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ADVANCED APPROACHES AND EVALUATION OF OCULAR DRUG DELIVERY SYSTEM

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ABSTRACT

Eye is the most exclusive organ of the body and various drug delivery systems are used to deliver drug into eye but there are various limitations like rapid precorneal drug loss of conventional systems. Ocular disposition and elimination of a therapeutic agent is dependent upon physicochemical, microbiological, pharmaceutical properties and ophthalmic irritancy properties of ocular dosage forms as well as the relevant ocular anatomy and physiology. To improve ocular drug contact time, bioavailability and residence time, and to reduce the patient discomfort, frequency of dose, as well as to slow down the elimination of the drug there are significant efforts concentrating towards newer drug delivery systems for ophthalmic administration. This review focuses on the various new drug delivery systems applied in eye like inserts, in-situ gel, the newly developed particulate and vesicular systems like liposomes, pharmacosomes and discomes, niosomes, nanoparticles, iontophorosis, corneal shields, drug embedded contact lenses, ocular wafers etc and the most recent advanced approaches of the ocular delivery systems like the delivery of the genes and proteins to the internal structures which were used in treating the diseases caused due to genetic mutation, along with safety evaluation of ocular drug delivery formulations with some case studies.

Key words: Ophthalmic disorders, advanced ocular therapy, control drug delivery systems, vesicular systems, safety evaluation.

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INTRODUCTION

Ocular drug delivery has remained as one of the most demanding task for pharmaceutical scientists. The unique structure of the eye does not allow the drug molecules at the required site of action.[1] Conventional drug delivery systems; which include solutions, suspensions, gels, ointments and inserts, suffer with the problems or disadvantages as well as advantages are mentioned in table1.[2]It leads to development of advanced techniques for ocular therapy those include particulate delivery system which improves the pharmacokinetic and pharmacodynamic properties of various types of drug molecules.[3] and novel controlled drug delivery systems such as dendrimers, microemulsions, muco-adhesive polymers, hydrogels, iontophoresis, collagenshelid, prodrug approaches. Other advanced approaches for the treatment of macular degeneration include intravitreal small interfering RNA (siRNA) and inherited retinal degenerations involve gene therapy. This system provides many advantages over conventional systems as they increase the efficiency of drug delivery by improving the release profile and also reduces drug toxicity. The rapid progress of the biosciences opens new possibilities to meet the needs of the posterior segment treatments. The examples include the antisense and aptamer drugs for the treatment of cytomegalovirus (CMV) retinitis and age-related macular degeneration, respectively, and the monoclonal antibodies for the treatment of the age-related macular degeneration. These reviews focus briefly on different drug delivery systems for ocular therapy along with their safety evaluation of ocular drug delivery formulations case studies.

OPHTHALMIC DISORDERS

According to location of diseases, ocular disorders are grouped as[4]

1. Periocular
2. Intraocular.

1. Periocular disorders:

a) Blepharitis: An infection of lid structures (usually by staphylococcus aureus) with concomitant seborrhoea, rosacea, a dry eye and abnormalities in lipid secretions

b) Conjunctivitis: The condition in which redness of eye and presence of a foreign body sensation are evident. There are many causes of conjunctivitis but the great majority are the result of acute infection or allergy.

c) Kertitis: The condition in which patient have a decreased vision ,ocular pain, red eye, and often a cloud / opaque cornea .It is mainly caused by bacteria ,viruses, fungi etc.

d) Trachoma: This is caused by the organism chalmydia trachoma is; it is the most common cause of blindness in North Africa and Middle East.

2. Intraocular disorders: These conditions are difficult to manage and include intraocular infections: i.e. infections in the inner eye, including the aqueous humour, iris, vitreous humour and retina.

Glaucoma [5-9]

Glaucoma is a group of disease of the eye characterized by damage to the ganglion cells and the optic nerve. If left untreated, these effects may lead to various degrees of loss of vision and blindness. Increased intraocular pressure (IOP) remains the most important risk factor for the development of glaucoma.

Types of Glaucoma: Glaucoma may be classified in a variety of ways, which describe causative factors, when known. Based upon the mechanism of obstruction of outflow of aqueous humor:

a. Glaucoma angle closure: Usually a more acute form of disease and is seen in 5 to 10% of all patients.

b. Open angle glaucoma: Occurs in 80% to 90% of cases .It can be further described as either high tension or normal tension (also known as low tension) glaucoma

c. Congenital glaucoma: This results from developmental ocular abnormalities and occurs in less than 2% of patients. Finally, glaucoma may be secondary to other ocular disorders, systemic disorders, or trauma, or may be seen with medication usage, or after intraocular surgery.

ROUTES OF DRUG ADMINISTRATION AND ELIMINATION FROM THE EYE[10]

Schematic presentation of the ocular structure with the routes of drug kinetics was illustrated in Figure.1. Disadvantages and complications associated with ocular drug delivery represented in Figure.2.

