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International Journal of Pharmaceutics 269 (2004) 1–14
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Review

Vesicular systems in ocular drug delivery: an overview

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Received 2 August 2002; received in revised form 24 July 2003; accepted 1 September 2003

Abstract

The main aim of pharmacotherapeutics is the attainment of effective drug concentration at the intended site of action for a sufficient period of time to elicit a response. Poor bioavailability of drugs from ocular dosage form is mainly due to the tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium. Though the topical and localized application are still an acceptable and preferred way to achieve therapeutic level of drugs used to treat ocular disorders but the primitive ophthalmic solution, suspension, and ointment dosage form are no longer sufficient to combat various ocular diseases. This article reviews the constraints with conventional ocular therapy and explores various novel approaches, in general, to improve ocular bioavailability of the drugs, advantages of vesicular approach over these and the future challenges to render the vesicular system more effective.

Keywords: Niosomes; Liposomes; Vesicular drug delivery; Ophthalmics

1. Introduction

Drug delivery in ocular therapeutics is a challenging problem and is a subject of interest to scientists working in the multi-disciplinary areas pertaining to the eye, including chemical, biochemical, pharmaceutical, medical, clinical, and toxicological sciences. Recently, increased attention has been focussed on two main objectives:

(A) To find or tailor make newer, effective, and safe drug molecules for various ocular conditions and diseases that are poorly controlled.
(B) To improve existing ocular dosage forms and exploit newer delivery systems for improving the ocular bioavailability of existing molecules.

Current trends in ocular therapeutics and drug delivery suggest that the existing dosage forms will be replaced by novel drug delivery systems that offer improved biopharmaceutical properties with the capability to deliver therapeutic agents more precisely to targeted receptors in the eye in a predictable manner (Reddy and Ganesan, 1996).

Drugs are commonly applied to the eye for a localized action on the surface or in the interior of the eye (Davies, 2000). A major problem in ocular therapeutics is the attainment of an optimal drug concentration at the site of action. Poor bioavailability of drugs from ocular dosage form is mainly due to the precorneal loss factors which include tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane (Shell and Baker, 1974; Le Bourlais et al., 1998; Kaur and Kanwar, 2002). Due to these physiological and anatomical constraints, only a small fraction of the administered drug,

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doi:10.1016/j.ijpharm.2003.09.016

effectively 1% or even less of the instilled dose is ocularly absorbed (Shell, 1984; Burstein and Anderson, 1985). This forces the clinician to recommend a frequent dosing at an extremely high concentration and pulse type dosing results in several side effects of ophthalmic products. In order to overcome the problems of conventional ocular therapy, such as short residence time, drug drainage, and frequent instillation; newer delivery systems are being explored, in general, to improve the ocular bioavailability of the drug.

Various approaches, like viscosity enhancement, use of mucoadhesive, particulate drug delivery, vesicular drug delivery, prodrugs, and other controlled systems, like ocuserts, are being explored (Sirbat et al., 2000; Kaur and Kanwar, 2002; Kaur and Smitha, 2002; Bourlais et al., 1998). In this review, the constraints with conventional topical dosage forms, possible newer approaches, and the need to develop vesicular delivery systems, shall be discussed.

2. Conventional ocular drug delivery constraints

For the ailments of the eye, topical administration is usually preferred over systemic administration so as to avoid systemic toxicity, for rapid onset of action, and for decreasing the required dose.

Though topical administration offers many advantages to treat disorders of anterior structures of the eye, it suffers from a serious disadvantage of poor bioavailability due to several biological factors (Fig. 1), which exist to protect the eye and consequently limit the entry of ocular drugs. The constraints in topical delivery of the eye are discussed below.

2.1. Pre-ocular retention

It has been estimated that the human eye can hold approximately 30 μl of an ophthalmic solution without overflow or spillage at the outer angle (Mishima et al., 1966), while the volume delivered by most commercial ophthalmic eye drop dispensers is approximately 50 μl. Thus, a large proportion of the drug is wasted due to administration of an excess volume. Following the removal of the excess solution from the front of the eye, a second mechanism of clearance prevails. The eye has an efficient system for tear turnover (∼1 μl/min). The two mechanisms of clearance result in a biphasic profile for an instilled solution with a rapid initial clearance phase due to removal of excess fluid followed by a slower second phase due to tear turnover (Chrai et al., 1973; File and Patton, 1980).

