high local concentrations of drug and they only have a short contact time with the eye.

Most eye-drops consist of an aqueous medium, to which can be added buffers (phosphate, borate, acetate and glucuronate), organic and inorganic excipients, emulsifiers, and wetting agents in order to accommodate a wide range of drugs with varying degrees of polarity. Vehicles may include water, aqueous mixtures of lower alkanols, vegetable oils, polyalkylene glycols, or petrolatum based jelly. Other excipients include ethylcellulose, ethyl oleate, carboxymethylcellulose and polyvinylpyrrolidone.

Contact time between the vehicle and the eye can be increased by the addition of polymers such as polyvinyl alcohol and methylcellulose, although generally the effects on drug absorption are not dramatic. Drainage from the cul-de-sac may also be reduced by punctual occlusion or simple eyelid closure, which prolongs the contact time of the drug with the external eye. This serves two purposes- first it maximises the contact of drug with the periocular tissues increasing absorption through the cornea and second, the systemic absorption is reduced.

Perfusion

Continuous and constant perfusion of the eye with drug solutions can be achieved by the use of ambulatory motor driven syringes that deliver drug solutions through fine polyethylene tubing positioned in the conjunctival sac. The flow rate of the perfusate through a minipump can be adjusted to produce continuous irrigation of the eye surface (3–6 $ml.min^{-1}$) or slow delivery (0.2 $ml.min^{-1}$) to avoid overflow. This system allows the use of a lower drug concentration than used in conventional eye-drops, yet will produce the same potency. Side effects are reduced and constant therapeutic action is maintained. This system is not used very often due to the inconvenience and the cost involved, but may find application for irritant drugs or for sight-threatening situations

Sprays

Spray systems produce similar results to eye-drops in terms of duration of drug action and side effects. Sprays have several advantages over eye-drops:

1. a more uniform spread of drug can be achieved
2. precise instillation requiring less manual dexterity than for eye-drop administration and is particularly useful for treating patients with unsteady hand movements
3. contamination and eye injury due to eye-drop application are avoided
4. spray delivery causes less reflex lacrimation.
5. Can be used by patients who have difficulty bending their neck back to administer drops.

The only disadvantage is that sprays are more expensive to produce than eye-drops so they are not widely used; however, several manufacturers have advanced spray systems at a pre-production stage. Prototype devices that force small volumes through a valving system look promising as delivery devices of the future. Recently, it has been demonstrated that one sixth of the conventional dose of pilocarpine hydrochloride delivered in this manner produces an equivalent miosis to the standard dose[12].

Use of polymers to increase viscosity

The viscosity of ophthalmic solutions is often increased to improve retention times of a drug on the corneal surface and hence bioavailability. Soluble polymers in aqueous solution are often used to extend the drug residence time in the cul-de-sac. The more commonly used viscolyzing agents include polyvinyl alcohol (PVA) and derivatives of cellulose. Cellulosic polymers, such as methylcellulose, hydroxyethylcellulose (HEC), hydroxypropyl-methylcellulose (HPMC) and hydroxypropylcellulose (HPC), are widely used as viscolyzers showing Newtonian properties. They have common properties: i). a wide range of viscosity (400 to 15000 cps); ii). compatibility with many topically applied drugs and iii). increased stability of the lacrimal film. There is a general relationship between increasing viscosity and improving bioavailability which would be expected since contact with the absorbing surface is prolonged; however, solutions that are so thick they require a force of more than 0.9 N to shear them markedly interfere with blinking.

Of the many naturally occurring polymers, sodium hyaluronate and chondroitin sulphate have been extensively investigated as potential ophthalmic drug delivery vehicles. Sodium hyaluronate is a high molecular weight polymer extracted by a patented process from sources including rooster coxcombs. The molecule consists of a linear, unbranched, non-sulphated, polyanionic glycosaminoglycan, composed of a repeating disaccharide unit (D-sodium glucuronate and N-acetyl-D-glucosamine). Sodium hyaluronate has an unusual rheological behaviour, undergoing a rapid transformation from a gel to a liquid on application of shear stress (pseudoplasticity). Hence, the viscosity is higher at the resting phase, so it provides a thickened tear film, with slow drainage and an improved distribution on the cornea during blinking. Furthermore, the carboxyl groups of hyaluronate form hydrogen bonds with hydroxyl groups of mucin in the eye, producing an intimate contact with the cornea. They demonstrate a considerably prolonged residence time when compared to saline (T 1/2=11.1 minutes at 0.2% concentration and 23.5 minutes at 0.3% compared to a T 1/2 of 50 seconds for the saline[13]).

