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REVIEW

Penetration Enhancers and Ocular Bioadhesives: Two New Avenues for Ophthalmic Drug Delivery

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ABSTRACT

This review is focused on the two avenues of development that promise a major impact on future ocular drug therapeutics: bioadhesives, including hydrogels and other agents like carbopols, polyacrylic acids, chitosan, etc., and penetration enhancers, including different surfactants, calcium chelators, etc. The capacity of some polymers to adhere to the mucin coat covering the conjunctiva and the corneal surface of the eye forms the basis for ocular mucoadhesion. These systems markedly prolong the residence time of a drug in the conjunctival sac, since clearence is now controlled by the much slower rate of mucus turnover rather than the tear turnover rate. But improving the corneal drug retention alone is inadequate in bringing about a significant improvement of drug bioavailability. Another approach consists of transiently increasing the pentration characteristics of the cornea with appropriate substances, known as penetration enhancers or absorption promoters. The main aim of this article is to give an insight into the potential application of mucoadhesives and corneal penetration enhancers for the conception of innovative opthalmic delivery appraoches, to decrease the systemic side effects, and create a more focused effect, which may be achieved with lower doses of the drug. Ophthalmic formulations based on these mucoadhesives and penetration enhancers are simple to manufacture and exhibit an excellent tolerance when administered into the cornea. The use of the former considerably prolongs the corneal contact time and the use of the latter increases the rate and amount of drug transport. The various corneal epithelial barriers along with the major routes of transport of drugs are discussed. The article includes a list of the various

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substances in use or under investigation for the aforementioned properties, along with their mechanisms of action. A fair appraisal of the subject with regard to these two therapeutic approaches and any expected ill effects has been made.

Key Words: *Absorption promoters; Mucoadhesives; Ocular drug delivery; Review*

INTRODUCTION

Successful delivery of drugs into the eye is extremely complicated because the eye is protected by a series of complex defense mechanisms which make it difficult to achieve an effective concentration of drug within the target area of the eye. These complex defense mechanisms lead to a poor bioavailability of drugs delivered in classical ophthalmic dosage forms (eye drops) into the lower cul-de-sac (Fig. 1). Drugs administered systemically for their ocular action also have a poor access to the eye tissue because of the blood–aqueous barrier, which prevents drugs from entering into the aqueous humor, and the blood–retinal barrier, which prevents drugs from entering into the extravascular retinal space and the vitreous body (1,2).

After topical administration of an ophthalmic drug solution, the drug is first mixed with the lacrimal fluid and thus diluted. Moreover, the contact time of the drug with ocular tissues is relatively short (1–2 min), mainly due to the spillage of instilled solution from the precorneal area. The latter is a consequence of induced lacrimation. Lacrimal secretion is the protective mechanism of the anterior segment of the eye, to maintain visual functions including clearing of the inner and outer surfaces of the eye and elimination of foreign substances. The major route by which most ophthalmic drugs enter the eye is traditionally believed to be the cornea, despite its protection by the tight barrier of the epithelium. However, a minor route, contiguous with the cornea and involving the conjunctiva and the sclera (the so-called non-corneal route), has also been reported (3–6).

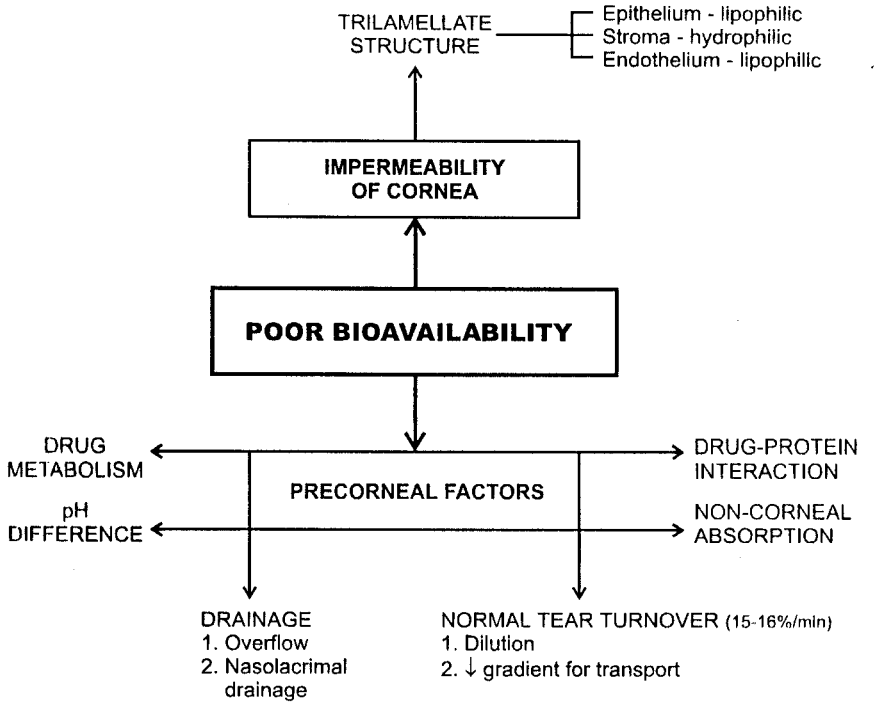

Figure 1. Factors affecting the poor bioavailability from ocular dosage forms.

The Corneal Barrier

The cornea, which is 1 mm thick at its edges and 0.5 mm thick at its center, is classically described as a heterogeneous tissue composed of (7):

1. the epithelium and its basal layer in contact with the Bowman's membrane;
2. the corneal stroma, which alone represents nine-tenths of the thickness of the cornea;
3. the Descemet's membrane and corneal endothelium.

The corneal epithelium in itself is also a layered structure, 50 to 100 μm thick, consisting of a deep layer of basal columnar cells, an intermediate layer of polyhedral cells, and a surface layer of squamous, polygonal-shaped cells. The outermost cells have skirting intercellular junctions, termed "tight junctions." These form a strong barrier to non-lipophilic substances, allowing the preferential penetration of non-ionized forms.

