CHAPTER II

ANATOMY AND PHYSIOLOGY OF THE EYE

The human eye has a spherical shape with a diameter of 23mm. Behind its visible portions are complicated arrays of delicate mechanisms that work in union to transmit an image to the brain. The amount of light entering the eye is controlled by pupil, which dilates and contracts accordingly. The structural components of the eye ball are divided into three layers:

1. The outer most coat comprises of the clear, transparent cornea and white, opaque sclera;

2. The middle layer comprises the iris anteriorly, the choroid posteriorly and ciliary body as intermediate part and

3. The inner layer is the retina, which is an extensive of the central nervous system.

The fluid system in the eye, the aqueous humor and vitreous humor, also play an important role. Cornea is an optically transparent tissue that acts as the principle refractive element of the eye. The corneal diameter is about 11.7 mm with a radius of curvature of the anterior surface of about 7.8mm. The corneal thickness is 0.5-0.7 mm and it is thicker in the centre than in limbus. The cornea is composed of epithelium, Bowman’s membrane, stroma, Descemet’s membrane and endothelium. The relative thickness of corneal epithelium (50-90µm), stroma and endothelium are about 0.1, 1.0, 0.01 µm, respectively. The cornea and lens, whose shape is adjusted by the ciliary body, focus the light on the retina, where receptors convert it into nerve signals that pass to the brain. The cornea is avascular, but a mesh of blood vessels in the choroid supplies the retina with oxygen and sugar. Lacrimal glands secrete tears that wash foreign bodies out of the eye and keep the cornea from drying out. Blinking compresses and releases the lacrimal sac, creating a suction that pulls excess moisture from the eye’s surface. The drugs in ophthalmic preparations reach the inside of the eye through the cornea. Since the structure of the cornea consists of epithelium –stroma –endothelium, which is equivalent to a fat-water- fat
structure, the penetration of non-polar compounds through the cornea depends on their oil/water partition coefficients (Stella et al., 1980; Kishida et al. 1973).

The blood–ocular barrier normally keeps most drugs out of the eye. However, inflammation breaks down this barrier, allowing drugs and large molecules to penetrate into the eye. As inflammation subsides, this barrier usually returns. The blood-ocular barrier is comprised of the following sites:

**Blood-aqueous barrier:** The ciliary epithelium and capillaries of the iris.

**Blood-retinal barrier:** Non-fenestrated capillaries of the retinal circulation and tight-junctions between retinal epithelial cell preventing passage of large molecules from choriocapillaries into the retina.

### 2.1 ABSORPTION OF DRUGS IN THE EYE

It is often assumed that drugs administered into the eye are rapidly and totally absorbed. However, contrary to this belief, the moment a drug is placed in the lower cul-de-sac of the eye several factors immediately begin to affect its bioavailability. Absorption of drugs takes place either through corneal or non-corneal routes. The non-corneal route involves absorption across the sclera and conjunctiva into the intraocular tissues. This route is however not productive as it restrains the entry of drug into aqueous humor. Maximum absorption thus takes place through the cornea, which leads the drug into the aqueous humor. The goal of ophthalmic drug delivery systems has traditionally been to maximize ocular drug absorption rather than to minimize systemic absorption.

### 2.2 DRUG ELIMINATION FROM LACRIMAL FLUID

Designing formulations and delivery systems for ophthalmic use has always intrigued the formulator as most of the instilled volume of liquid dosage forms like solution, suspensions and liposomes is either drained from conjunctival sac into nasolacrimal duct or is cleared from pre-corneal area (Chrai et al., 1974; Zaki et al., 1978) resulting in poor bioavailability of drugs.

Drugs are mainly eliminated from the pre-corneal lacrimal fluid by solution drainage, lacrimation and nonproductive absorption by the conjunctiva of the eye. These factors and the corneal barrier limit the penetration of the topically administered drug into
the eye. Only a few percentage of applied dose is delivered into intraocular tissue, while the major part (50-100%) of the dose is absorbed systemically (Larivinen et al., 1995). Precorneal constraints include.

**Spillage of drug by overflow**

The normal resident volume of tear is 7µl (Maurice, 1973; Mishima et al., 1966; Ethlers, 1965) and if blinking doesn’t occur the human eye can accommodate 30 µl without spillage from the palpebral fissure. With an estimated drop volume of 50 µl, 70% of administrated dose is expelled from the eye by over flow and if blinking occurs only the residual volume, approximately 10µl is left (Wright and Meger, 1962) indicating that 90% of the does is expelled.

**Dilution of drug by tear turns over**

Tear turn over has a major share in removing drug solution from conjunctiva cul-de-sac. Normal human tear turn over is approximately 16% per minute, which is stimulated by many factors like drug entity, pH, tonicity of dosage form (Kupferman et al., 1974; Seig and Robinson, 1977; Conard et al., 1978) and formulation adjuvant. These factors render topical applications of ophthalmic solutions into the cul-de-sac extremely inefficient.