2.2. Corneal absorption

The main route for intraocular absorption is across the cornea (Ahmed and Patton, 1987). Two features, which render the cornea an effective barrier to drug

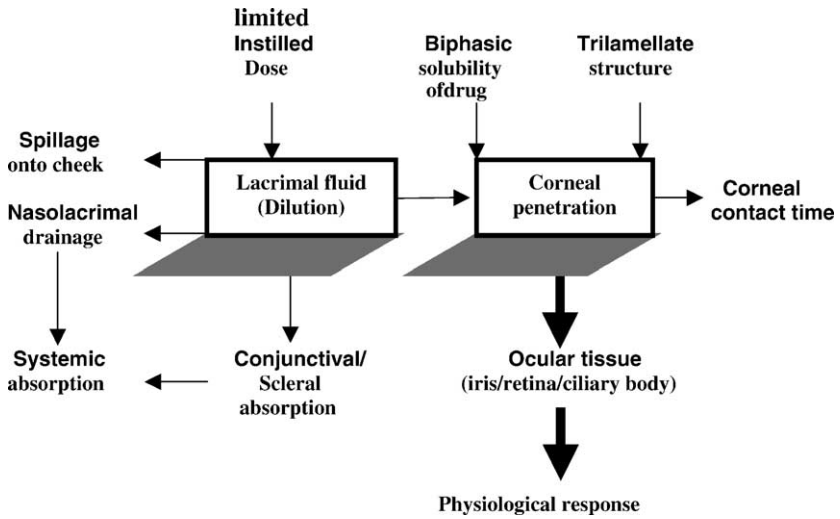

Fig. 1. Factors attributing to poor bioavailability of an ophthalmic formulation.

absorption, are its small surface area and its relative impermeability. In contrast, the area of conjunctiva, which is a vascular thin mucous membrane covering the inside of the eyelids and the anterior sclera, in humans is approximately 17-fold larger than the cornea. Moreover, it is also between 2 and 30 times more permeable to drugs than cornea (Watsky et al., 1988; Wang et al., 1991). Thus, following topical administration to the pre-ocular area, conjunctival drug absorption is an important loss factor that competes with corneal absorption (Lee and Robinson, 1979).

Secondly, in terms of drug delivery, the cornea can be considered to be comprised of three layers, which account for its poor permeability characteristics: (i) the outer epithelium, which is lipophilic in nature; (ii) the stroma, which constitutes approximately 90% of the thickness of cornea and is hydrophilic; and (iii) the inner endothelium consisting of a single layer of flattened epithelium-like cells. Since, the cornea has both hydrophilic and lipophilic structures, it presents an effective barrier to the absorption of both hydrophilic and lipophilic compounds.

Another serious route for the elimination of topically applied drugs from the precorneal area is the nasal cavity, with its larger surface area and a high permeability of the nasal mucosal membrane as compared to that of the cornea. The ocular drugs are prone to absorption into systemic circulation through the nasal mucosal lining, which is continuous with the conjunctival sac (Desai and Blanchard, 1994).

3. Formulation approaches to improve ocular bioavailability

Various approaches that have been attempted to increase the bioavailability and the duration of therapeutic action of ocular drugs can be divided into two categories. The first is based on use of the drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves, maximizing corneal drug absorption and minimizing precorneal drug loss.

The typical pulse entry type drug release behavior observed with ocular aqueous solutions (eye drops), suspensions, and ointments can be replaced by a more controlled, sustained, and continuous drug delivery, using a controlled release ocular drug delivery system. These systems can achieve therapeutic action with a smaller dose and a fewer systemic and ocular side effects. Such systems include implantable systems (Kunou et al., 1995), ocuserts (Saettone and Salimen, 1995), collagen shields (Poland and Kaufman, 1988; Friedberg et al., 1991), etc., but the limitations of these systems include poor patient compliance, need of surgery, and difficulty in self-insertion.

Other approaches include increased viscosity of vehicle, which is based on the fact that by increasing the contact time between the drug and the ocular surface, the bioavailability of the applied drug can be enhanced. Studies to date indicate, that this approach has only limited value, as the formulations are liquid and, therefore, subject to elimination from the eye by all the factors discussed earlier (Grass and Robinson, 1984).

Particulate drug delivery systems, like nanoparticles and microspheres, can also be used to improve the residence time of the drug (Kreuter, 1990). Upon administration to the eye, the particles reside at the delivery site and the drug is released from the particles through diffusion, chemical reaction, polymer degradation, or ion-exchange mechanism. Smaller particles are better tolerated by the patients than larger particles and hence microspheres and nanoparticles represent very comfortable prolonged action ophthalmic drug delivery systems. However, some workers observed that nanoparticles consisting of poly(alkyl cyanoacrylate) damaged the corneal epithelium by disrupting the cell membrane (Zimmer and Kreuter, 1991; Marchal-heussler et al., 1993; Calvo et al., 1994).

Capacity of some polymers to adhere to the mucin coat covering the conjuctiva and the corneal surfaces of the eye by a non-covalent bond (Hui and Robinson, 1985) has been exploited to provide an intimate contact between the drug and the absorbing tissue, which may result in high drug concentration in the local area and hence, drug flux through the absorbing tissue (Park and Robinson, 1984). Common disadvantage observed is that the adhesive often detaches itself from the rate controlling drug delivery device and causes premature release of drugs.