The numbers refer to following processes: 1) transcorneal permeation from the lacrimal fluid into the anterior chamber, 2) non-corneal drug permeation across the conjunctiva and sclera into the anterior uvea, 3) drug distribution from the blood stream via blood-aqueous barrier into the anterior chamber, 4) elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and Sclemm's canal, 5) drug elimination from the aqueous humor into the systemic circulation across the blood-aqueous barrier, 6) drug distribution from the blood into the posterior eye across the blood-retina barrier, 7) intravitreal drug

administration, 8) drug elimination from the vitreous via posterior route across the blood-retina barrier, and 9) drug elimination from the vitreous via anterior route to the posterior chamber.

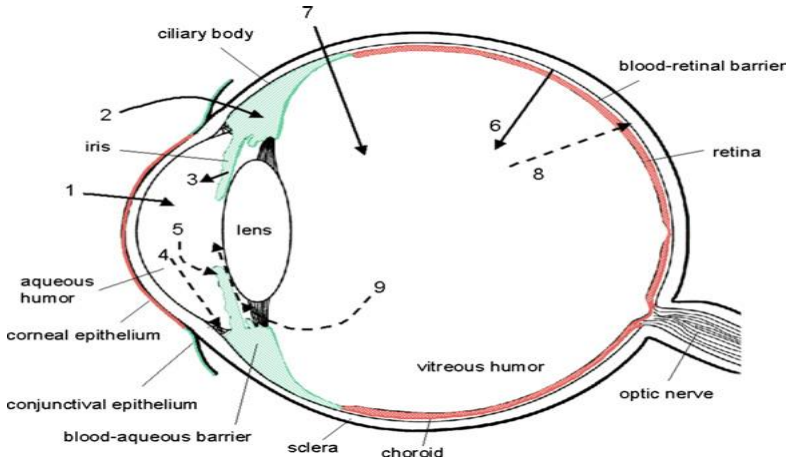

Figure. 1. Schematic presentation of the ocular structure with the routes of drug kinetics illustrated.

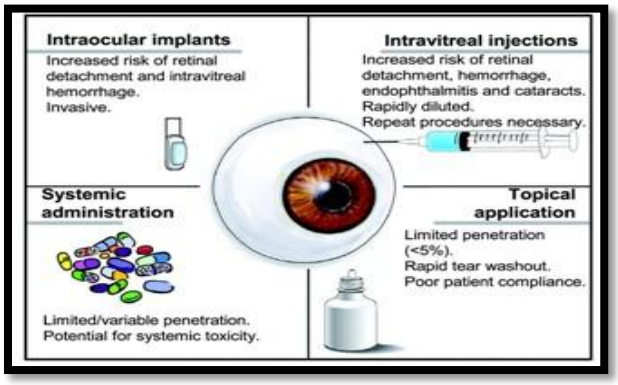

Figure.2. Disadvantages and complications associated with ocular drug delivery.[11]

BARRIERS TO OCULAR DRUG DELIVERY[12]

1.Cornea as a barrier

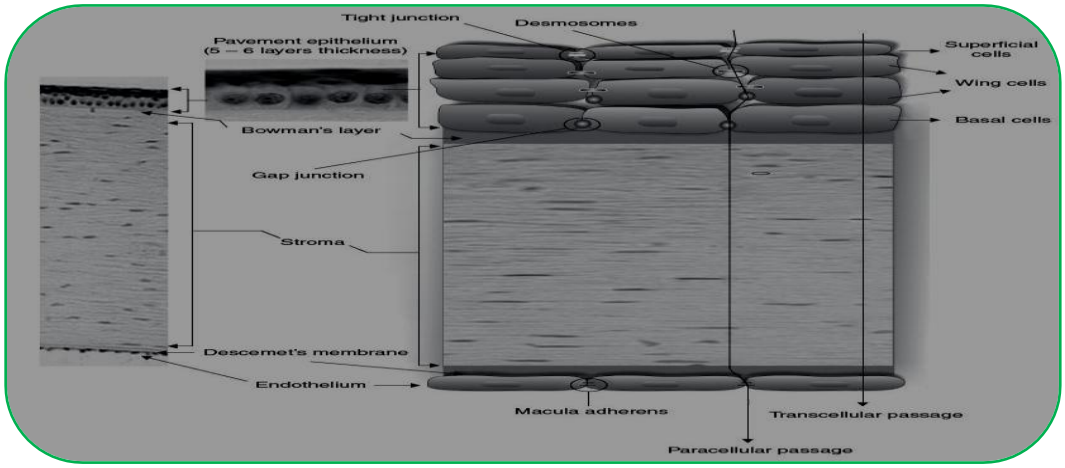

Figure3:Topical administration to retina[13]