The Bowman's membrane separating the stroma from the epithelial tissue is composed of a layer of collagen fibers, 8 to 14 μm thick, forming a tough and relatively impermeable barrier. The corneal stroma is composed essentially of collagen and represents approximately 90% of the thickness of the cornea. It is highly hydrophilic, porous, and an open-kit, thus allowing the free passage of hydrophilic substances but acting as a barrier to lipophilic molecules. Descemet's membrane covers the posterior surface of the stroma. It is a single-cell layer, 5 to 10 μm thick, and is also composed of collagen. The corneal endothelium, a single-cell layer lining the posterior surface of the stroma, is rich in phospholipids, permeable to lipid-soluble materials, and almost impermeable to ions.

Almost all of the ophthalmic drugs that have been studied so far appear to cross the cornea by simple diffusion involving the paracellular and the transcellular pathways. The paracellular pathway anatomically involves the intercellular space and is the primary route of passive ion permeation (8). Considering that the total surface area of the cornea exposed to the tears, attributable to the paracellular pathway, is rather small, it is supposed that most of the drugs would probably opt for the transcellular pathway in crossing the cornea. The principal drug properties governing drug absorption via this pathway are: (i) the lipophilicity of the drug, as reflected by its *n*-octanol/buffer partition coefficient (PC), the optimum PC being in the range 10–100; (ii) pK_a, which determines the proportion of drug in its preferentially absorbed form at a given pH; and (iii) solubility, drugs that are both lipid- and water-soluble pass through the cornea readily.

Alternative Route for Ocular Delivery

The conjunctiva, a thin mucous membrane lining the inside of the eyelids and the anterior sclera, contributes to the non-corneal ocular absorption (3). Its surface area is an order of magnitude greater than that of the cornea (9), and it is two to three times (depending on the drug) more permeable than the cornea (6,10). Since the conjunctiva is highly vascularized, the blood circulation removes most of the drug before it can enter the inner ocular tissues.

IMPROVING THE AVAILABILITY OF DRUGS USING VARIOUS FORMULATION APPROACHES

A major challenge in ocular therapeutics is the improvement of ocular bioavailability from less than 1–3% to at least 15–20%. The last three decades have witnessed continued efforts in this direction, and investigations are being pursued along the following lines.

1. To increase the transcorneal passage of drugs by incorporating absorption promoters/penetration enhancers into the drug formulations.
2. To optimize of formulation vehicles for prolonged drug retention in the precorneal area and an increased contact time between the administered drug and the conjunctival and corneal epithelia by the following methods.
   - Addition of water-soluble, natural, synthetic, or semi-synthetic viscolizers.
   - Use of drug carrier systems such as nanoparticles, microspheres, liposomes, etc., which would remain in the cul-de-sac for a longer period of time, thus giving a sustained action.
   - A newer approach involving the utilization of the mucoadhesive property of polymers to improve the ocular absorption of poorly-absorbed drugs.

Since a lot has already been published about the use of viscolizers and the already-existing drug carrier systems, along with their application to ophthalmics, the main focus of this review will be on penetration enhancers and the use of bioadhesive polymers to improve the ocular bioavailability to a sufficient extent that an ocularly-delivered drug can elicit its biological action. The aim of this article is to give an insight into the potential application of mucoadhesives and corneal penetration enhancers for the conception of innovative ophthalmic delivery approaches, to decrease the systemic side effects, and create a more pronounced effect, which may be achieved with lower doses of the drug. Ophthalmic formulations based on these mucoadhesives and penetration enhancers are simple to manufacture and exhibit an excellent tolerance when administered into the cornea. The use of the former considerably prolongs the corneal contact time and the use of the latter increases the rate and amount of drug transport.

Use of Penetration Enhancers

This approach consists of increasing transiently the permeability characteristics of the cornea with appropriate substances, known as penetration enhancers or absorption promoters. It bears a strict analogy to techniques aimed at facilitating drug penetration through the skin and other epithelia such as the buccal, nasal, intestinal, or rectal.

There are two possible modes of action of the absorption promoters (Fig. 2). Surface-active absorption enhancers are believed to increase the permeability of the cell membranes, while calcium chelators act mainly on the tight junctions. However, most enhancers have been shown to affect both the cell membranes and the tight junctions.

The stratified corneal epithelial cell layer can be characterized as a "tight" ion-transporting tissue (11) because of the high resistance (12–16 $k\Omega\,cm^2$) exhibited by the paracellular pathway (12). The paracellular pathway consists of the intercellular space with intercellular junctions. Further, the intercellular junction consists of the zonula occludens or tight junction at the most apical zone of contact. Adjacent to the tight junction is the adherens junction. Below these two junctions are the spotlike contacts of desmosomes and gap junctions (13,14). Adherens junctions are formed by the transmembrane protein cadherin that is responsible for Ca^{2+}-dependent cell–cell adhesion, while the desmosomes are structures that bind cells to one another. Gap junctions include connexons and contain porous channels for communication between cells. These structures do not provide any barrier for drug penetration.

The entry of molecules through the paracellular pathway is primarily restricted by tight junctions.

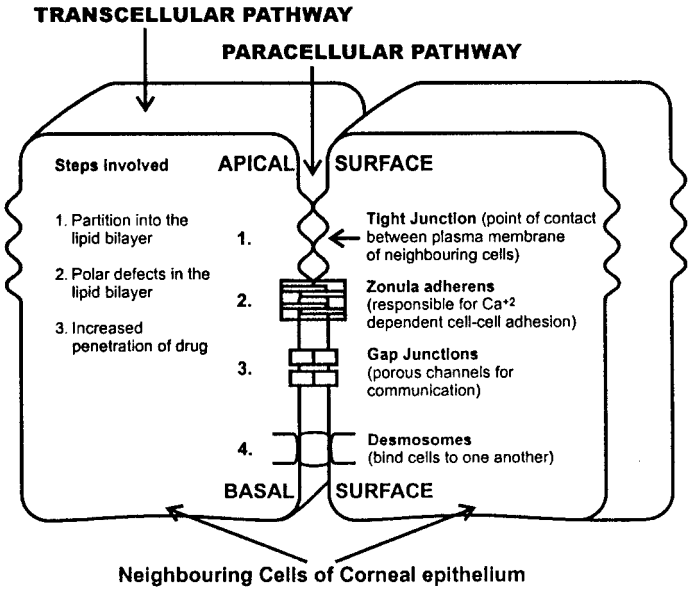

Figure 2. Schematic illustration of the transcellular and paracellular modes of action of penetration enhancers.