Increasing the permeability of the corneal epithelial membrane can maximize the transport characteristics across the cornea (Lee, 1993; Liaw and Robinson, 1993; Sasaki et al., 1995). Penetration enhancers or the absorption promoters can thus be used to increase

the permeability of cell membrane or loosen the tight junctions or both (Kaur and Smitha, 2002). Large numbers of enhancers, like actin filament inhibitors, surfactants, bile salts, chelators, and organic compounds, have been used. However, the unique characteristics and great sensitivity of the corneal conjuctival tissues impose great caution in the selection of enhancers with regard to consideration of their capacity to effect the integrity of the epithelial surfaces. There is evidence that penetration enhancers themselves can penetrate the eye and may, therefore, lead to unknown toxicological complications, e.g. benzalkonium chloride (BAC) was found to accumulate in the cornea for days (Green et al., 1987). EDTA was found to reach the iris–cilliary body in concentrations high enough to alter the permeability of the blood vessels in the uveal tract indirectly accelerating drug removal from aqueous humor (Grass and Robinson, 1984). Bile salts and surfactants were found to cause irritation of the eye and nasal mucosa (Green, 1993; Merkus et al., 1993).

- Even though various drug delivery systems mentioned above offer a numerous advantages over conventional drug therapy but still they are not devoid of pitfalls, including
- Poor patient compliance and difficulty of insertion as in ocular inserts,
- Tissue irritation and damage caused by penetration enhancers and collagen shields,
- Toxicity caused by insertion of foreign substances, like albumin and polybutylcyanoacrylate, as in case of nanoparticles and microspheres, and
- Change in pharmacokinetic and pharmacodynamics of the drug as caused by altering the chemical structure of the drug (prodrug approach).

In order to overcome these problems, the researchers (Gregoriadis and Florence, 1993; Le Bourlias et al., 1995; Saettone et al., 1996; El-Gazayerly and Hikal, 1997; Vyas et al., 1998) have come up with the concept of vesicular drug delivery systems as applied to corneal delivery. Vesicular delivery is a means of prolonged and controlled delivery. Drug enclosed in the lipid vesicles allows for an improved solubility and transport through the cornea (Table 1).

4. Vesicular drug delivery systems

Vesicular systems not only help in providing prolonged and controlled action at the corneal surface but also help in providing controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface. Moreover, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, but will localize and maintain drug activity at its site of action. The penetration of drug molecules into the eye from a topically applied preparation is a complex phenomenon. The rate of drug penetration depends not only on the physicochemical properties of the drug itself, such as its solubility (Hanna, 1980), and particle size, in case of suspensions (Schoenwald and Stewart, 1980) but

Table 1
Liposome-encapsulated drugs studied for ophthalmic administration

S. No.	Drug	Vesicular system	Result	Reference
1	Oligonucleotide	Liposomes	Better control of release rate	Bochot et al. (1998)
2	Acetazolamide	Liposomes	Produced a marked decrease in IOP	El-Gazayerly and Hikal (1997)
3	Pilocarpine HCl	Liposomes	Increased miotic response and ocular bioavailability of the drug	Khalil et al. (1992)
4	Cholramphenicol	Liposomes	Higher (twice) drug concentration obtained	De Laval et al. (1992)
5	Inulin	Liposomes	Increased ocular concentration of drug	Ahmed and Patton (1987)
6	Dehydrostreptomycin	Liposomes	No significant advantage	Singh and Mezei (1984)
7	Triamcinolone acetonide	Liposomes	Significantly higher concentration of drug in ocular tissues	Singh and Mezei (1983)
8	Iodoxuridine	Liposomes	Improved efficacy in treatment of Herpes simplex keratitis	Smolin et al. (1981)
9	Cyclopentolate	Niosomes	Promotes ocular absorption of the drug	Saettone et al. (1996)
10	Timolol maleate	Discomes	Entrapped comparatively higher amount of drug than niosomes	Vyas et al. (1998)

also on those of its vehicle (Kupferman et al., 1981). In vesicular dosage forms, the drug is encapsulated in lipid vesicles, which can cross cell membrane. Vesicles, therefore, can be viewed as drug carriers and as such they change the rate and extent of absorption as well as the disposition of the drug. Vesicular drug delivery systems used in ophthalmics broadly include liposomes and niosomes.

4.1. Liposomes

Liposomes are the microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 80 nm to 10 μm. Liposomes (also called phospholipid vesicles) were first described by Bangham et al. (1965). Such vesicles (Fig. 2) composed of one or more phospholipid bilayer membranes can entrap both hydrophilic and hydrophobic drugs, depending on the nature of the drug and hence, it is possible to apply water-insoluble drugs in liquid dosage form. According to their size, liposomes are known as either small unilamellar vesicles (SUV) (10–100 nm) or large unilamellar vesicles (LUV) (100–3000 nm). If more than one bilayers are present, then they are referred to as multilamellar vesicles (MLV).