2.Sclera as a barrier: Static barriers

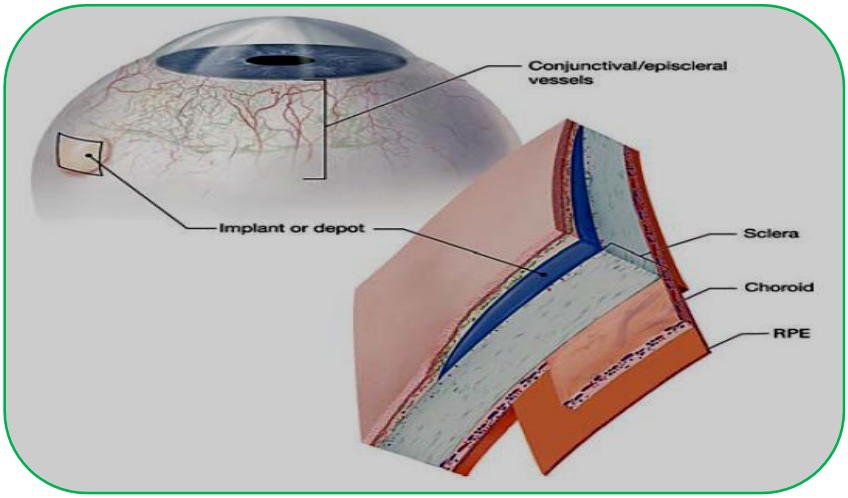

Figure 4:Transcleraldelivery[14]

3.RPE as a barrier: It includes

a) Blood Retinal Barrier (OBRB):

Formed by the retinal pigment epithelium (RPE) to limit transport of molecules from choroidalcirculation into the retina. Tight junctions are expressed between the RPE cells.

b) Inner blood retinal barrier (iBRB)

DIFFERENT DRUG DELIVERY SYSTEMS FOR OCULAR DISORDER TREATMENT

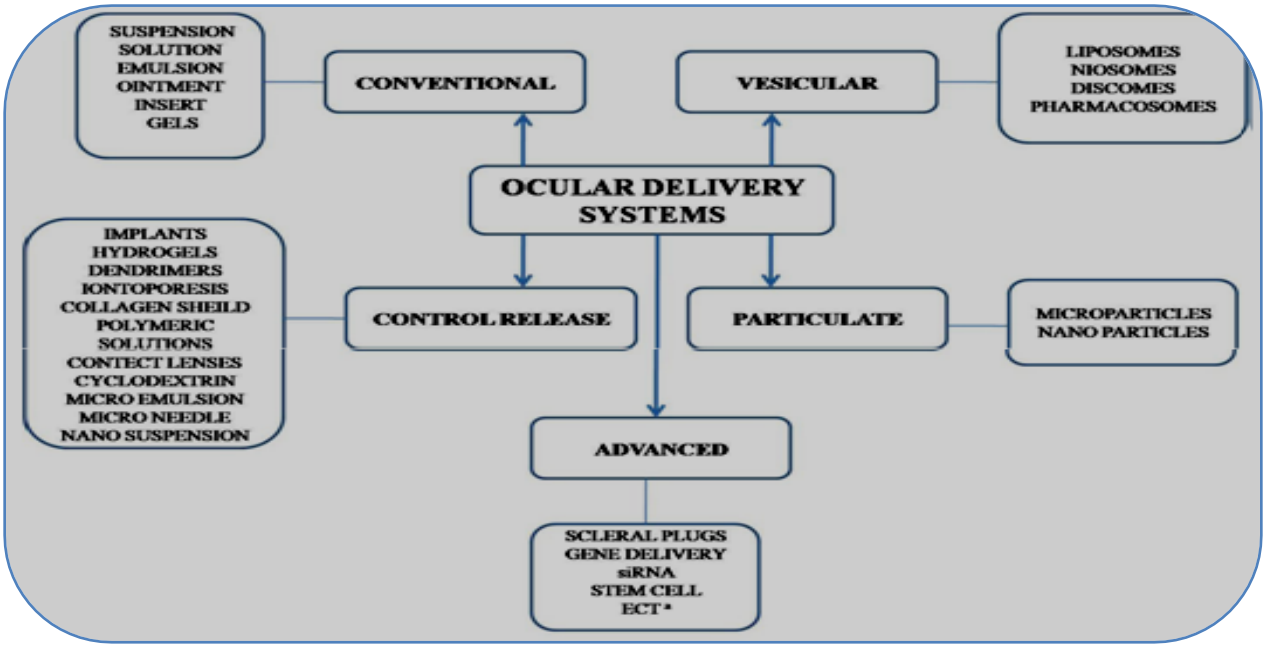

Figure5: Different drug delivery systems for ocular therapy[15]

A .CONVENTIONAL DELIVERY SYSTEMS:

Solutions, Suspensions, Emulsions: Various properties of eye drops like hydrogen ion concentration, osmolality, viscosity and instilled volume can influence retention of a solution in the eye .[16] Less than 5 Percent of the dose is absorbed after topical administration into the eye. Ocular absorption is limited by theocorneal epithelium, and it is only moderately increased by prolonged ocular contact.

Sprays: Although not commonly used, some practitioners use mydriatics or cycloplegics alone or in combination in the form of eye spray. These sprays are used in the eye for dilating the pupil or for cycloplegic examination.