The latter are bandlike structures in which the plasma membranes of neighboring cells are brought into close apposition and completely encircle the superficial epithelial cells. Both the assembly and barrier properties of tight junctions are influenced by the classical second messenger and signaling pathways, including tyrosine kinases, Ca^{2+}, protein kinase C, G proteins, calmodulin, cAMP, and phospholipase C (15,16). Tight-junction permeability has been shown to depend on a number of factors (17), including:

- degree of maturation of epithelia;
- response to physiological requirements;
- change in environmental conditions such as osmolarity and ionic strength;
- presence of drugs, vitamins, and hormones. Among these, a few agents play a central role, e.g., the concentration of calcium. Drugs that disrupt the organization of the actin network, such as EDTA (ethylenediaminetetraacetic acid) and cytochalasin B, may also cause an increase in corneal permeability, while those stabilizing it, i.e., phalloidin and kinetin, have the reverse effect. Maintenance of the tight junction requires an unknown appropriate level of Ca^{2+} bound to or in the vicinity of the plasma membrane (18). Thus, the chelation of Ca^{2+} can lead to the disruption of these tight junctions.

Classes of Penetration Enhancers

Calcium Chelators

The calcium chelators such as EDTA have been reported to loosen the tight junctions between the superficial epithelial cells, thus facilitating paracellular transport (19,20). The Ca^{2+} depletion does not act directly on the tight junctions, rather it induces global changes in the cells including: (i) the disruption of actin filaments; (ii) the disruption of adherent junctions; (iii) diminished cell adhesion of actin filaments; and (iv) the activation of protein kinases (21). The association of actin filaments inside the cell with the junctional complex also seems to be disrupted by the low extracellular Ca^{2+} (22,23). Grass and Robinson (24) were among the first to emphasize the positive effect of chelating agents on corneal drug absorption. They found that 0.5% EDTA doubled the ocular absorption of topically-applied glycerol and cromolyn sodium. Similarly, 0.5% EDTA also showed an enhancing effect for timolol and atenolol, but reduced the transcorneal flux of levobunolol (25,26). Sasaki et al. (27) reported small but significant permeability increases for timolol and for the more lipophilic befunolol with 0.5% EDTA: EDTA acts on the tight junctions producing ultrastructural changes in the corneal epithelium, resulting in a water influx and a decrease of the overall lipophilic characteristics (19). This effect may account for the permeability reduction observed in the case of more lipophilic drugs. The studies in our laboratory indicated that there was an increase in the IOP-lowering effect of acetazolamide in the presence of 0.5% EDTA (28).

Surfactants

Unlike the chelators, surfactant enhancers have been suggested to increase drug and peptide permeability through the cell membranes or via the transcellular pathway (29). Epithelial cells are surrounded by an outer cell membrane composed of a phospholipid bilayer with the protein molecules embedded in the lipid membrane. When present at low concentrations, these surfactants are incorporated into the lipid bilayer, forming polar defects which change the physical properties of the cell membranes. When the lipid bilayer is saturated, mixed micelles begin to form, resulting in the removal of phospholipids from the cell membranes and hence leading to membrane solubilization. It has therefore been suggested that a dose-dependent increase in the permeability of the cell membrane is responsible for surfactant-induced increases in permeability across different epithelia. Recent research suggests that many surfactants increase the paracellular penetration of drugs by affecting the tight junctions (30–32). Palmitoyl carnitine, a zwitterionic surfactant, has been shown to enhance drug absorption without causing significant changes to the morphology of intestinal cells both in vivo and in vitro, suggesting that this surfactant increases the paracellular penetration via the tight junction (31). Disruption of tight-junction integrity has also been reported using other surfactants, such as sodium caprate and sodium dodecyl sulfate (32). Various classes of surfactants have been reported in the literature to act as corneal penetration enhancers, including the following.

Non-ionic surfactants The corneal permeability of atenolol, timolol, and betaxolol was increased

significantly by 0.05% Brij® 35, Brij® 78, and Brij® 98, respectively (19). The effect of sodium deoxycholate, poly oxyethylene-9-lauryl ether (non-ionic surfactant), and L-α-lysophosphatidylocholine on sulfadicramide dialysis was studied through synthetic membranes and through the animal cornea (in vitro). Polyoxyethylene-9-lauryl ether was found to be the most effective surfactant among these (33). The promoting effects induced by polyoxyethylene ethers depend on the number of oxyethylene groups in a molecule, also on the HLB number, and a significant effect is obtained if many such groups are present in the molecule. The use of this group to enhance the biological availability of various drugs applied to the eye (34,35) consists of a combination of two mechanisms: loosening the cell packing through local disturbances of Ca^{2+} metabolism and increasing the permeability of a given tissue by washing out proteins from the membranes (36,37).

Bile acids and salts Bile salts are amphipathic molecules that are surface-active and self-associate to form micelles in aqueous solutions. These agents, e.g., deoxycholate, taurodeoxycholate, and glycocholate, act by changing the rheological properties of biological membranes (27). Owing to their mucolytic properties these agents can increase diffusion of drug molecules through the membrane by inducing a transient change in its structure and permeability (33). The exact mechanism of the enhancement of corneal permeability by bile salts is still unclear, though it has been indicated that they promote the transport of hydrophilic and macromolecular compounds, mainly through a porelike route, such as intercellular channels (38).