**Nasolacrimal drainage/systemic drug absorption**

Most of the administered drug is lost through nasolacrimal drainage immediately after dosing. The drainage allows drug to be systemically absorbed across the nasal mucosa and the gastrointestinal tract leading to multifarious effects. One such drug is timolol, a mixed β1 and β2 antagonist used in glaucoma therapy, which entails serous risk of systemic absorption. It was reported that 450 cases of serious side effects of timolol resulting in deaths of 32 patients due to bronchospasm (Fraunfelder and Meyer, 1987) and cardiovascular effects, have occurred.
Fig. 2.1: Factors and corneal barrier limitation for penetration of topically administered drug.
Conjunctival absorption:

Another mechanism that competes for the drug absorption into the eye is the superficial absorption of drug into palpebral and bulbar conjunctiva with concomitant removal from the ocular tissues by peripheral blood stream.

Enzymatic metabolism:

Enzymatic metabolism may operate in the precorneal space or in the cornea (Lee et al., 1980; Lee et al., 1982), which results in further loss of drug entities possessing labile bonds. Competing with the foregoing forms of drug removal is the transcorneal absorption, the route that effectively brings the drug through absorption into the aqueous humor. Clearly, the physiological barriers restraining the entry of the drug into eye are formidable, restraining the bioavailability to 1-3 % of the instilled dose (Mikkelson et al., 1973). In order to overcome this, frequent doses of drugs at very high concentration are recommended. This type of pulsed dosing not only results in extreme fluctuations in ocular drug concentration but also leads to untoward side-effects.

2.3 TRANSCORNEAL PENETRATION

Transcorneal penetration of drug is mainly affected by

a. Corneal barriers

b. Physicochemical properties of drugs and

c. Active ion transport systems present at cornea.

Corneal Barriers:

Corneal epithelium is the main barrier to drug absorption into the eye (Maurice and Mishima, 1984). Compared to other epithelial tissues (intestinal, nasal, bronchial, tracheal) corneal epithelium is relatively impermeable, but it is more permeable compared to the stratum corneum of the skin (Rojanasakul et al., 1992).

The stratified corneal epithelium acts as a protective barrier against invasion by foreign molecules and also as a barrier to ion transport. The corneal epithelium consists of
a basal layer of columnar cells, two or three layers of wing cells and one or two outer most layers of squamous, polygonal shaped superficial cells. In a healthy corneal epithelium intercellular tight junctions (zonula occludens) completely surround the most superficial cells; nevertheless the intercellular spaces are wider between wing cells and basal cells. This allows the paracellular diffusion of large molecules through these layers of cells only (Rojanasakul et al., 1990; Tonjum 1974). Tight junctions serve as selective barrier of small molecules and they completely prevent the diffusion of macromolecules via the paracellular route. It was found by perfusion studies that small molecules (Glycerol, PEG 200 and 400) are able to penetrate through intercellular spaces of corneal epithelium, but inulin (M.W.5000) and horseradish peroxide (M.W. 40,000) are molecules that are too large for paracellular penetration across the epithelium.

Corneal stroma is a highly hydrophilic tissue containing mostly water. Due to its relatively open structure, drugs with molecular size up to 50,000 can diffuse in normal stroma. Hydrophilic corneal stroma is a rate limiting barrier for ocular absorption of most lipophilic drugs (Maurice and Mishima 1984; Schoenwald, 1990). The corneal endothelium is responsible for maintaining normal corneal hydration and it has been estimated that drugs with molecular dimensions up to about 20nm can diffuse across normal endothelium.

2.4 PHYSICOCHEMICAL PROPERTIES OF DRUG

Transcellular or paracellular pathway is the main route for drugs to penetrate across corneal epithelium. Hydrophilic drugs penetrate primarily through the paracellular pathway, which involves passive or altered diffusion through intercellular spaces while lipophilic drugs prefer the transcellular route. For topically applied drugs, passive diffusion along their concentration gradient, either transcellular or paracellular permeation is the main permeation mechanism. On the contrary, Na⁺, K⁺ and ATPase pump is involved in corneal transport of lysine that requires carrier mediated transport system. While for drug loaded nanoparticles of poly (ε - Caprolactone), nanocapsules and insulin have been reported to follow endocytic pathway (Rojanasakul et al., 1990).

Lipophilicity, solubility, molecular size and shape, charge and degree of ionization also affect the route and rate of permeation in cornea. Chemical equilibrium between ionized and unionized drug in eye drop and in lacrimal fluid affect the penetration of
ionizable drugs e.g. weak acid and weak bases. Unionized species usually penetrate the lipid membranes more easily. For example pilocarpine (free base) and timolol base penetrate better than their ionized form.