Products based on hyaluronates are widely used in intraocular surgery as a substitute for vitreous humor and as an adjuvant to promote tissue repair. Hyaluronates protect the corneal endothelium and other delicate tissues from mechanical damage by providing a stabilised hydrogel. These unique properties give hyaluronates great potential in the ocular drug delivery.

Chondroitin sulphate is a glycosaminoglycan with a repeat unit containing

ß-D-glucoronic acid and ß-D-N-acetyl galactosamine. It is similar to hyaluronic acid except for modification of the position of a hydroxyl group and the addition of sulphate groups to the galactosamine residue. Chondroitin sulphate has a good affinity to the corneal surface, preventing premature break-up of the tear film between blinks[14]. Thus, formulations containing chondroitin have been used for the treatment of dry eye and they are superior to hyaluronic acid particularly in severe cases.

Carbomers (carbopols) are polyacrylic acid polymers widely used in pharmaceutical and cosmetic industries. They have several advantages, including high viscosities at low concentrations, strong adhesion to mucosa without irritation, thickening properties, compatibility with many active ingredients, good patient acceptability and low toxicity profiles. These properties have made carbomers very valuable in the field of ophthalmic formulations, particularly for the treatment of dry eye since they have long ocular residence times.

Gelling polymers

At high concentrations, carbomers form acidic, low viscosity, aqueous solutions that transform into stiff gels when the pH is raised. Although these materials gel in the conjunctival sac upon instillation, at this concentration they can cause irritation to the eye due to their high acidity which cannot always be neutralized by the buffering action of the tear fluid. At low concentrations, the carbopol gels show long retention increasing the contact time of solutes and suspended solids.

Gelling systems that make the transition from a liquid phase to a gel or solid phase in response to a specific trigger, such as pH, temperature or concentration of ions, have been used to deliver drugs to the eye. They have the advantage over viscous solutions in that the material can be dispensed from the bottle or tube easily, and only thickens on contact with the tear film gelling *in situ,* usually in the eye cul-de-sac. The polymers used are natural (such as gellan gum) or synthetic such as cellulose acetate phthalate or a pluronic.

Gellan gum is an anionic polysaccharide formulated in aqueous solution, which forms clear gels, the strength of which increases proportionally to the amount of either monovalent or divalent cations present. Gelation occurs in the eye due to the concentration of sodium present in human tears (~2.6 $\mu g.\mu l^{-1}$). The reflex tearing, which usually leads to a dilution of ophthalmic solutions, in this case further enhances the viscosity of the gellan gum by providing cations needed for gelation. Gellan gum based formulations (0.6% w/v) do demonstrate prolonged ocular retention in man[16 17].

Other gels that form *in situ* are characterised by a high polymer concentration, such as 25% pluronics and 30% cellulose acetophthalate (CAP) which were found to cause discomfort. To reduce the total polymer content in the system, polymers were combined to improve gelling properties. A system was explored which contained carbopol, which demonstrates pH-mediated phase transitions, and methylcellulose, which exhibits thermal gelation. Such system could be formulated as a liquid at a specific pH and room temperature but would gel on exposure to the physiological conditions of the surface of the eye i.e. pH 7.4 and 34°C[18].

Ointments

Ointments are not as popular as eye drops since vision is blurred by the oil base, making ointments impractical for daytime use. They are usually applied for overnight use or if the eye is to be bandaged. They are especially useful for paediatric use since small children often wash out drugs by crying. Ointments are generally non-toxic and safe to use on the exterior of the surface of the eye. However, ointment bases such as lanolin, petrolatum and vegetable oil are toxic to the interior of the eye, causing corneal oedema, vascularization, scarring and

endothelial damage. Intraocular contamination with these vehicles should therefore be avoided. Formulations based on white petrolatum-mineral oil have a residence half-time of over an hour in man[19].

Antibiotics such as tetracyclines are used in the form of an ointment, producing effective antibacterial concentrations in the anterior chamber for several hours, whereas an aqueous solution of tetracycline is ineffective for intraocular infections. A problem with extremely lipophilic drugs, including corticosteroids, is that the therapeutic agent may not be released as it partitions into the oil base. For these drugs, alternative systems such as water-soluble inserts may be preferable.