Liposomes can act as carriers for a wide variety of drug molecules, proteins, nucleotides, and even plasmid endowing them with a great potential for their application in ophthalmics (Kurz and Ciulla, 2002). The potential of liposomes in topical ocular drug delivery was first exploited by Smolin et al. (1981), followed by Schaeffer and Krohn (1982) and Schaeffer et al. (1982). Liposomes offer advantages over most ophthalmic delivery systems in being completely biodegradable and relatively non-toxic. Another potential advantage of liposomes is their ability to come in an intimate contact with the corneal and conjunctival surfaces, thereby, increasing the probability of ocular drug absorption (Schaeffer et al., 1982; Dharma et al., 1986). This ability is especially desirable for drugs that are poorly absorbed, for example, the drugs with low partition coefficient, poor solubility or those with medium to high molecular weights (Megaw et al., 1981; Al-Muhammed et al., 1996), and enzymes, like cholinesterase (Shek and Barber, 1987). Liposomes were used by Dean et al. (1999) for nuclear targeting of plasmid DNA in human corneal cells. They found that a small sequence of DNA mediating nuclear localization of plasmids is active in cationic liposome transfected cells and leads to increased gene expression, thus improving efficiency of ocular gene transfer in vivo. Similarly, Bochot et al. (2000) used liposomes for intravitreal administration of oligonucleotides for treatment of ocular viral infections, like Herpes simplex virus or Cytomegalovirus (CMV). Antisense oligonucleotides are poorly stable in biological fluids and their intracellular penetration is limited and hence a system that is able to permit a protection of oligonucleotides against degradation and their slow delivery into the vitreous would be more favorable for improving patient compliance. Bochot et al. (2000, 2002) found the use of liposomes for intravitreal administration very promising since these lipid vesicles are able to protect oligonucleotides against degradation by nucleases. Further, they increase the retention time of many drugs in the vitreous. Liposomes can also be used as promising dosage forms for topical administration of immunosuppressive compounds for the treatment of ocular immune-mediated diseases (Pleyer et al., 1993). Pleyer et al. (1993) found that liposomes containing immunosuppressive compound (FK506) were effective in delivering significantly higher drug concentrations ($P < 0.05$) to all ocular tissues and particularly aqueous humor and vitreous humor as compared to the oil formulation of the agent. Further, liposomes can be use to protect drug molecules from the attack of metabolic enzymes present at the tear/corneal epithelium interface, e.g. liposomes have

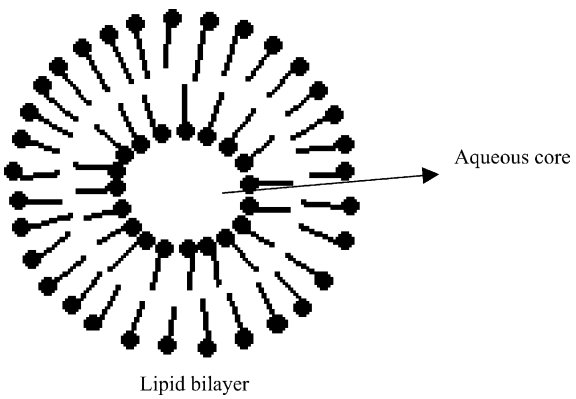

Fig. 2. Basic structure of vesicular systems.

been found to give better retention for lipophilic prodrugs in comparison to the parent drug molecule (Kawakami et al., 2001). They improved the retention time of tilisolol in the precorneal area or vitreous body, by preparing liposomes incorporating the *O*-palmitoyl prodrug of tilisolol. They found that at 24 and 48 h after intravitreal injection of *O*-palmitoyltilisolol liposomes, the tilisolol concentration in the vitreous body was significantly higher compared with the concentration after intravitreal injection of tilisolol liposomes.

The effectiveness of liposomes in ocular drug delivery depends on a number of factors, including drug encapsulation efficiency, size (Elorza et al., 1993), and charge of liposomes, distribution of a drug within liposomes, stability of liposomes in the conjunctival sac and ocular tissues, their retention in the conjunctival sac, and the affinity liposomes exhibit towards the corneal surface. Liposomes may be equated to colloidal particles, and are expected to be subject to the same clearance mechanisms, as other foreign bodies, that may come in contact with the ocular surface, and tend to be washed away by reflex tearing. Larger particles are more likely to be entrapped under the eyelids or in the inner can thus and so remain in contact with the corneal and conjunctival epithelia for extended periods. However, for patient comfort, it is considered that solid particles intended for ophthalmic use should not exceed 5–10 μm diameter (Burrow et al., 2002).