Ointment and Gels: Prolongation of drug contact time with the external ocular surface can be achieved using ophthalmic ointment vehicle but, the major drawback of this dosage form like, blurring of vision and matting of eyelids can limits its use.

Inserts[17, 18]

a Lacriserts: The lacrisert is a sterile rod shaped device made of hydroxylpropyl cellulose without any preservative is used for the treatment of dry eye syndrome. This device was introduced by Merck, Sharp and Dohme in 1981.It weighs 5 mg and measures 12.7 mm in diameter with a length of 3.5mm.Lacrisert is useful in patients with keratitis sicca whose symptoms are difficult to treat with artificial tear alone. It is inserted in to inferior fornix where it imbibes water from the conjunctiva and cornea.

b SODI/ Wafers:Soluble ocular drug insert (SODI) is a small oval wafer which was developed by Soviet scientists for cosmonauts who could not use eye drops in weightless conditions. The unit is made from acrylamide N-vinylpyrrolidine and ethylacrylate designed as ABE. It is in the form of sterile thin films of oval shape weighing 15 to 16 mg. After introduction into cul de sacs where wetted by tear film it softens in 10-15 seconds and assumes the curved configuration of the globe. During the following 10-15 min; the film turns into viscous polymer mass thereafter in 30-60 min it becomes a polymer solution. The advantages and disadvantages are of above all conventional dosage forms are mentioned in Table 1.

Table.1Advantages and disadvantages of conventional dosage forms[2]

DOSAGE FORMS	ADVANTAGES	DISADVANTAGES
1.Solutions	Convenience, rapid pre-corneal elimination	Loss of drug by drainage, no sustained action
2.Suspensions	Patient compliance. Best for drugs with slow dissolution.	Loss of both solution and suspended solid
3.Emulsions	Prolonged release of drug from vehicle, enhanced pulsed entry.	Patient non compliance, blurred vision, possible oil entrapment
4.Gels	Comfortable, less blurred vision than ointment., flexibility in drug choice. Improved drug	No rate control on diffusion, matted eyelids after use
5.Ointment	stability Increased tissue contact time, inhibition of dilution by tears, resistance to naso-lachrymal drainage	Sticking of eyelids, blurred vision, no true sustained effect. drug choice limited by partition co-efficient.
6.Erodible inserts	Sophisticated and effective delivery system., flexibility in drug type and dissolution rate need only be introduced into eye and not removed	Patient discomfort, requires patient insertion, movement of system around eye can cause abrasion
7.Non-erodible inserts	Controlled rate of release Prolonged delivery	Patient discomfort „h Irritation to eye Patient placement and removal

B.VESICULAR SYSTEM:

Liposomes:

Liposomes are biocompatible and biodegradable lipid vesicles made up of natural lipids and about 25–10 000 nm in diameter. They are having an intimate contact with the corneal and conjunctival surfaces which is desirable for drugs that are poorly absorbed, the drugs with low partition coefficient, poor solubility or those with medium to high molecular weights and thus increases the probability of ocular drug absorption.[19] To the corneal epithelium which is thinly coated with negatively charged mucin, positively charged surface of the liposomes may combine.

Limitations:

The major limitations of liposomes are chemical instability, oxidative degradation of phospholipids, cost and purity of natural phospholipids.

Niosomes and Discomes:

Niosomes are chemically stable as compared to liposomes and can entrap both hydrophobic and hydrophilic drugs. They are non toxic and do not require special handling techniques. Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic or amphiphilic drugs. Vyas and co workers reported that there was about 2.49 times increase in the

ocular bioavailability of timolol maleate encapsulated in niosome as compared to timolol maleate solution.[20] Non-ionic surface active agents based discoidal vesicles known as (discomes) loaded with timolol maleate were formulated and characterized for their in vivo parameters. In vivo studies showed that discomes released the contents in a biphasic profile if the drug was loaded using a pH gradient technique. Discomes may act as potential drug delivery carriers as they released drug in a sustained manner at the ocular site.[21]

Pharmacosomes:

This term is used for pure drug vesicles formed by the amphiphilic drugs. Any drug possessing a free carboxyl group or an active hydrogen atom can be esterified (with or without a spacer group) to the hydroxyl group of a lipid molecule, thus generating an amphiphilic prodrug. The amphiphilic prodrug is converted to pharmacosomes on dilution with water. The pharmacosomes show greater shelf stability, facilitated transport across the cornea, and a controlled release profile.[22]

C.CONTROL DELIVERY SYSTEMS:

Mechanism of controlled sustained drug release into the eye: [23-30]

1. The corneal absorption represents the major mechanism of absorption for the most conventional ocular therapeutic entities.

2. Passive Diffusion is the major mechanism of absorption for nor-erodible ocular insert with dispersed drug.

3. Controlled release can further regulated by gradual dissolution of solid dispersed drug within this matrix as a result of inward diffusion of aqueous solution.