The effect of taurocholic acid and taurodeoxycholic acid on the in vitro rabbit corneal permeability of hydrophilic molecules and macromolecular compounds was studied. Taurodeoxycholic acid markedly increased the corneal permeability of these penetrants at 2 and 10 mM concentration (39). Taurocholic acid has been reported to show a significant increase in the conjunctival absorption of β-blockers. However, it had a lower promoting activity on the corneal permeability (27). Further, it has been reported that sodium taurocholate (Tc-Na) marginally increased the corneal permeabilities of hydrophilic compounds such as 6-carboxy fluorescein and glutathione and macromolecular compounds such as FITC-dextran and insulin, whereas sodium taurodeoxycholate (TDC-Na) produced a marked effect (38). A difference in the physicochemical properties of these bile salts, e.g., their solubilizing activity, lipophilicity, and Ca^{2+} sequestration capacity, is probably related to their permeability-enhancing effects. The critical micellar concentrations (CMCs) of dihydroxy bile salts are generally lower, and their aggregation numbers larger than those of trihydroxy salts. Thus, the solubilizing activity of TDC-Na is higher than that of Tc-Na. Moreover, TDC-Na also has a higher lipophilicity and calcium ion sequestration activity (39), leading to a greater loosening of the corneal epithelial barrier.

Lysophosphatidilo lipids These are amphiphilic surfactants produced in a natural way from phospholipids by phospholipases. Their mechanism of action as a promoter is not fully understood. It is supposed that, like other surfactants, they can affect intracellular proteins and polar groups of phospholipids in intercellular spaces, which may favor the formation of channels permitting the penetration of water and substances dissolved therein (33).

Preservatives

Many researchers have demonstrated that some preservatives significantly increase the corneal permeability of ophthalmic drugs (40–43).

Benzalkonium chloride (BAC) Benzalkonium chloride shows the highest promoting effect on corneal drug penetration from amongst the currently used preservatives. Camber and Edman (42) demonstrated that exposing the isolated porcine cornea to 0.017% BAC or 0.5% chlorbutanol for 4 hr almost doubled the transcorneal flux of pilocarpine. The addition of BAC (0.005%) enhanced 2.5-fold the transport percentage and P_{app} of S-1033, a novel prostaglandin derivative used as an antiglaucoma medication (44). Podder et al. (45) reported that BAC increased the ocular absorption of timolol in rabbits by approximately 80%, but that systemic timolol absorption was also increased and no change was observed in the absorption ratio between the eye and systemic circulation. Smolen et al. (46) demonstrated that BAC and another cationic surfactant, diethylamino-ethyl dextran, enhanced the miotic response of topically-applied carbachol. Instillation of 0.01% BAC shows no ocular irritation according

to the Draize score, and at a concentration of 0.004–0.01% BAC had no influence on the epithelial aerobic metabolism (47). Corneal exposure to multiple drops of BAC leads to epithelial accumulation but no penetration into the anterior chamber (39). However, 0.01% BAC has been reported to cause cells of the corneal epithelium to peel at their borders (48). Benzalkonium chloride has also been found to enlarge the intercellular spaces in the superficial cells of the cornea (49,50). It has been established that rabbits are more sensitive to single doses of preservatives than humans, and it was found that 0.01% BAC increased the anterior chamber fluorescence level in rabbits but not in humans (40).

Cetylpyridinium chloride Inclusion of 0.02% cetylpyridinium chloride with pilocarpine nitrate applied topically to the rabbit eye was found to cause a miotic effect 10 times that obtained in the absence of cetylpyridinium (51). In another study, cetylpyridinium chloride was shown to enhance penicillin penetration across the isolated rabbit cornea (52) with an intact epithelial layer. The penetration was even more than that shown by the de-epitheliazed corneas.

Glycosides

Some of the glycosides with surface activity have been used successfully as penetration enhancers. For example, the following.

Saponin Saponin is a type of glycoside widely distributed in plants. It is an amphiphilic compound that has surface activity. Purified quillaja saponin has been reported to possess an ability to promote the nasal absorption of gentamicin antibiotics in mice and rats, and the nasal or ocular absorption of insulin in rats (52). At 0.5% concentration, saponin was found to enhance the corneal penetration of β-blockers (27). It increased the ocular permeability of hydrophilic β-blockers markedly, but the increase was slight in the case of lipophilic β-blockers. At 1.0% concentration, saponin has been reported to act as a promoter of systemic absorption of insulin administered via eye drops (53). It is not known at present if the saponins act by simple detergent action to make the epithelial membrane more permeable to intermediate-sized peptides, or if there is a selective interaction between certain moieties of the excipient and certain sites on the epithelial surface that play a regulatory role in controlling peptide absorption (54).

Digitonin Digitonin (a non-ionic surfactant) possesses certain detergent characteristics and has the ability to permeabilize membranes in a wide variety of cells (55). In the cornea, digitonin has been found to selectively solubilize membrane cholesterol and cause exfoliation of the epithelium, layer by layer (56). Digitonin has greatly increased the corneal absorption, in vitro, of a series of different molecular weight polyethylene glycols, but also led to a significant alteration or complete removal of the epithelial layer (57).

Fatty Acids

These agents act as absorption promoters by affecting both the cell membranes and the tight junctions, and by forming ion-pair complexes with cationic drugs (58). Muranishi (59) demonstrated that fatty acids perturb the membrane structural integrity by their incorporation into the plasma membrane. The effect of capric acid on the paracellular pathway has been related to the Ca^{2+}-dependent contraction of the perijunctional actomyosin ring, as well as the chelation of Ca^{2+} around the tight junction. Caprylic acid was reported to interact mainly with proteins, whereas capric acid interacted with both proteins and lipids (60). Capric acid has been reported to increase the ocular delivery of hydrophilic β-blockers, but causes only a slight improvement in the ocular delivery of lipophilic β-blockers (27). Kato and Iwata (58,61) also reported that fatty acids significantly increased the corneal permeability of bunazosin, but this enhancing effect was attributed to ion-pair formation rather than membrane disturbance.

Miscellaneous

A miscellaneous group of substances have been quoted in the literature to act as ocular promoters. These include the following.

Azone Newton et al. (62) reported that azone, a transdermal absorption promoter, increased the ocular delivery of instilled cyclosporin and enhanced its immunosuppression activity. Four penetration enhancers [azone (laurocapram), hexamethylene lauramide, hexamethylene octanamide, and decylmethyl sulfoxide] were studied for cimetidine and all of them enhanced the corneal penetration of

cimetidine. The effect of azone on the corneal permeability of a series of structurally-unrelated drugs, ranging from hydrophilic to lipophilic, was also studied. Azone enhanced the transcorneal penetration of hydrophilic drugs but retarded the apparent drug permeation of lipophilic drugs across the cornea (63). The mechanism of these penetration enhancers may have to do with the changes in the structure and fluidity of the barrier membrane because of their high lipophilicity and sequestration in the epithelium. It is also possible that the enhancer, when highly concentrated in the epithelium, may loosen the epithelial cell junctions and facilitate the influx of water and hydrophilic compounds but retard the movement of lipophilic molecules by creating a more hydrated barrier (63).