It has been reported (Ahmed and Patton, 1987) that the drug levels of inulin in cornea were higher when it was encapsulated in liposomes as compared to its aqueous solution. Liposomal encapsulation was shown to cause up to a 15-fold increase in inulin concentration in cornea and conjunctiva. Similar results were also obtained by Stratford et al. (1983a). This increased uptake of inulin by conjunctiva and cornea has been attributed to the physical adsorption of lipid vesicles onto the epithelial surface of the membrane (Stratford et al., 1983b; Lee et al., 1984).

The behavior of liposomes as an ocular drug delivery system has been observed to be, in part, due to their surface charge. Positively charged liposomes seem to be preferentially captured at the negatively charged corneal surface as compared with neutral or negatively charged liposomes. According to Felt et al. (1999a,b), cationic vehicles are expected to slow down drug elimination by the lacrymal flow both by increasing solution viscosity and by interacting with the negative charges of the mucus. The binding affinity of liposomes to the cornea suggests that the interaction is probably electrostatic in nature (Mezei and Gulasekharam, 1982; Schaeffer and Krohn, 1982; Singh and Mezei, 1984; Lee et al., 1985; Lee, 1993). A successful, topical ocular formulation of acetazolamide using liposomes as vehicle has been reported in the literature (El-Gazayerly and Hikal, 1997). Acetazolamide is an anti-glaucoma drug but due to its low solubility and permeability characteristics; it is administered orally resulting in numerous side effects. However, by using liposome approach these workers developed a topical formulation of acetazolamide, which produced a marked decrease in intraocular pressure. Furthermore, they evaluated neutral, positively charged and negatively charged liposomes for their entrapment efficiency and drug release behavior. The percent entrapment efficiency was 29.27, 41.06, and 49.58% for negatively, neutral, and positively charged liposomes, respectively. This behavior was accounted for by the fact that since acetazolamide is a weak acid it undergoes an electrostatic attraction with the positively charged stearylamine. The proportion of drug released after 9 h was 13.36, 33.8, and 26.7%, for negatively, neutral, and positively charged liposomes, respectively. This was explained on the basis that the charged lipids serve to tighten the molecular packaging of the vesicle bilayer resulting in the decreased drug release from charged liposomes compared to the neutral ones. Moreover, it was found that positively charged liposomes have a higher binding affinity to the corneal surface than neutral and negatively charged vesicles as a result of interaction of positively charged liposomes with the polyanionic corneal and conjunctival mucoglycoprotiens. Fresta et al. (1999) found that acyclovir was able to interact with both positively and negatively charged membranes via electrostatic or hydrogen bonds. They observed no interaction with neutral membranes made up of dipalmitoylphosphatidylcholine. Different liposome preparation procedures were carried out to encapsulate acyclovir. The workers reported that the drug encapsulation was mainly dependant on the amount of water, which the liposome system is able to entrap. In the case of MLVs, charged systems showed the highest encapsulation

efficiency. No particular difference in the encapsulation efficiency was observed for oligolamellar vesicles prepared with the reverse-phase evaporation technique. Oligolamellar liposomes showed the highest acyclovir encapsulation parameters and had release profiles similar to those of multilamellar liposomes. In vivo experiments using male New Zealand albino rabbits were carried out to evaluate the aqueous humor concentration of acyclovir and hence its ocular bioavailability. The most suitable ophthalmic drug delivery system was the oligolamellar system made up of dipalmitoylphosphatidylcholine–cholesterol–dimethyldioctadecylglycerol bromide (7:4:1 molar ratio), which presented the highest encapsulation capacity and was able to deliver greater amounts of the drug into the aqueous humor than a saline acyclovir solution or a physical liposome/drug blend. Schaeffer et al. (1982) worked on indoxole and penicillin G and reported that liposome uptake by the cornea is greatest for positively charged liposomes, less for negatively charged liposomes, and least for neutral liposomes, suggesting that the initial interaction between the corneal surface and liposomes is electrostatic adsorption. Positively charged unilamellar liposomes enhanced transcorneal flux of penicillin G across isolated rabbit cornea more than fourfold. The findings suggest that liposomes enhance corneal penetration of drug by being adsorbed onto the corneal surface, with direct transfer of drug from liposomal to epithelial cell membranes. Similar results were also obtained by McCalden and Levy (1990) and Law et al. (2000). By observing the morphology of corneal surface treated with liposomes, Law et al. (2000) suggested that positively charged liposomes formed a completely coated layer on the corneal surface. These liposomes bind intimately on the corneal surface leading to an increase of residence time, therefore, leading to an increase in the corneal absorption time. Further, it has been observed that the degree of liposome–cell interaction can be improved by increasing the degree of positive surface charge using stearylamine (Schwendener et al., 1984). Schaeffer and Krohn (1982) examined the corneal uptakes of ^{14}C-phosphotidylcholine from labeled liposomes and concluded that the degree of association of liposomes with the corneal surface decreased in the order $MLV^{+} > SUV^{+} > MLV^{-} > SUV^{-} > MLV, SUV$ (where the superscript indicates the charge carried by the vesicles), an observation which they attributed to the negative charge on the corneal epithelium at the physiological pH. In vivo clearance of radiolabeled liposomes formulation by gamma-scintigraphy was measured and it was reported that MLVs had a prolonged retention compared to SUVs of the same lipid composition. Controversial results were, however, reported by Lee and Carson (1986). They prepared positively charged, multilamellar liposomes of inulin and found that in spite of their affinity for ocular surfaces, these liposomes provided 5 and 100 times lower ocular concentrations of inulin at 30-min post-dosing than aqueous solutions and neutral liposomes, respectively, and failed to sustain inulin concentrations in any anterior segment tissues over 120 min. According to them, this negative effect of positively charged liposomes on the ocular bioavailability of inulin can be attributed to a twofold faster disappearance rate from the tear pool, which more than offsets their intrinsic ability to increase corneal drug permeability. Recently, Monem et al. (2000) prepared liposomes of pilocarpine hydrochloride and inferred that neutral MLVs displayed the most prolonged effect with respect to negatively charged MLVs and free drug. Using phase transition and light scattering technique, they found that the storage stability of pilocarpine hydrochloride liposomes was at least 15 months and hence suitable for commercial use.