Particulates

Poorly soluble drugs for ophthalmic administration are frequently formulated as micronised suspensions. Larger particles theoretically provide prolongation of effect due to the increased size of the reservoir; however, an increase in particle size is associated with irritation giving rise to an increased rate of removal, assisted by agglomeration of particles and ejection. The relationship between particle size and retention is poorly understood and size is probably not the only determining factor, with parameters such as zeta potential and surface chemistry also being important. Increasing the size of particles to 25 μm in diameter will increase retention time to around 12 hours in a rabbit, compared to 3 μm particles which disappear almost as fast as aqueous solutions[20]. However, increasing the particle size of 0.1% 3H-dexamethasone suspension, from 5.75, to 22 μm demonstrated that clearance of the larger particle size from the eye is faster than complete dissolution[21]. Similarly, increasing the concentration of fluorometholone solids in a suspension did not increase the aqueous humour drug concentration-time profile[22]. Hence, small particle sizes generally improve patient comfort and acceptability of suspension formulations.

Interestingly, submicron or nanosphere formulations demonstrate therapeutic advantages over aqueous solutions, although one would expect rapid clearance from the eye. For example, pilocarpine (2% w/v) adsorbed onto a biocompatible latex of average size 0.3 μm will maintain a constant miosis in the rabbit for up to 10 hours compared to 4 hours with pilocarpine eye drops[23]. Other nanoparticle systems have been investigated for the prolongation of contact time in order to increase the ocular absorption. Betaxolol-poly-e-caprolactone nanoparticles produce a significantly greater reduction in intra-ocular pressure compared to the commercial eyedrops[24]. Similar improvements were obtained with carteolol (a ß-blocker) which caused a better penetration of the drug in the nanosphere formulation.

Sustained release devices

Although some particulate systems could be classified as sustained release devices, the term is applied here only to macroscopic devices such as inserts. Provision of a matrix to sustain drug release in the eye can be achieved in several ways. For example, a hydrophilic (soft) contact lens can serve as a drug reservoir. The drug is incorporated into the lens by either instilling drops on the lens when in the eye, or pre-soaking the lens in a solution of the drug. Other systems are not placed on the cornea, but are inserted under the eyelid, such as insoluble inserts of polyvinyl alcohol or soluble collagen which dissolve in lacrimal fluid or disintegrate while releasing the drug. Soluble inserts are made of such substances as gelatin, alginates, agar and hydroxypropylmethylcellulose. These systems have been developed as a method for delivering larger amounts of drugs to the eye over a long period.

Ocuserts® (Alza Corporation, U.S.A.) are insoluble inserts containing pilocarpine used in the treatment of glaucoma, and have a one-week duration of action. The major advantages of this system include longer duration of drug action, avoidance of

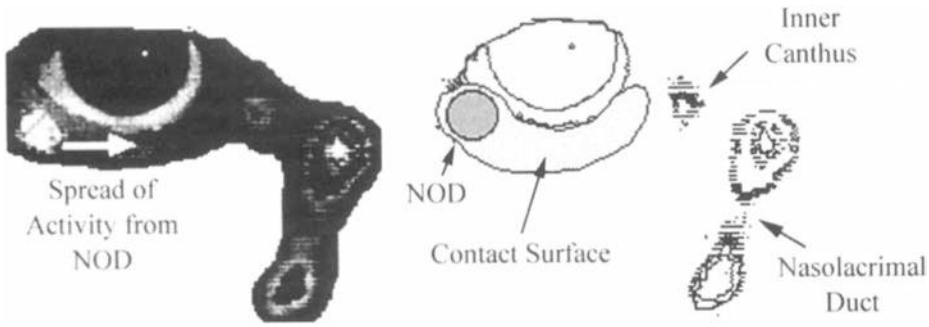


Figure 11.11 Combined photographic and scintigraphic image of the eye (left); illustration of contact area (right)

accommodative spasms in younger patients and better patient compliance. However, 20% of all patients treated with the Ocusert® lose the device without being aware of the loss. For this reason, patients fitted with the device should be checked regularly.

Collagen shields have proven useful as the basis of a drug delivery device. The shield is made from porcine scleral collagen that is moulded into a contact lens-like shape. This is hardened and crosslinked by exposure to ultraviolet light. The shield conforms to the shape of the eye and lubricates the cornea as it dissolves. The dissolution time depends on the degree of crosslinking which can be controlled to release drug over a period from approximately 12 to 72 hours. Drugs can be incorporated into the collagen matrix during manufacture, adsorbed into the shield during rehydration, or topically applied over the shield in the eye. As the shield dissolves, the drug is released gradually into the tear film, maintaining high concentrations on the corneal surface and increasing drug permeation through the cornea[25]. Studies suggest that drug delivery by collagen shields may be helpful in the early management of bacterial keratitis, in preference to frequent administration of topical antibiotics, subconjunctival injection, or topical administration over a soft contact lens bandage.

