OCULAR DELIVERY OF PEPTIDES AND PROTEINS

RICHARD ADDO, R.Ph., Ph.D
ASSOCIATE PROFESSOR
UNION UNIVERSITY SCHOOL OF PHARMACY
JACKSON TENNESSEE USA
Structure of eye and different pathways of ocular administration

Challenges for ocular delivery of proteins/peptides

Formulation considerations

Peptide transport systems in the eye

Ocular administration for topical delivery of proteins/peptides

Ocular administration for systemic delivery of proteins/peptides

Strategies for ocular delivery of proteins/peptides
STRUCTURE OF THE EYE

Outermost coat: Clear, transparent cornea and white, opaque sclera

Middle layer: iris anteriorly, choroid posteriorly and intermediate ciliary body

Inner layer: retina

Topical administration with trans-corneal permeation

Topical administration with non-corneal permeation across the conjunctiva and sclera

Drug distribution from the blood through the blood-aqueous barrier into the anterior chamber

Drug distribution from the blood-retina barrier into the posterior chamber

Intra-vitreal drug administration route

Sub-tenon injection
BARRIERS TO ABSORPTION

- Basal layer, 2-3 layers of wing cells and 1-2 outermost layers of squamous cells
- Outermost layers
  - Intercellular tight junctions surround the most superficial layers and restrict passage of peptides and proteins
  - Absorption relies on transcellular passage or strategies that can modulate the tight junctions
- Wing cells and basal cells
  - Intercellular spaces are wider and permit paracellular diffusion
- Negatively charged corneal epithelium offers greater resistance to negatively charged compounds as compared to positively charged ones
CHALLENGES TO OCULAR DELIVERY OF PROTEINS/PEPTIDES

- **Barriers for locally delivered drugs**
  - Loss of drug from ocular surface
  - Lacrimal-fluid barrier
  - Blood-ocular barrier
- Low drug contact time
- Tear production and turnover
- Consequent dilution

Schematic presentation of the different barriers for ocular delivery of proteins and peptides

- **Pre-corneal restrictions/barriers**
  - Size and Hydrophilicity
    - Reduced permeation/absorption across corneal epithelium
- **Post-corneal restrictions/barriers**
  - Enzymatic Degradability
    - Low ocular bioavailability
  - Systemic Absorption
    - Low ocular bioavailability and systemic side-effects
FORMULATION CONSIDERATIONS

Aggregation

Is induced by shaking, prolonged storage, heating, freezing, lyophilization

Can lead to

- Reduced bioactivity
- Immunogenic reactions
- Blockage of tubing, membranes or pumps in an infusion set
- Unacceptable physical appearance such as opalescence

Example:

- Insulin can undergo self-association/aggregation due to the hydrophobic regions of the molecule
- Human epidermal growth factor (hEGF) undergoes pH and concentration dependent aggregation

Can be prevented by

- Use of appropriate formulation excipients; example: mannitol, trehalose
- Proper care in processing of formulation
- Synthesizing a resistant derivative
FORMULATION CONSIDERATIONS

Formulation Additives

- **Protease Inhibitors:**
  - Used if the protein/peptide is likely to degrade upon ocular administration
  - Aminopeptidase inhibitors: bestatin, amastatin, puromycin, p-chloromercuribenzoate

- **Sugars:** Exert a protective effect on proteins by changing the solvent structure around the protein

- **Cyclodextrins:** Act by molecular encapsulation of amino acid chains thereby preventing hydrophobic interactions
Epithelial cells express nutrient transporters and receptors on their surface which help the movement of vitamins and amino acids across cell membranes.

Proton coupled receptors help translocation of di- and tripeptides across the epithelium.

Transporters are classified as PepT1, PepT2 and peptide/histidine transporters (PHT1 and PHT2).

Expression of PHT1 in bovine and human retinal pigment epithelial cells (BRPE and HRPE), ARPE-19 cells (human RPE cell type), bovine and human neural retina cells has been reported.

PepT2 and PHT 2 expression reported in bovine and human retina.