It has been suggested that the delivery of aqueous drugs to cells can be improved using LUVs because of their greater internal volume. Similarly, Fitzgerald et al. (1987) concluded that positive surface charge was found to significantly affect the liposomal drainage rate whereas increase in size restricted drainage from the inner canthal region. As compared to the solutions, the liposomes were found to restrict solution drainage. Assessment of ocular irritability of neutral or positively charged liposomes by the Draize test, histological examination, and the rabbit blinking test has also been reported in the literature (Taniguchi et al., 1988). The mean total score (MTS) of the Draize test was found to show a slight increase immediately following instillation of liposome preparations. However, it did not exceed the “practically nonirritating level”, and the MTS rapidly became less than the “nonirritating level”. No corneal histological alteration was observed by optical microscopy following instillation of each liposome preparation.

Although the neutral liposome preparation failed to increase the rabbit blinking count, the positively charged liposome preparation did so to a significant degree. The neutral liposome preparation was confirmed to be nonirritating. However, the positively charged liposome preparation may cause an initial pain or unpleasantness following instillation. Thus, it can be concluded from all these reports that vesicle size and charge can be used to increase the liposome uptake by the cells. In general, positive charge helps in improving the contact time with the cornea but at same time it can lead to irritation and also release rate of the drug is found to be more in case of neutral liposomes. In addition, increased size also restricts solution drainage, thus prolonging contact time of the drug but it can be increased within the limits of not inducing any irritancy.

Other than charge and size, use of bioadhesive polymers (e.g. a polyacrylic acid, chitosan, hyaluronic acid) to prolongs the residence time of an ocular preparation in the precorneal region (Robinson, 1990; Zimmer et al., 1995; Kaur and Smitha, 2002; Baeyens et al., 2002) is another approach which can further improve liposomal drug delivery. Kaufman et al. (1994) developed a new drug delivery system, collasomes, liposomes coupled to collagen matrices. Collasomes immensely increased the bioadhesive ability of the liposomes, were well tolerated, and since the collagen particles are suspended in carrier vehicles, they could be instilled safely and effectively by patients in much the same fashion as ointments or drops. Yerushalmi and Margalit (1994) also demonstrated that liposomes, coated with collagen layer bound to cell monolayer with higher affinity. Few other approaches were used to increase the contact time of ocular liposomes, like Bochot et al. (1998) achieved prolonged retention of liposomal suspension of oligonucleotide by dispersing liposomes within a medium which would be able to form a gel in situ after administration. They used poloxamer 407, which is a copolymer of polyoxyethylene and polyoxypropylene and has a unique property of reversible thermal gelation. This allows instillation of fluid solution, which formed a semi solid gel at physiological temperature in the eye. They concluded that poloxamer gels presented an interesting system to control the release of a drug compared to a simple gel.

Schaeffer et al. (1982) investigated as yet another method to enhance the retention of drug bearing liposomes at the corneal surface under the conditions of tear flow. They incorporated mixed brain gangliosides into the membranes of phosphatidyl choline liposomes to provide receptor sites for wheat germ agglutinin, a plant lectin that binds strongly to both human and rabbit corneal epithelium. Ganglioside-containing liposomes showed a 2.5-fold increase in their binding to rabbit cornea in vitro when corneas were pretreated with wheat germ agglutinin (500 μg/ml), suggesting that the lectin mediates specific binding of these liposomes to the corneal surface. The data support the potential use of liposomes as a vehicle for topical drug flux enhancement. Nicholls et al. (1997) also used lectins as ligands to selectively bind particulates to the required area of the precornel region for extended periods. Similarly, immunoliposomes of antiviral drugs, like acyclovir and iododeoxyuridine, using monoclonal antibodies have also been reported (Norley et al., 1986, 1987). It was reported that these site-specific and sustained release immunoliposomes can act as improved vehicle for drug delivery in treatment of ocular Herpes simplex virus infection.