A PVA gel which hydrates on contact with the eye clears with a mean half-life of 8 minutes in normal subjects[26] but it increases the bioavailability of pilocarpine by 16 times compared to conventional formulations. This is due to presentation of the drug in the nonionised form; and to sustained high concentration in the lower marginal tear film. Presentation of drug was confined to the sclera with little spread to cornea (Figure 11.11). As the diffusion path from sclera to ciliary body is relatively short, this should result in high concentrations of drug in the lower hemisphere interior with much lower concentrations in the upper segment.

Intraocular drug delivery

Over the last two decades, treatment of intraocular conditions and diseases, such as endophthalmitis, has been attempted mainly by intravenous, topical or subconjunctival administration of suitable drugs. Sub-therapeutic intravitreal concentrations of the drugs following each of these routes frequently resulted in poor recovery of vision. Topical application of drugs for the treatment of posterior segment disorders is severely limited by the highly efficient clearance mechanisms. Improving the precorneal residence time of topically applied drugs by viscosity enhancing agents, gelling agents, or mucoadhesive

polymers has failed to provide sufficient concentrations in the vitreous humor. Similarly, subconjunctival injection of antibiotics and antivirals is also unsuccessful in achieving therapeutic concentrations in the vitreous humor. The intraocular penetration of drugs following intravenous administration is hampered by the presence of tight junctional complexes of the retinal pigment epithelium and the retinal blood vessels forming the blood-retinal barrier.

Most diseases affecting the posterior segment are chronic in nature and require prolonged drug administration. Intravitreal injections remain the main route of delivery in order to avoid the concomitant side effects seen with systemic administration. An intravitreal injection provides therapeutic concentrations of the drug adjacent to the intended site of activity and only a small dose of drug is required. However, possible retinal toxicity of the injected dose must be taken into account. Usually an intravitreal injection is restricted to a volume of 0.1–0.2 ml administered following both anterior chamber and vitreal taps. Following injection, the drug diffuses through the vitreous gel with little restriction. For most drugs the diffusion coefficient through the vitreous humor is close to that through water

Once distributed throughout the vitreous humor, rapid elimination of the drug is observed via two routes:

1. anteriorly by simple diffusion to the posterior chamber, followed by removal to the systemic circulation along with the aqueous humor drainage
2. posteriorly across the retina where it is removed by active secretion.

Drugs lost primarily by anterior chamber diffusion have a half life in the vitreous humor of 20–30 hours, while drugs lost via the trans-retinal route, such as the penicillins, have typically much shorter half lives of 5–10 hours. Unfortunately,these time scales are still shorter than required and depot device which deliver over days to weeks are being developed. Ocular inflammation results in the breakdown of the blood-retinal barrier and increases the elimination of non-transported drugs from the vitreous humor. In contrast, drugs that are removed by the active transport systems reside longer in the vitreous following ocular inflammation due the failure in the transport mechanism. Rate of drug loss is also enhanced in vitrectomised and lensectomised eyes.

Liposomes

Liposomal encapsulation has the potential not only to increase the activity and prolong the residence of the drug in the eye, but also to reduce the intraocular toxicity of certain drugs. For example, liposome-encapsulated amphotericin B produces less toxicity than the commercial solubilized amphotericin B formulation when injected intravitreally. The main drawbacks associated with liposomes are their short shelf life and difficulty in storage, limited drug loading capacity and instability on sterilization and finally, transient blurring of vision after an intravitreal injection. Despite these disadvantages, they have potential as drug delivery systems as they are composed of substances that are non-toxic and totally biodegradable.

Microparticulates and nanoparticles

Microspheres and nanoparticles are retained for extended periods within the eye and can provide slow, sustained release of drugs. The delivery systems are especially attractive because of the ease of manufacturing and improved stability compared to liposomes. The polymers used in the manufacture can be *erodible,* in which case the drug release is due to the polymer degradation, or *non-erodible,* where the drug is released is by diffusion through the polymer.

Intraocular devices

The administration of medications by implants or depot devices is a very rapidly developing technology in ocular therapeutics. These overcome the potential hazards associated with repeated intravitreal injection such as clouding of the vitreous humor, retinal detachment and endophthalmitis.