Cytochalasins Cytochalasins are a group of small, naturally-occurring heterocyclic compounds that bind specifically to actin microfilaments, the major component of the cell cytoskeleton, and alter their polymerization (64,65). In addition to its normal role in regulating cell contractibility, mobility, and cell-surface receptors, the cytoskeleton has been shown to participate in regulation of epithelial tight-junction permeability (66,67). The actin microfilament has been found to play a role in positioning junctional strands, through its association with plasma membrane components, and influencing the degree of opening of the occluding junctions (68). Among the cytoskeleton-active agents, the specificity and efficacy of cytochalasin B on tight-junction permeability make it a very useful agent in peptide delivery. It has been shown to be effective in enhancing the corneal permeability with minimal membrane damage (69).

Ionophores Mitra (70) showed that an ionophore such as lasalocid can also be used to enhance the corneal permeability of pilocarpine.

Effect of Absorption Promoters on Conjunctival Membranes

An absorption promoter can control the extent and pathway of the ocular and systemic absorption of instilled drug solution by altering the corneal and conjunctival drug penetration. Most of the agents discussed above show different effects on the corneal and conjunctival membranes. These different responses of corneal and conjunctival barriers to absorption promoters can be exploited and used to control the extent and pathway of the ocular and systemic absorption of drugs instilled into the eye. The mechanisms of action of absorption promoters and the barrier properties of membranes are the two factors which define drug permeability.

The conjunctiva, consisting of a thin mucous membrane and a highly vascularized tissue lining the inside of the eyelids and the anterior sclera, contributes mainly to the non-corneal ocular absorption and systemic absorption. It has a non-keratinized, stratified, squamous epithelium, overlying a loose, highly-vascular connective tissue. The superficial conjunctival epithelium has tight junctions that are the main barrier for drug penetration across this tissue. However, the intercellular spaces in the conjunctival epithelium are wider than those in the corneal epithelium. Therefore, the richness of the paracellular route makes the conjunctiva more permeable than the cornea. Taurocholic acid had the most potent effect on the conjunctival penetration of β-blockers. It has even been reported to increase the conjunctival penetration of macromolecules such as insulin (71). Meanwhile, EDTA showed only a slight promoting activity on the conjunctival penetration of β-blockers (27). Saponin, although a strong surfactant, also had a slight effect on conjunctival penetration. In contrast, capric acid did not affect conjunctival penetration at all.

In corollary to the above observations, penetration enhancers are being employed to increase the ocular absorption of peptide drugs, e.g., insulin, for their systemic effects. The ocular route is a simple, non-invasive, more accurate and less expensive alternative for the systemic delivery of peptides compared to the buccal, nasal, rectal, vaginal, or dermal routes. It also avoids: (i) pain in comparison to administration by parenteral injections; and (ii) gastrointestinal degradation since the drug enters the circulation directly (72,73). Chiou and Chuang (53) and Yamamoto et al. (74) demonstrated that insulin was well absorbed and showed a significant hypoglycemic effect after its instillation (in combination with several absorption enhancers). Saponin was found to be the best enhancer of insulin absorption via this route (53), while EDTA, fusidic acid, bile salts, and some surfactants also showed an enhancing effect on insulin absorption (37,75,76). Furthermore, insulin instilled with ophthalmic preservatives, especially BAC and paraben, showed a significant hypoglyemic response (77).

Other Applications

In addition to the use of penetration enhancers in eye solutions, both for improving the corneal and conjunctival (systemic) delivery of drugs, the use of these agents has also been extended to their incorporation into other ocular delivery systems. For example, solid ocular inserts made of polyvinylalcohol (PVA) containing sulfadicramide and some absorption promoters, e.g., polyoxyethylene-9-lauryl ether, L-(lysophosphatidylocholine), and deoxycholic acid sodium salt, have been reported. Such inserts showed an increase in the penetration of the drug through the animal cornea in in vitro studies (78).

Furthermore, addition of a penetration enhancer to the vehicle of an ophthalmic solution has been used to reduce the size of the drop instilled and, since this reduction in size results in a decreased systemic drug loss with a decreased potential for systemic toxicity, at the same time improve the ocular absorption of poorly-absorbed drugs (79). As an advantage, an equivalent or even improved ocular bioavailability and therapeutic response are obtained. Moreover, the small drop being instilled ensures less lacrimation and hence a decreased dilution and drainage. The decrease in drop size prevents losses (due to increased lacrimation) and hence makes the product more cost-effective.

Limitations to Practical Applicability

In spite of the citation of a large number of virtual applications of penetration enhancers in the literature, the unique characteristics and high sensitivity of the corneal/conjunctival tissues require great caution to be applied in their selection with regard to their capacity to affect the integrity of the epithelial surfaces. There is evidence that penetration enhancers themselves can penetrate the eye and may therefore lead to unknown toxicological implications, e.g., BAC was found to accumulate in the cornea for days (80). Similarly, EDTA was found to reach the iris–ciliary body in concentrations high enough to alter the permeability of the blood vessels in the uveal tract, indirectly accelerating drug removal from the aqueous humor (81). A repeated application of 0.5% EDTA was observed to significantly alter the corneal epithelial architecture, although a single application was well tolerated (41). Azone, at concentrations of 0.1% or higher, has also been reported to be irritating, discomforting, and/or toxic to the eyes (82). Even saponin has been reported to cause eye irritation at the 0.5% level (53). Bile salts and surfactants were shown to cause irritation of the eye and the nasal mucosa (43,83). Rojanasakul et al. (69) used laser scanning confocal microscopy and electrophysiological techniques to confirm that even though most of the enhancers (in particular, EDTA, digitonin, and sodium deoxycholate) significantly increased corneal permeability, they may lead to severe cellular membrane damage.