Implants:

For chronic ocular diseases like cytomegalovirus (CMV) retinitis, implants are effective drug delivery system. Earlier non biodegradable polymers were used but they needed surgical procedures for insertion and removal. Presently biodegradable polymers such as Poly Lactic Acid (PLA) are safe and effective to deliver drugs in the vitreous cavity and show no toxic signs.[31]

Iontophoresis:

In Iontophoresis direct current drives ions into cells or tissues. Positively charged of drug are driven into the tissues at the anode and vice versa. Ocular Iontophoresis delivery is not only fast, painless and safe but it can also deliver high concentration of the drug to a specific site. Iontophoretic application of antibiotics in eye not only increases their bactericidal activity but

also reduce the severity of disease. Similarly application of anti-inflammatory agents can reduce vision threatening side effects.[32-33]

Dendrimers:

Dendrimers are successfully used for different routes of drug administration and have better water-solubility, bioavailability and biocompatibility. Vandamme and co workers have developed and evaluated poly (amidoamine) dendrimers containing fluorescein for controlled ocular drug delivery. They determined the influence of size, molecular weight and number of amine, carboxylate and hydroxyl surface groups in several series of dendrimers. The residence time was longer for the solutions containing dendrimers with carboxylic and hydroxyl surface groups.[34]

Cyclodextrins:

Cyclodextrins (CDs) are cyclic oligosaccharides capable of forming inclusion complexes with many guest molecules. This complexation of CD does not interrupt the biological membrane compared to conventional permeation enhancer like benzalkonium chloride. Due to inclusion, the free drug is not available, so drugs with inherent irritant properties can be successfully delivered by this approach. CD molecules are inert in nature and were found to be non irritant to the human and animal eye.[35]

Contact lenses:

For prolongation of ocular residence time of the drugs, hydrophilic contact lenses can be used. Greater penetration of fluorescein has been reported by Bionite lens made from hydrophilic polymer (2-hydroxy ethyl methacrylate) in human.[36]

Collagen Shield:

Collagen shield basically consist of cross linked collagen, fabricated with foetal calf skin tissue and developed as a corneal bandage to promote wound healing. Topically applied antibiotic conjugated with the shield is used to promote healing of corneal ulcers. Tear fluid makes these devices soft and form a thin pliable film which is having dissolution rate up to 10, 24 or 72 hours. Because of its structural stability, good biocompatibility and biological inertness, collagen film proved as a potential carrier for ophthalmic drug delivery system.[37]

Microemulsion:

Microemulsion is dispersion of water and oil stabilized using surfactant and cosurfactant to reduce interfacial tension and usually characterized by small droplet size (100 nm), higher thermodynamic stability and clear appearance.Optimization of these components results in

significant improvement in solubility of the drug molecule e.g. indomethacin, chloramphenicol for eye diseases.[38]

Nanosuspensions:

Nanosuspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because they enhanced not only the rate and extent of ophthalmic drug absorption but also the intensity of drug action with significant extended duration of drug effect. For commercial preparation of nanosuspensions, techniques like media milling and high pressure homogenization have been used.[39]

Microneedles:

Microneedles are developed to deliver drug to posterior segment. Microneedle had shown prominent in vitro penetration into sclera and rapid dissolution of coating solution after insertion while in vivo drug level was found to be significantly higher than the level observed following topical drug administration like pilocarpine.[40]

Prodrugs:

The ideal prodrugs for ocular therapy not only have increased lipophilicity and a high partition coefficient, but it must also have high enzyme susceptibility to such an extent that after corneal penetration or within the cornea they are either chemically or enzymatically metabolized to the active parent compound.

Penetration Enhancers:

Penetration enhancers increases the permeability through corneal epithelial membranes and finally increases transport of drug across the cornea. Examples of enhancers include actin filament inhibitors, surfactants, bile salts, chelators, and organic compounds. But penetration enhancers themselves can penetrate the eye and may lead to unknown toxicological complications e.g., benzalkonium chloride (BAC) was found to accumulate in the cornea for days.[41]

Mucoadhesive Polymers:

They are basically macromolecular hydrocolloids with plentiful hydrophilic functional groups, such as hydroxyl, carboxyl, amide and sulphate having capability for establishing electrostatic interactions.[42]A mucoadhesive drug formulation for the treatment of glaucoma was developed using a highly potent beta blocker drug, levobetaxolol (LB) hydrochloride and partially neutralized poly acrylic acid (PAA).

Phase Transition Systems/In-situ gel system:

Phase transition of the formulation from the liquid form to the gel or solid phase occurs when these systems instilled into the cul-de-sac of eye lead to increase the viscosity of a drug formulation in the precorneal region results in increased bioavailability, due to slower drainage from the cornea. These systems can be influenced by pH, temperature or by ion activation. A sol to gel system with mucoadhesive property to deliver the steroid fluorometholone to the eye was prepared by Middleton and Robinson.[43]

D.PARTICULATE DRUG DELIVERY SYSTEM

Nanoparticles

Nanoparticles are prepared using bioadhesive polymers to provide sustained effect to the entrapped drugs. An optimal corneal penetration of the encapsulated drug was reported in presence of bioadhesive polymer chitosan. Similarly Poly butyl cyanoacrylate nanoparticles, containing pilocarpine into collagen shields, showed greater retention and activity characteristics with respect to the controls.