Implantable devices have been developed that serve two major purposes. First, to release of drug at zero order rates, thus improving the predictability of drug action, and second, to release of the drug over several months, reducing dramatically the frequency of administration.

Depot devices, have been developed to treat proliferative vitreoretinopathy and retinitis associated with cytomegalovirus. Various implantable devices, such as a gentamicin osmotic minipump, a polyvinyl alcohol/ethylene vinyl acetate cup containing ganciclovir, a polysulfone capillary fibre with daunomycin in tristearin and ganciclovir intraocular implant have been suggested.

Vitrasert® is a commercially available sustained release intraocular device for ganciclovir approved for use in-patients suffering from cytomegalovirus. Apart from the anticipated problems of endophthalmitis and retinal detachment, dislocation of implant and poor intravitreal drug levels due to its placement into the suprachoroidal space has been observed.

Iontophoresis

Iontophoresis (see transdermal chapter) facilitates drug penetration through the intact corneal epithelium. The solution of the drug is kept in contact with the cornea in an eye-cup bearing an electrode. A potential difference is applied with the electrode in the cup having the same charge as the ionized drug, so that the drug flux is into the tissue. This method of administration is very rarely used, except under carefully controlled conditions. Iontophoresis allows penetration of antibiotics that are ionised and therefore do not penetrate by other methods, for example, polymyxin B used in the local treatment of infections. Although the technique is found to be suitable for a range of compounds like NSAIDS, antivirals, antibiotics, anaesthetics and glucocorticoids, its acceptability as a routine drug delivery system is limited by the lack of information on side effects from repeated or multiple applications on the same or different site.

Commonly reported toxic effects include slight retinal and choroidal burns and retinal pigment epithelial and choroidal necrosis, corneal epithelial oedema, persistent corneal opacities and polymorphonuclear cell infiltration. Other disadvantages of iontophoresis include side effects such as itching, erythema and general irritation.

Systemic administration

Drugs are usually administered systemically for the treatment of diseases involving the optic nerve, retina and uveal tract. The blood/aqueous barrier only allows drugs to pass into the anterior chamber of the eye by one of two processes, both of which are very slow:

(1) secretion from the ciliary body
(2) diffusion from the capillaries of the iris.

Most drugs are unable to reach the anterior chamber in therapeutically active concentrations because they are either not sufficiently lipid soluble or they are bound to plasma proteins and hence cannot pass through the blood vessel wall,

Some drugs such as acetazolamide are ineffective when given topically and although it can be administered parenterally this is impractical for chronic administration. A

sustained-release oral preparation of acetazolamide is valuable for patients with glaucoma since it produces consistent and sustained levels of drug. Better carbonic anhydrase inhibitors such as dorzolamide may decrease enthusiasm for acetazolamide. Recently, the sublingual route has been explored for the delivery of timolol to decrease intraocular pressure in patients with ocular hypotension.

CONCLUSIONS

Increasing the residence time of an ophthalmic formulation on the corneal surface increases the drug bioavailability and therefore reduces frequency of administration. Although recent advances have been made in ocular drug delivery systems, eye drops are still the most commonly used formulations as they are the least expensive preparations, are easy to use, and do not interfere with vision. However, frequent administration is necessary.

The retention of a drug on the corneal surface is determined by the amount of tear flow and by the blink frequency, which can be stimulated by different factors. The most important factor influencing the retention of a drug on the corneal surface appears to be the properties of the drug itself. If a drug irritates the eye, it is difficult to obtain a long retention. If the drug is non-irritant, retention time can be increased by instillation of small drops, by adjustment of the osmolarity, tonicity, pH and by choosing the appropriate preservatives and adjuvants.