Although penetration enhancers promise superior therapeutic efficacy, this approach should be introduced for clinical use only after considering the balance of risks and benefits. Further, information on the different mechanisms of drug penetration, ocular metabolism, ill effects, and the influence of ocular diseases on the specific drug absorption-enhancement techniques is required to determine the nature of this balance.

Bioadhesives

If we extend the approach of effective drug delivery a step further, then it may be stated that another necessary condition for the activity of the dosage form is the guarantee to keep it in place for a sufficient length of time. Conventional aqueous solutions topically applied to the eye have an inherent disadvantage in that most of the instilled drug is lost within the first 15–30 sec after instillation, due to reflex tearing and drainage via the nasolacrimal duct. Hence, one of the goals in ophthalmic research has been to increase the duration of contact time. The first major step in this direction has been to enhance the precorneal retention of ophthalmic solutions by the incorporation of viscosity-building agents such as PVA and methyl cellulose. Nevertheless, viscosity alone cannot significantly prolong the residence time. This can be considered, in part, as the premise of using bioadhesive polymers to enhance drug absorption.

Bioadhesion refers to the process of attachment of a drug carrier system to a specific biological location. The biological surface can be epithelial tissue, or it can be the mucous coat on the surface of a tissue. If the adhesive attachment is to a mucous coat, the phenomenon is referred to as mucoadhesion. The mucosal layer lines a number of regions of the body, including the gastrointestinal tract, the urogenital tract, the airways, the ear, nose, and eye. The capacity of some polymers to adhere to the

mucin coat covering the conjunctiva and the corneal surfaces of the eye by non-covalent bonds forms the basis of ocular mucoadhesion. These bioadhesive polymeric systems significantly prolong the drug residence time, since the clearance is now controlled by a much slower rate of mucus turnover than the tear turnover rate (84). Mucoadhesives thus increase the residence time and, in addition, also provide an intimate contact between the drug and the absorbing tissue which may result in a high drug concentration in the local area and hence a high drug flux through the absorbing tissue.

Mucin Coat of the Eye

Mucin forms the bottom layer of the tear film lying adjacent to and wetting the corneal epithelium. This lowermost layer of tear film forms a bridge between the hydrophobic corneal epithelial surface and the aqueous layer of the tear film lying immediately above it. The composition of mucus varies widely depending upon animal species, anatomical location, and the normal or pathological state of the organism (85). Its major constituents are the high molecular weight glycoproteins capable of forming slimy and viscoelastic gels containing more than 95% water (86,87). These glycoproteins form disulfide as well as ionic bonds and also physical entanglements, and consist of a peptide backbone, a major portion of which is covered with carbohydrates (grouped in various combinations) such as galactose, fucose, *N*-acetyl glucosamine, *N*-acetyl galactosamine, and sialic acid. Mucin may have a different charge density depending on the pH, because of the differences in the dissociation constant of functional groups on the carbohydrate moiety and the amino acids of the polypeptide backbone. At physiological pH of tears the mucus network usually carries a significant negative charge because of the presence of sialic acid and sulfate residues (88).

Mechanism

The phenomenon of bioadhesion involves a succession of events. The first step is the establishment of an intimate contact between the bioadhesive and the corneal membrane. This may occur either from a good wetting of the bioadhesive surface, i.e., mucin layer, or from the swelling of the bioadhesive. Further, a sufficient spreading is also necessary to guarantee a contact at the molecular level between the bioadhesive and the membrane. Once contact is established, the second stage is the penetration of the bioadhesive into the crevices of the tissue surface. This may also sometimes entail the interpenetration of chains of the bioadhesive with those of the mucus. This further increases the area of contact (89).

At the molecular level, mucoadhesion can be explained on the basis of attractive interactions arising from Van der Waal's forces, electrostatic attraction, hydrogen bonding, and hydrophobic interactions. Repulsive interactions can also occur because of the electrostatic and steric repulsion. However, for mucoadhesion to occur, the attractive interactions should be greater than the non-specific repulsion (90).

Polymers Used

The most commonly used bioadhesives are macromolecular hydrocolloids with numerous hydrophilic functional groups capable of forming hydrogen bonds (91) (such as carboxyl, hydroxyl, amide, and sulfate groups). Typically, these polymers consist of high molecular weight molecules which cannot cross biological membranes. The bioadhesive polymers can be either natural, synthetic, or semi-synthetic, in nature. Further, they can be either water-soluble polymers with linear chains or water-insoluble polymers that are swellable networks joined by cross-linking agents. Polymers commonly used in ophthalmics for their mucoadhesive properties, under the various groups, include: hydroxypropyl cellulose, hydroxypropyl methylcellulose (HPMC) as the non-ionics; chitosan, DEAE-dextran as the polycationics; polyacrylic acid (PAA) derivatives, e.g., carbopols, polycarbophils, carboxymethylcellulose (CMC), etc., as the polyanionics. According to Park and Robinson (92), cationic and anionic polymers bind more effectively with the corneal epithelium than the neutral polymers. Moreover, they found that polyanionics are better than polycationics in terms of binding/potential toxicity. But this cannot be generalized, because the positively-charged polymeric hydrogels could possibly develop additional molecular-attractive forces due to the electrostatic interactions with negatively-charged mucosal surfaces and hence show improved efficacy (93).

The bioadhesive power of a polymer is affected not only by the nature of the polymer but also by

the nature of the surrounding media (85,89,94,95). The polymer-related factors include the molecular weight, the concentration of the active polymer, the flexibility of the polymer chains, and their spatial conformation. Meanwhile the pH, initial contact time, swelling, etc., form the environment-related factors. The physiological variables include mucin turnover and diseased state. Further, it has been postulated that other physicochemical properties of the polymer, such as surface spreading and absorptive characteristics, may also exert an influence (85,87). It has been proposed (88,96) that mucoadhesive polymers, when incorporated into viscous solutions (an earlier method of improving residence time in the cul-de-sac), can further improve the precorneal retention of these solutions and also the bioavailability of incorporated drug.