Microparticles Microspheres of poly lacto gylcolic acid (PLGA) for topical ocular delivery of a peptide drug vancomycin were prepared by an emulsification/ spray-drying technique.[44]

E. ADVANCED DRUG DELIVERY SYSTEM

Scleral Plug therapy:

Scleral plug can be implanted using a simple procedure at the pars plana region of eye, made of biodegradable polymers and drugs, and it gradually releases effective doses of drugs for several months upon biodegradation. The plugs are effective for treating vitreoretinal diseases such as proliferative vitreoretinopathy, cytomegalovirus retinitis responds to repeated intravitreal injections and for vitreoretinal disorders that require vitrectomy.[45]

Gene Therapy:

Gene therapy approaches are used to treat blindness arising from corneal diseases, cataract as the leading cause of vision loss. Several kinds of viruses including adenovirus, retrovirus, adeno-associated virus, and herpes simplex 140 virus, have been manipulated for use in gene transfer and gene therapy applications.[46]Topical delivery to the eye is the most expedient way of ocular gene delivery. However, the challenge of obtaining substantial gene expression following topical administration has led to the prevalence of invasive ocular administration. Retroviral vectors have been widely used due to their high efficacy; however, they do not have the ability to transduce nondividing cells, leads to restrict their clinical use. The advanced delivery systems

that prolong the contact time of the vector with the surface of the eye may enhance transgene expression, thereby facilitate non-invasive administration.[47]

siRNA therapy:

Feasibility of using siRNA for treatment of choroidal neovascularisation has been demonstrated using siRNA directed against vascular endothelial growth factor (VEGF) or VEGF receptor 1 (VEGFR1), and both of these approaches are being tested in clinical trials. Topical delivery of siRNAs directed against VEGF or its receptors has also been shown to suppress corneal neovascularisation. siRNA has become a valuable tool to explore the potential role of various genes in ocular disease processes. It appears that siRNAs may be valuable in the pathogenesis and development of new treatments for several ocular diseases, based on in vivo and in vitro studies.[48] However, its use in vivo remains problematic, largely due to unresolved difficulties in targeting delivery of the siRNA to the tumor cells. Viral gene delivery is very efficient however it currently lacks adequate selectivity for the target cell type. New encapsulated siRNA have been developed using liposomes, coupled-antibodies or others polymer vesicles. Therapeutic approach using siRNA provides a major new class of drugs that will shed light the gap in modern medicine.

Stem cell Therapy:

The most successful ocular application has been the use of limbal stem cells, transplanted from a source other than the patient for the renewal of corneal epithelium. The sources of limbal cells include donors, autografts, cadaver eyes, and (recently) cells grown in culture. Stem-cell Therapy has demonstrated great success for certain maladies of the anterior segment.[49]

Cell Encapsulation:

The entrapment of immunologically isolated cells with hollow fibres or microcapsules before their administration into the eye is called Encapsulated Cell Technology (ECT) which enables the controlled, continuous, and long-term delivery of therapeutic proteins directly to the posterior regions of the eye. The polymer implant containing genetically modified human RPE cells secretes ciliary neurotrophic factor into the vitreous humour of the patients' eyes. ECT can potentially serve as a delivery system for chronic ophthalmic diseases like neuroprotection in glaucoma, anti-angiogenesis in choroidal neovascularisation, anti-inflammatory factors for uveitis.[50]

Protein and Peptide therapy:

The intravitreous injection of ranibizumab is an example for the delivery of therapeutic proteins/ peptides. For designing of optimized methods for the sustained delivery of proteins and to predict the clinical effects of new compounds to be administered in the eye, the basic knowledge of Protein and Peptide is required. However, several limitations such as membrane permeability, large size, metabolism and solubility restrict their efficient delivery. Poor membrane permeability of hydrophilic peptides may be improved by structurally modifying the compound, thus increasing their membrane permeability. Ocular route is not preferred route for systemic delivery of such large molecules. Immunoglobulin G has been effectively delivered to retina by trans scleral route with insignificant systemic absorption.[51]

Oligonucliotide therapy:

Oligonucleotide (ON) therapy is based on the principle of blocking the synthesis of cellular proteins by interfering with either the transcription of DNA to mRNA or the translation of mRNA to proteins. Among several mechanisms by which antisense molecules disrupt gene expression and inhibit protein synthesis, the ribonuclease H mechanism is the most important. A number of factors have been determined to contribute to the efficacy of antisense ON. One primary consideration is the length of the ON species. Lengths of 17– 25 bases have been shown to be optimal, as longer ONs have the potential to partially hybridize with nontarget RNA species. Biological stability is the major barrier to consider when delivering both DNA and RNA oligonucleotides to cells. Protection from nuclease action has been achieved by modification of phosphate backbones, sugar moiety, and bases.[52]

Aptamer:

Aptamers are oligonucleotide ligands that are used for high-affinity binding to molecular targets. They are isolated from complex libraries of synthetic nucleic acid by an iterative process of adsorption, recovery, and reamplification. They bind with the target molecules at a very low level with high specificity. Pegaptanib sodium (Macugen; Eyetech Pharmaceuticals/Pfizer) is an RNA aptamer directed against VEGFb165, where VEGF isoform primarily responsible for pathological ocular neovascularisation and vascular permeability.[53]

Ribozyme therapy:

RNA enzymes or ribozymes are a relatively new class of single-stranded RNA molecules capable of assuming three dimensional conformations and exhibiting catalytic activity that induces site-specific cleavage, ligation, and polymerization of nucleotides involving RNA or DNA. They function by binding to the target RNA moiety through Watson-Crick base pairing

and inactivate it by cleaving the phosphodiester backbone at a specific cutting site. A disease named, Autosomal dominated retinitis pigmentosa (ADRP) is caused by mutations in genes that produce mutated proteins, leading to the apoptotic death of photoreceptor cells. Lewin and Hauswirth have worked on in the delivery of ribozymes in ADRP in rats shows promise for ribozyme therapy in many other autosomal dominant eye diseases, including glaucoma.[52]

EVALUATION OF OCULAR DRUG DELIVERY SYSTEM[53]

A. *In-Vitro* evaluation methods

Bottle method

In this method, dosage forms are placed in the culture bottles containing phosphate buffer at pH 7.4. The culture bottles are shaken in a thermostatic water bath at 37°C. A sample of medium is taken out at appropriate intervals and analyzed for drug contents.

Diffusion method

An appropriate simulator apparatus is used in this method. Drug solution is placed in the donor compartment and buffer medium is placed in the receptor compartment. An artificial membrane or goat cornea is placed in between donor and receptor compartment. Drug diffused in receptor compartment is measured at various time intervals.

Modified rotating basket method

In this method, dosage form is placed in a basket assembly connected to a stirrer. The assembly is lowered into a jacketed beaker containing buffer medium. The temperature of system is maintained at 37°C. A sample of medium is taken out at appropriate time intervals and analyzed for drug content.

Modified rotating paddle apparatus

In this method, diffusion cells (those that are used for analysis of semi-solid formulations) are placed in the flask of rotating paddle apparatus. The buffer medium is placed in the flask and paddle is rotated at 50 rpm. The entire unit is maintained at 37$\pm$0.5° C. Aliquots of samples are removed at appropriate time intervals and analyzed for drug content.

Flow through devices

There are obvious and insurmountable limitations to the official dissolution testing apparatus concerning maintenance of sink condition for drugs that saturate rapidly in large volumes of medium. The in- homo genicity of the solution in the rotating basket and poor reproducibility led to enhanced use of flow through devices. A constant fluid circulation apparatus is used as a flow through device. The apparatus consist of a glass dissolution cell, a continuous duty oscillating

pump, a water bath and a reservoir. The dosage form is placed in the reservoir of the dissolution medium. The whole assembly is maintained at the temperature of 37°C. The dissolution medium is circulated through the apparatus. Sampling of medium is done at various time intervals and analyzed for drug content.

B. *In-vivo* methods

- The drug delivery systems can be evaluated for its pharmacokinetic and pharmacodynamic profiles.
- The main objective of the pharmacokinetic studies is to determine the drug release from the dosage form to the eye.
- Rabbit is used as an experimental animal because of a number of anatomical and physiological ocular similarities and also due to larger size of the eye.
- Pharmacokinetic studies are performed by measuring drug concentration in various eye tissues eg. lens, cornea, iris, ciliary body, retina, sclera, aqueous and vitreous humour in rabbits.

The intraocular pressure of the eye is measured with a tonometer

- Ocular pharmacokinetic studies can also be carried out by tear fluid sampling, which is a non-invasive technique.
- Usually, disposable glass capillaries of 1ml capacity are used for sampling. The samples are collected from the marginal tear strip of the rabbits.
- Extreme care must be taken to avoid any corneal contact and possible induced lacrimation.
- To withdraw aqueous humour, rabbits are anaesthetized with ketamine and aqueous humour about 200ml is withdrawn from the anterior chamber using 1ml syringe with 26 gauge needle.
- Vitreous samples are also obtained with 20 gauge needle. The entire cornea, lens, and iris-ciliary body are also removed and analyzed for the drug content