Various esterases, peptidases, proteases and other enzymes are present in the ocular tissue including cornea. Consequently, many topically applied drugs are metabolized during or after absorption (e.g. Pilocarpine, levobunolol, epinephrine). In some cases, enzymatic transformation of ocular prodrugs in the corneal epithelium can be utilized for releasing the active parent drug from the inactive prodrug. In addition to passive corneal diffusion of ions via the paracellular pathway, ions can be actively transported across the corneal epithelium and endothelium. The corneal epithelium contains ionic channels that are selective for cations over anions and also contains an outwardly rectifying anion channel in the apical membrane and highly conductive potassium channel in the basal cells. Sodium penetrates from the tears into epithelium via passive diffusion, but it is actively transported from the epithelium to stroma.

2.5 NON-CORNEAL ABSORPTION

Apart from the corneal route topically applied ocular drugs may be absorbed through non- corneal route. This route involves drug penetrating across the bulbar conjunctiva and underlying sclera into the uveal tract and vitreous humor. This route is important for hydrophilic and large molecules, such as insulin and p-aminolclididine, which have poor corneal permeability.

Tight junction of the superficial conjunctival epithelium is the main barrier for drug penetration. Conjunctival permeabilities for hydrophilic drugs are typically an order of magnitude greater than their corneal permeability. The limiting molecular size for conjunctival penetration is between 20,000 and 40,000.

Topically applied drugs penetrate across the sclera through perivascular spaces, through the aqueous media of gel-like mucopolysaccharides or through empty spaces within collagen network. Sclera is more permeable in comparison to cornea. The scleral permeability for some β-blockers, sucrose, inulin and polyethylene glycols (mean molecular weights of 229-1056) is higher than corneal permeability.
2.6 PHARMACOKINETICS OF OCULAR DRUG ADMINISTRATION

Considering the eye as two compartments, precorneal and the aqueous humor, rate at which drug disappears from the precorneal compartment can be expressed mathematically as follows (Schoenwald, 1990):

\[
\frac{dC_T}{dt} = -q_T C_T - \left( K_p S_c / h_c \right)(C_T - C_{AH}) - \frac{V_D e^{-K_{nl} t}}{V_{o} + V_D}
\]

and the rate at which drug appears in aqueous humor compartment can be expressed as:

\[
\frac{dC_{AH}}{dt} = \frac{K_p S_c (C_T - C_{AH}) - K_{eAH} C_{AH}}{V_{AH} h_c} - \frac{C_{AH}}{V_{AH}}
\]

Where

- \( C_T \): Drug concentration in the tear fluid;
- \( K_p \): Specific transcorneal permeability rate,
- \( S_c \): Surface area of cornea,
- \( h_c \): Thickness of cornea,
- \( C_{AH} \): Drug concentration in aqueous humor,
- \( V_D \): Drop size of the drug solution instilled,
- \( K_{nl} \): \((0.25=0.0113 V_d) \text{ min}^{-1}\)
- \( V_{o} \): Normal resident tear volume
- \( C_{AH} \): Volume of aqueous humor and
- \( C_D \): Volume of drug pool in the pre-corneal area after instillation drug.
2.7 REFERENCES


CHAPTER III

DRUG SPECIFIC REVIEW

3.1 MOXIFLOXACIN HYDROCHLORIDE

Introduction

The quinolones are one class of antibiotics that has been used to treat the ocular infections, since their introduction into the ophthalmic community in 1991 (Leibowitz HM, 1991 and Leibowitz HM, 1991). The fluoroquinolones are formed by the addition of fluorine and other groups to nalidixic acid. They are divided into generations based on their antibacterial spectrum. The earlier generation agents are narrower spectrum than the later ones.

First generation quinolone started with the discovery of nalidixic acid, the quinolone prototype introduced in 1962. The early quinolones achieved only minimal serum levels and were effective only against gram-negative bacteria. They had no activity against Pseudomonas (Kowalski RP et al 2001)

Second-generation fluoroquinolones such as norfloxacin, ofloxacin and ciprofloxacin showed vastly increased gram-negative activity and greater systemic activity. Ciprofloxacin still remains the most effective agent against Pseudomonas (Kowalski RP et al 2001)

Third generation drugs such as levofloxacin showed expanded activity against gram-positive bacteria, atypical pathogens and increased solubility.

Finally the forth generation 8 methoxy fluoroquinolone drugs showed vastly increased gram positive coverage, combined with the significant activity against anaerobes and atypical pathogens.

For ophthalmic indications the fourth generation fluoroquinolone Moxifloxacin appears to offer considerable advantages over second and third generation predecessors. These fourth generation agents possess broader spectrum bactericidal activity, including effective coverage against gram-positive, anaerobic and atypical pathogens (Kowalski RP, et al 2002, Perry CM, et al 2002 and Mather R et al 2002). Also the newer fluoroquinolone showed excellent bioavailability, low toxicity, safety, significantly improved ocular...