Usually the polymeric solutions exhibiting pseudoplastic behavior are preferred, mainly because they do not influence the viscosity characteristics (pseudoplastic behavior) of the precorneal tear film (97). Viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear rate should thus be preferred (98), e.g., hyaluronic acid, polyacrylic acid, chitosan.

The various mucoadhesive agents which are being employed in ophthalmic vehicles for improving and extending the action of different drugs are discussed below.

Hyaluronic Acid (HA)

Hyaluronic acid is a high molecular weight biological polymer consisting of linear polysaccharides present in the extracellular matrix. In the eye, HA is present in the vitreous body and in low concentrations in the aqueous humor (99). In addition to its mucoadhesive properties, sodium hyaluronate is an attractive ophthalmic drug-delivery vehicle because of its high water-binding capacity, non-irritancy, increased viscosity, and pseudoplastic behavior (100–102). Camber et al. (103) observed a two- to threefold increase in the absorption of 1% pilocarpine hydrochloride solution into the albino rabbit eye when delivered in 0.2% and 0.75% sodium hyaluronate (SH) solutions. Gamma scintigraphic studies showed that the residence time for 0.2% and 0.3% SH solutions is significantly longer in keratoconjunctivitis sicca patients (104). Ludwig and Van Ooteghem (105) used the tracer fluorescein to show an increased precorneal residence time with 0.25% HA solution. Gurny et al. (106) showed further that SH allowed maintenance of 50% of the drug activity even up to 20 min after administration. The molecular weight of the HA polymer influences directly the retention potential at the ocular surface (107). Gentamicin, when formulated with 0.25% HA, showed increased gentamicin bioavailability on the ocular surface (108). Dosage forms based on the benzyl esters of HA have also been proposed for ophthalmic sustained-release preparation of methyl prednisolone (109). In some studies, films and microspheres were also prepared from HA (110,111).

Carboxymethylcellulose

The ocular concentration of timolol improved three- to ninefold in the presence of sodium CMC, compared with the non-viscous eye drops (112). The improved ocular penetration was probably due to the longer corneal contact. A decreased rate of systemic absorption was also reported, and attributed to the slower spreading of the solution on the nasal mucosa. Acetazolamide formulated in CMC, when compared with the saline solution of the drug in patients with unilateral open-angle glaucoma, indicated a longer duration of action. However, the results were not spectacular and were significant only when using high drug concentrations (113). Sodium CMC has been defined as the most mucoadhesive polymer by Smart and Kellaway (114).

Polyacrylic Acid Derivatives

These include (a) carbopols and (b) polycarbophils. Both are polymers of acrylic acid differing in the extent of cross-linking and the cross-linking agent used. Carbopols are cross-linked with allyl sucrose and polycarbophils with divinyl glycol (115). Many workers have suggested that polyacrylic acid is one of the most pseudoplastic polymers, with important bioadhesive properties (116,117).

Several of the carbopols are appropriate for use in the pharmaceutical industry. For example, carbopol 934P is lightly cross-linked, has a molecular weight of approximately 3,000,000 Da, and is readily soluble in aqueous solutions. Davies et al. (96) demonstrated that the precorneal retention of the PAA solution was significantly greater than that of the PVA solution, as indicated by the miotic response intensity of pilocarpine 1 hr after administration. Comparable experiments have been carried

out by Saettone et al. with pilocarpine in the rabbit eye (118,119). Jarvinen et al. (120) observed that equiviscous solutions of two strongly bioadhesive polymers, CMC and PAA, decreased the systemic absorption of timolol in rabbits. The ocular bioavailability of a 0.5% timolol solution in rabbits, when compared with 0.5% timolol in isoviscous PVA and PAA solution, showed that the bioadhesive and non-newtonian PAA polymer produced lower ocular concentrations. This could be because of the slower release of timolol from PAA and the longer retention of the vehicle in the conjunctival sac by mucoadhesion (121). When compared with the commercially-available aqueous timolol solution (0.5%), 0.1% timolol in PAA hydrogel showed the same efficacy in lowering intraocular pressure in healthy volunteers (122). Betaxolol, formulated with PAA, showed a more constant release of the drug in comparison with a 0.5% solution of betaxolol in rabbits (123). Fucidic acid, an antibiotic, formulated in PAA (fucithalmic) gives a long-lasting antibiotic efficiency (124). Le Bourlais et al. (125) have tested PAA polymeric gels in aqueous/non-aqueous solvents incorporating the immunosuppressive agent cyclosporin and showed a significantly improved drug corneal penetration. Polyacrylic acid was studied as a controlled-release drug-delivery vehicle for ribozyme, an oligonucleotide potentially able to prevent HIV proliferation (126). The formulation promoted ribozyme-based therapeutics to gain entry into a diseased tissue and cell.

Polycarbophil is an anionic synthetic polymer consisting of particles that swell but are insoluble in water. Its swelling characteristics in water depend on the pH and ionic strength of the test solution, with the swelling increasing as pH increases. At low pH (1–3), polycarbophil absorbs approximately 15–35 mL of water per gram of resin, whereas in neutral or basic media it can absorb up to 100 mL per gram. The swelling in water permits entanglement of the polymer chains with mucus on the surface of the corneal tissue. The unionized carboxylic acid groups bind to the mucin molecule by means of hydrogen bridges (127). While hydrogen bonding is the primary force, interpenetration and physical entanglement of the polymer chains and mucin may also play an important role in the establishment of bioadhesive bonds. Gentamicin, when formulated in polycarbophil, showed increased (two times) uptake of gentamicin by the bulbar conjunctiva, compared with an aqueous control formulation (128). A polycarbophil bioadhesive vehicle with in situ gelling properties has been formulated for ocular fluorometholone and could be administered as a drop that would gel in the precorneal area (129,130). Hui and Robinson (130) tested the delivery of ^{14}C-labeled progesterone, in Noveon AA-IUSP polycarbophil-based gel, to the precorneal pockets of rabbits. Results showed a prolonged presence of the drug, and higher tissue concentrations vs. the control.