C. Safety Evaluation of Ocular Drug Delivery Formulations: Case Studies[11]

Injectable Therapies

Most injectable therapies are used intravitreally, and there is a plethora of literature on the safety and pharmacokinetics of these formulations by this route, mostly conducted in rabbits. These intravitreal biologics produce ocular inflammation in animals at high doses, as detected by ophthalmoscopic and/or microscopic examination. There is often no prediction of which species

are more sensitive to ocular inflammation induced by high doses of intravitreal biologics; rabbits are usually more sensitive to oligonucleotides than other species (Doug Kornbrust, personal communication, Preclinsight, Reno, NV). Both intravitreal fomiversen and pegaptanib cause ocular inflammation at lower doses in rabbits compared to monkeys and/or dogs. This may be because of the smaller vitreal volume, decreased retinal vascularity, and/or decreased aqueous humor outflow from the uveoscleral pathway in rabbits compared to larger species, which increase drug half-life. Toll-like receptors (TLRs) play a crucial role by recognizing proteins or DNA/RNA sequences belonging to infectious agents, and activation of TLRs results in the production of proinflammatory mediators and cytokines and links innate adaptive responses under pathological conditions, including the various regions and diseases of the eye. Therefore, it is possible that TLRs play a role in inflammation induced by biologicals injected in the eye. In addition, the influence of biologicals—for example, small interfering RNAs—on inflammatory responses depends on the contemporaneous administration of vehicles and the mode of delivery.

Monkeys were more sensitive to ocular inflammation than rabbits at high intravitreal doses of ranibizumab, with no apparent correlation between the degree of ocular inflammation and the appearance of serum antibodies to ranibizumab.

Microparticles: Localized foreign-body reaction has been observed after intravitreal injection of microspheres loaded with Ganciclovir, PLA, or PLGA particles loaded with inert fluorochromes.

Nanoparticles: Functionally, however, the intravitreal nanoparticle injections do not affect the ERG.The disadvantages of microparticles and nanoparticles is the risk of injection and that intraocular injections may cause vitreous clouding and periocular injections may cause a foreign-body response in the case of microparticles.

Liposomes: Intravitreally injected liposomes of antibiotics, antivirals, antifungals, and antimetabolic agents are less toxic than the free form because there is less free drug in contact with tissues. Liposomes also protect poorly stable drugs from degradation, such as phosphodiester antisense oligonucleotides and peptides.

Ocular Implants

Insertion of implants by incision is slightly more invasive compared to injection, and the normal wound-healing process takes place with both procedures. Pars plana incisions and injections have been studied in monkey eyes from 6 to 13 years following the procedure, and the scars were found to consist of fibrous tissue and blood vessels that extended from the epislera into the

vitreous. There were quantitative but no qualitative differences between the two procedures, and there were ultrastructural features of mature scar tissue.

Nonbiodegradable Implants

The ocular safety of nonbiodegradable implants of ganciclovir (Vitrasert) was studied in rabbits 80 days following implantation. No evidence of ocular inflammation by indirect ophthalmoscopy was observed, but lens opacification, cataracts, and retinal-detachment ERG changes were observed. There was no evidence of drug-related effects microscopically, although a chronic inflammatory reaction with multinucleated giant cells around the silk suture used to secure the implant to the sclera was observed. Although Vitrasert is an approved, marketed drug in the United States, the pharmacology review is unavailable on the FDA Web site.

Biodegradable Implants

Scleral plugs of biodegradable PLGA to deliver ganciclovir, doxorubicin, and fluconazole to the vitreous have been evaluated for ocular toxicity up to 24 weeks following implantation in rabbits. Slit-lamp biomicroscopy showed no ocular inflammatory reactions, and no substantial changes were observed by ERG. Histology showed no abnormalities in the rabbit retinal tissue adjacent to the implant site and the posterior pole. Inflamatory cells infiltrated the matrix pore, and fibrous tissue closed the sclerotomy site. An intramscleral PLA implant of betamethasone evaluated for ocular toxicity for up to 16 weeks following implantation in rabbits showed no ERG changes and complete degradation of the implant at 16 weeks, with replacement by loose connective tissue and a few multinucleated giant cells with no histological retinal changes. Similarly, there was no ocular toxicity for up to 3 months following insertion of a PLGA cyclosporine implant into the anterior chamber in rabbits. Clinical safety evaluation of a PLGA rod implant of dexamethasone DDS following intravitreal insertion by sclerotomy in a phase 2 study in patients with persistent macular edema showed a mild increase in incidence of adverse events in the dexamethasone DDS treatment groups compared to the observation groups on day 8 that were expected as a result of the surgical procedure (hyperemia, pruritis, vitreous hemorrhage, and anterior chamber cells and flare. The rate of ocular adverse events after day 8 and up to study termination at day 180 was similar between treatment and observation groups, including no corticosteroid-induced cataract formation or increases in IOP.

CONCLUSION:

Novel ocular drug-delivery formulations and methods for localized, sustained delivery may provide the solution for treating serious intraocular diseases. The latest available targeted drug

delivery systems focus on the localised delivery of the drugs as well as certain macromolecular substances like proteins, genes like DNA, siRNA to the internal parts of the eye. Species and strain differences in ocular pigmentation and anatomy, retinal vasculature, and vitreal volume are important for designing and interpreting ocular toxicity studies and human risk assessment. Novel ocular drug formulations and sustained delivery are generally well tolerated, and inflammatory effects appear to be more pronounced in animals than humans. Although regulatory guidance is meagre for ocular drugs, review of FDA Pharmacology Reviews and selected literature can provide insights.

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