The successful application of liposomes as a topical ophthalmic drug delivery device requires knowledge of vesicle stability in the presence of tear fluid (Barber and Shek, 1986, 1990). The release of 5-carboxyfluorescein from large unilamellar liposomes in the presence of rabbit tear fluid was studied in vitro as a function of bilayer cholesterol content. Reverse evaporation vesicles were prepared from egg phosphatidylcholine, stearylamine, and varying amounts of cholesterol. Both the rate and the extent of fluorescent dye release were significantly increased in the presence of rabbit tear fluid at all cholesterol levels. However, by incorporating increasing amounts of cholesterol in the vesicle bilayers, tear-induced leakage was reduced. The release kinetics reported in this study are similar to those observed in the presence of human serum. While serum-induced leakage is attributed to high-density lipoprotein-mediated destabilization, reported differences in tear protein composition suggest some other, as yet unidentified, factors.

Despite the above discussed factors, which make liposomes a potentially useful system for ocular delivery they are not very popular because of their short shelf life, limited drug capacity, and problems in sterilization. The latter problems can be taken as a

challenge to establish liposomes as an effective means of ocular delivery.

4.2. Niosomes

Niosomes are the non-ionic surfactant vesicles and like liposomes are bilayered structures, which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane, made up of lipids (Carafa et al., 1998). Niosomes are widely studied as an inexpensive alternative of non-biological origin to liposomes or perhaps as carrier systems physically similar to liposomes, in vivo, with particular properties, which can be exploited to attain different drug distribution and release characteristics. They have all the advantages of liposomes but the low cost, greater stability, and resultant ease of storage has led to the exploitation of non-ionic surfactants (niosomes) as alternatives to phospholipids. Theoretically, niosome formulation requires presence of a particular class of amphiphile and an aqueous system. Cholesterol is added in order to prepare vesicles, which are less leaky. In addition, stabilizers may be included to prevent vesicle aggregation by repulsive, steric, or electrostatic effect.

Niosomes in topical ocular delivery are preferred over other vesicular systems because: (i) they are chemically stable as compared to liposomes; (ii) can entrap both lipophilic and hydrophilic drugs; (iii) have low toxicity because of their non-ionic nature; (iv) unlike phosholipids, handling of surfactants requires no special precautions and conditions; (v) they exhibit flexibility in their structural characterization, e.g. in their composition, fluidity, and size; (vi) can improve the performance of the drug via better availability and controlled delivery at a particular site; (vii) they are biodegradable, biocompatible, and non-immunogenic (Carafa et al., 2002).

The non-ionic surfactant vesicles have been reported successfully, as ocular vehicle for cyclopentolate (Saettone et al., 1996). These vesicles were obtained by sonication of equimolar mixture of polysorbate 20 and cholesterol. The formulation was buffered at two pH values (7.4 and 5.5). In the in vitro study, the pH 5.5 non-ionic surfactant vesicle formulation (independent of molar concentration of components) promoted transcorneal permeation of cyclopentolate with respect to a reference buffer solution while opposite effect was observed at pH 7.4. In the in vivo study, the niosomes, independent of their pH, significantly improved the ocular bioavailability of cyclopentolate, with respect to reference buffer solution. Based on this they concluded that non-ionic surfactant vesicles may promote absorption of cyclopentolate by preferentially modifying the permeability characteristics of the conjunctival and scleral membranes. No irritation with the niosomal formulation (as indicated by Draize scoring scale) was an additional advantage.

Vyas et al. (1998) reported that there was about 2.48 times increase in the ocular bioavailability of timolol maleate (a water-soluble drug) encapsulated in niosomes as compared to timolol maleate solution. Though this was not found to be the case with liposomes. Singh and Mezei (1984) stated that since water-soluble drugs (dihydrostreptomycin sulfate) produced a lower ocular concentration in liposomal form than in its solution form and hence these workers concluded that liposomes are favorable as carrier system only for hydrophobic drugs, further emphasizing the claim that niosomes are a suitable delivery system for both hydrophilic and lipophilic drugs. An increased ocular bioavailability of water-soluble drugs, entrapped in niosomes, may be due to the fact that surfactants (chief constituents of niosomes) also act as penetration enhancers as they can remove the mucus layer and break junctional complexes (Green and Downs, 1975; Keller et al., 1980; Burstein, 1984; Kaur and Smitha, 2002). Also, the irritation power of surfactants decreases in the following order: cationic > anionic > ampholytic > non-ionic (Van Abbe, 1973), so the non-ionic surfactants are preferred. The modified form of niosomes known as discomes is also used in ophthalmics. Discomes are large (12–60 μm) structures derived from niosomes on addition of the non-ionic surfactant, i.e. Solulan C24. Discomes are capable of entrapping water-soluble solutes. They have a special advantage in case of ocular drug delivery where their large size can prevent their drainage into the systemic pool as well as disc shape could provide for better fit in the cul-de-sac of the eye.