Chitosan

A polycationic biopolymer obtained by alkaline deacetylation of chitin is a bioadhesive vehicle suitable for ophthalmic formulations since it exhibits several favorable biological properties such as biodegradability (131), non-toxicity (132), and biocompatibility (133,134). Chitosan solutions show pseudoplastic and viscoelastic properties (135,136) and, as a consequence, have been quoted as a potential vehicle for the instillation of drugs to the eye. Formulations containing chitosan showed prolonged residence not only by increasing solution viscosity but also because of the mucoadhesive property. Lehr et al. (93) demonstrated that the mucoadhesive performance of chitosan was significantly higher in a neutral or slightly alkaline medium, e.g., tear fluid (pH range from 7.0 to 7.4) (137). A suspension of bioadhesive microspheres made of chitosan has been reported to be a promising means of topical administration of acyclovir to the eye (138). The precorneal residence time of chitosan formulations containing tobramycin was investigated by gamma scintigraphy and compared with the commercial solution of the drug. The presence of the polysaccharide significantly prolonged the corneal contact time regardless of the concentration and molecular weight of chitosan in solution (139).

Others

Recently, xanthane and carrageenan have been described as bioadhesive polysaccharides showing sustained-release properties and adequate ocular compatibility (140,141).

Increase in Bioavailability

A successful ophthalmic formulation should not only have a prolonged contact time with the ocular surface and a reduced elimination of the drug but

also an improved bioavailability, especially with respect to the drug release, as has been stressed from time to time in different studies. Copolymerization of the bioadhesive polymer PAA with pluronic, a thermally-induced, phase-separating graft polymer, has been reported to yield a bioadhesive vehicle with a prolonged residence time plus a prolonged drug-release period in contact with mucosal surfaces such as the eye (142). Newer polymeric gels, with a biphasic activity of accomplishing both ocular drug retention and prolonged release of drug from the vehicle, have also been reported (91). Liposomes have been well evaluated as an ocular drug delivery system, and their reduced drainage attributed to their high affinity for the conjunctival membrane (rather than the corneal epithelium) (143). One of the methods to provide liposomes as well as other particulate carriers with the necessary site adherence and site retention to achieve carrier and drug targeting in topical ocular therapy is to endow them with the ability to be mucoadhesive. The ability of hyaluronic acid to express mucoadhesion at neutral pH (108,144) indicates the potential of targeting with this natural polymer, when used in conjunction with a drug carrier. This has been among the considerations behind the selection of hyaluronic acid as one of the liposome surface-bound agents for bioadhesive liposomes (145,146). These combinatorial drug-delivery systems are a newer trend in ophthalmic research, with the great potential of combining the advantages of different systems and overcoming their limitations. For example, the use of polymer (carbopol 1342)-coated liposomes increased both the residence time and the bioavailability of the entrapped drug (147,148). However, recently Henriksen et al. (149) evaluated the possibility of using chitosan-coated liposomes containing ^{125}I-labeled BSA for topical ocular administration. Comparing the retention of simple liposomes and chitosan-coated liposomes after in vivo administration to anesthetized rats, they observed that retention of the radioactive marker was not significantly improved with chitosan-coated liposomes. Davies et al. (147) have also reported that mucoadhesive polymer (carbopol 934P and carbopol 1342)-coated vesicles failed to significantly increase the bioavailability of entrapped tropicamide compared with uncoated vesicles and aqueous solution, even though the precorneal retention was significantly improved over the non-coated vesicles. The addition of the bioadhesive polymer PAA as a coating for nanoparticles has been shown to prolong the residence time of pilocarpine in the rabbit (150,151). Le Bourlais et al. (125) developed a nanocapsule gel which combined the adhesive properties of the PAA polymer with encapsulated cyclosporin into nanocapsules. The combination of the particles with the bioadhesive polymer reinforced the nanosphere adhesion to the precorneal/conjunctival mucin layer and hence led to a prolongation of the residence time of the drug in the eye. Topical administration of gentamicin using a long-acting soluble bioadhesive ophthalmic drug insert (BODI) improved treatment due to a decrease in the number of applications, a decrease in insert expulsion, and an effective level of antibiotic in tears controlled by the device (152). Rod-shaped mucoadhesive ophthalmic inserts fitting the upper or lower conjunctival fornix using PAA or polymethacrylic acid have also been reported (153).

Although a very promising approach, a word of caution is required regarding the use of such combinations of delivery systems. These combinations increase the complexity of the formulations, as well as increasing the difficulty of understanding the mechanism of action of the drug-delivery systems.

Challenges and Future Perspectives

As explained throughout this article, several significant advances have already been made in ophthalmic mucoadhesive research, but many problems remain to be dealt with and many challenges await in this area. Of particular importance are: (i) the development of standard evaluation methods; (ii) the determination of the exact nature of interactions occurring at the tissue–mucoadhesive interface; and (iii) the development of ideal mucoadhesives for practical applications. The mucoadhesives presently available are non-specific for mucin, i.e., they bind to the other substrates also. Further, these polymers need to be biodegradable (e.g., starch, hyaluronic acid, or fibrin) for their proper elimination. If not biodegradable then either they should be removed by the natural mucin turnover, or another approach would be to cross-link these polymers with enzyme-digestible cross-linking agents (154). Another area which needs to be delved into is the knowledge of the structure of mucin in the cornea. Once these structures are established, computational chemistry can be applied to tailor-make new and better ophthalmic mucoadhesives. The ideal mucoadhesive should thus be biodegradable, non-toxic, durable for

a certain period of time, and if possible may itself function as an absorption enhancer. A multidisciplinary approach is however desirable and necessary to overcome these challenges in the field of ophthalmic bioadhesives.

CONCLUSION

Although very few ophthalmic formulations containing bioadhesives or penetration enhancers are commercially available in the market, research in this area has provided a new impetus and dynamism, as never before, for the development of modified or novel ophthalmic formulations, with the promise of new and exciting directions in the field of formulation technology. The use of bioadhesives considerably prolongs the corneal contact time, whereas the absorption promoters increase the rate and amount of drug transport. Combining the two approaches would theoretically assure an increase in the bioavailability. However, more exhaustive clinical studies need to be performed to provide further information and insight into these approaches. Further, the optimization of ophthalmic drug delivery may be achieved by the rational fusion of the already-existing physical and chemical techniques.

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