REFERENCES

1. Wolff E. Mucocutaneous junction of lid-margin and distribution of tear fluid. *Trans. Opthal. Soc. UK 1946–66:291–*308,
2. Prydal JI, Kerr-Muir MG, Dilly PN. Comparison of tear film thickness in three species determined by the glass fibre method and confocal microscopy. *Eye* 1993; 7:472–475.
3. Tiffany JM, Winter N, Bliss G. Tear film stability and tear surface tension. 1989; 8:507–515.
4. Lee VHL, Robinson JR. Disposition of pilocarpine in the pigmented rabbit eye. *Int. J. Pharmaceut.* 1982; 11:155–165.
5. Lee VHL. Esterase activities in adult rabbit eyes. *J. Pharmaceut. Sci.* 1983; 72:239–244.
6. Ahmed I, Gokhale RD, Shah MV, Patton TF. Physicochemical determinants of drug diffusion across the conjunctiva, sclera and cornea. *J. Pharmaceut. Sci.* 1987; 76:583–586.
7. Chien DS, Homsy JJ, Gluchowski C, TangLiu DS. Corneal and conjunctival/scleral penetration of p-aminoclonidine, AGN190342, and clonidine in rabbit eyes. *Cur. Eye Res.* 1990; 9:1051–1059.
8. Brown RH, Lynch MG. Drop size of commericial glaucoma medications. *Am. J. Ophthalmol* 1986; 102:673–674.
9. Reducing the size and toxicity of eyedrops. 10th National science writers seminar in ophthalmology: 1988; Arlington.
10. Whitson JT, Love R, Brown RH, Lynch MG, Schoenwald RD. The effect of reduced eyedrop size and eyelid closure on the therapeutic index of phenylephrine. *Am. J. Ophthalmol.* 1993; 115:357–359.
11. Lal A, Kumar V. The therapeutic response to topical instillation of different drop volume of tropicamide in humans. *Afro-Asian J. Opthalmol.* 1995; 14:15–18.
12. Martini LG, Embleton JK, Malcolmson RJ, Richard J, Wilson CG. The use of small volume ocular sprays to improve the bioavailability of topically applied ophthalmic drugs. *Europ. J. Pharmaceut. Biopharm.* 1997; 44:121–126.
13. Snibson GR, Greaves JL, Soper NDW, Prydal JI, Wilson CG, Bron AJ. Precorneal residence times of sodium hyaluronate solutions studied by quantitative gamma scintigraphy. *Eye* 1990; 4:594–602.
14. Silver FH. Biomaterials used in ophthalmology. *Biomaterials, Medical Devices and Tissue Engineering.* London: Chapman and Hall, 1993:120–152.

15. Wilson CG, Zhu YP, Frier M, Rao LS, Gilchrist P, Perkins AC. Ocular contact time of a carbomer gel, Geltears®, in human. *Br. J. Ophthalmol.* 1998; 82:1131–1134.
16. Greaves JL, Wilson CG, Rozier A, Grove J, Plazonnet B. Scintigraphic Assessment of an Ophthalmic Gelling Vehicle in Man and Rabbit. *Curr. Eye Res.* 1990; 9:415–420.
17. Meseguer G, Buri P, Plazonnet B, Rozier A, Gurny R. Gamma scintigraphic comparison of eyedrops containing pilocarpine in healthy volunteers. *J. Ocular Pharmacol. Therapeut.* 1996; 12:481–488.
18. Joshi A, Ding S, Himmelstein KJ. U.S. Patent 5 252 318. 1993.
19. Greaves JL, Wilson CG, Birmingham AT. Assessment of the precorneal residence of an ophthalmic ointment in healthy subjects. *Br. J. Clin. Pharmacol.* 1993; 35:188–192.
20. Sieg JW, Triplett JW. Precorneal retention of topically instilled micronized particles. *J. Pharmaceut. Sci.* 1980; 69:863–864.
21. Schoenwald RD, Stewart P. Effect of particle size on ophthalmic bioavailability of dexamethasone suspensions in rabbits. *J. Pharmaceut. Sci.* 1980; 69:391–394.
22. Olejnik O, Weisbecker CA. Ocular bioavailability of topical prednisolone preparations. *Clin. Therapeut.* 1990; 12:2–11.
23. Gurny R. Preliminary study of prolonged acting "drug" delivery system for the treatment of glaucoma. *Pharm. Acta. Helv.* 1981; 56:130–132.
24. Marcha-Heussler L, Sirbat D, Hoffman M, Maincent P. Poly (epsilon-caprolactone) nanocapsules in carteolol ophthalmic delivery. *Pharmaceut. Res.* 1993; 10:386–390.
25. Shofner RS, Kaufman HE, Hill JM. New Horizons in ocular drug delivery. In: *Ophthalmol. Clin. N. Am.* Kooner TJ (ed) Philadelphia. W.B.Saunders Co., 1989.
26. Greaves JL, Wilson CG, Birmingham AT, Richardson MC, Bentley PH. Scintigraphic studies on the corneal residence of a New Ophthalmic Delivery System (NODS): Rate of clearance of a soluble marker in relation to duration of pharmacological action of pilocarpine. *Br. J. Clin. Pharmacol.* 1992; 33:603–609.