Vyas et al. (1998) prepared both niosomes and discomes of water-soluble drug timolol maleate and found that discomes entrapped comparatively a higher amount of drug (25% as compared to 14% in case of niosomes). Moreover, an increase in ocular bioavailability was found to be approximately 3.07-fold

compared to 2.48-fold in case of niosomes with respect to timolol maleate solution. Time corresponding to peak biological response was significantly delayed by about 5 h in case of discomes as compared to 3 h recorded for niosomes and 1.5 h for plain solution of timolol maleate. Hence, they concluded that discomes of timolol produce a better ocular hypotensive activity, both in terms of percentage reduction as well as duration of activity. They further stated that discomes can be a potential delivery system for controlled ocular administration of water-soluble drugs. Progressive incorporation of Solulan C24 into the vesicular dispersion leads to the partitioning of this soluble surfactant into the lipid bilayer till a critical level is reached with the net result that spherical structures are no longer favored and large flattened disc-like structures (discomes) are formed.

5. Further developments to render the vesicular systems more effective

Since, vesicular systems offer a great deal of advantages over the conventional systems, various pharmaceutical approaches can be tried to render their final formulation more effective. The best way to achieve this would be to enhance the precorneal retention. One such approach is combinatorial drug delivery. Combinatorial drug delivery systems are a new trend in ophthalmic research, with the great potential of combining the advantages of various systems and overcoming their limitations.

- Use of mucoadhesive polymers: one of the method to provide vesicles 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 indicates the potential of targeting with natural polymers when used in conjunction with drug carrier. It was found that hyaluronic acid modified liposomes when bound to topical model system (A431 cell line) are well retained at their sites even when vigorously and continuously flooded with fluid. It is also reported that liposomes coated with mucoadhesive polymer (carbopol 1342) showed no significant effect (Davies et al., 1993).
- Another approach can be to use either the viscosity increasing agent with vesicles and/or the use of penetration enhancers along with the vesicles in the formulation. Viscosity imparting agents prolongs the corneal contact time whereas penetration enhancers increase the rate and amount of drug transport. While using these agents it must be ensured that the stability of the system shall not be compromised and inclusion of such compounds shall not result in leaching.
- Grammer et al. (1996), used collagen corneal shields impregnated with liposomes and studied the effects of surface charge and bilayer fluidity of liposomes on their uptake and release by collagen corneal shields. They concluded that surface charge and bilayer fluidity were of minor importance for interaction with collagen corneal shields and since the release kinetics of a liposome-encapsulated hydrophilic or lipophilic substances are similar to release of non-encapsulated drug, they inferred that the combination of liposomes with collagen shield will be useful for drugs which do not penetrate the ocular surface as well as to prolong the corneal contact time of the liposomes.
- Another interesting approach can be entrapment of drug-cyclodextrin complex within vesicles. It has been found by some workers in routes other than eyes, that complexation of drugs with cyclodextrin can increase the entrapment of drug in non-ionic surfactants and hence improve activity. Additionally, this approach can act as a mean to control the duration of drug action in situ in case where dissociation constant of the complex can be tailored (Oommen et al., 1999; McCormack and Greogoriadis, 1994, 1998).

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 system.

6. Conclusion

Although eye drops represent 90% of all ophthalmic dosage forms, there is a significant effort directed

towards new drug delivery systems for ophthalmic administration. It is the consensus of most clinicians that the patient prefers a solution form of ocular drug delivery system provided that extended duration can be accomplished by these forms. Most of the formulation efforts aim at maximizing ocular drug absorption through prolongation of the drug residence time in the cornea and conjunctival sac as well as to slow drug release from the delivery system and minimize precorneal drug loss. The vesicular system fulfils all the requirements and in addition, it has the advantage of drug to be administered in the form of a drop, which increases patient compliance. In the vesicular systems, niosomes and discomes seem to be promising candidates for an ocular drug therapy though controlled clinical studies are necessary to provide more information regarding their long-term safety, stability, and effects on bioavailability. Further, it is of concern to check that the increased residence does not enhance the systemic concentration of the drug and hence its side effects. Newer concepts of exploiting the use of cyclodextrins in vesicular systems also need to be evaluated for ocular therapy. The authors feel an immense scope for developing suitable vesicular delivery systems for both hydrophilic and lipophilic drugs. However, indepth knowledge about the physicochemical characteristics of the drug molecule and expected interaction and implications of entrapping the same into a vesicular system is important.

Acknowledgements

The authors wish to acknowledge the financial support provided by Council of Scientific and Industrial Research (CSIR), New Delhi, India.

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