Drugs with poor ocular bioavailability can be suitably modified by design to facilitate recognition and uptake by peptide transporters.
Topical delivery is considered to be the best option for treatment of most ocular disorders. Several peptides have been identified for treatment of ocular disorders like dry eye disease, age-related macular degeneration, proliferative diabetic retinopathy, etc.

Loss to systemic circulation must be minimized.

- Phenylephrine used as a vasoconstrictor to minimize systemic absorption.
- Use of mucoadhesive polymer to improve ocular absorption.

Adverse physicochemical properties or enzymatic degradation of peptides might render them less effective.

- Loading them in a carrier system like liposome or nanoparticle may limit some of these problems.
Growth Factors

Human Epidermal Growth Factor (hEGF) stimulates cell proliferation in the corneal epithelium thus causing epithelialization during wound healing.

EGF can be produced biotechnologically in a commercially feasible manner.

It can thus be a suitable therapeutic agent for corneal trauma and during intraocular surgery.

Tissue Plasminogen Activator

tPA can be used to achieve clot lysis after surgery for cataract and/or glaucoma.

Since tPA is present in aqueous humor and other ocular tissues, its use is like a supplementation of body function.

Cyclosporin A

It has immunosuppressive, anti-fungal and anti-inflammatory activity.

Primary use is inhibition of kidney graft rejection.

Instillation in eye can inhibit rejection of corneal grafts.
STUDIES SHOWING OCULAR DELIVERY OF EGF

EGF Incorporated in Cationized Gelatin Hydrogel

EGF Incorporated in Beta Cyclodextrin Complex

Fig. 6.
rhEGF concentrations in tears after ocular administration of poloxamer gels. The poloxamer gel was composed of P407/F185 (16/14) and rhEGF (0.5%) or rhEGF/HP-β-CD complex (0.5%). Each point represents the mean±S.E. (n=3).


**In vitro transcorneal permeation**

**Concentration in aqueous humor after instillation in rabbit eye**

*Figure 2.* In vitro transcorneal permeation of GCV liposome preparation and solution ($\overline{X} \pm SD$, n = 5). GCV indicates ganciclovir.

*Figure 4.* Concentration-time profiles of GCV in aqueous humor after instillation of 1.0 mg/mL GCV liposome preparation and GCV solution in rabbit (ng/mL, $\overline{X} \pm SD$, n = 5). GCV indicates ganciclovir.
OCULAR ADMINISTRATION FOR SYSTEMIC DELIVERY

- Occurs because of contact of instilled solution with conjunctival and nasal mucosae

**Advantages:**
- Relative ease and low cost of formulating and administering eye drops (compared to injections)
- Relative insensitivity of eye towards immunological reactions (compared to lung and gut)
- Absence of first pass metabolism

**Challenges:**
- Reproducible delivery
- Low bioavailability
OCULAR ADMINISTRATION FOR SYSTEMIC DELIVERY

Insulin:
When administered to the eye, a sustained lowering of blood glucose was observed.

Use of absorption enhancers may often be required to enhance absorption of peptides through the eye.

Absorption enhancers must be safe and non-irritating to the eye.

Order of efficacy: Saponin > Fusidic Acid > BL-9 = EDTA > Glycocholate > Decamethonium = Tween 20

Aminopeptidase inhibitors or peptide analogs that are resistant to enzymes also help to improve bioavailability.

Systemic absorption of insulin (± SEM; n=5) following the ocular instillation of a 0.25% insulin solution containing Brij-78 as an enhancer. Data generated following a b.i.d. administration of eyedrops over a three-month period (• - blood insulin concentration; o - blood glucose levels).
Table 1—Summary of the Efficacy of 0.5-, 1-, and 2-mg Insulin Ocular Delivery Systems.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Insulin (mg)</th>
<th>Brij-78 (μg)</th>
<th>Area Above the Curve (% h) mean ± SD</th>
<th>Duration of BGC &lt;80% of Initial (h) mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyedrop 1</td>
<td>0.5</td>
<td>20</td>
<td>54 ± 12</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Device 1</td>
<td>0.5</td>
<td>0</td>
<td>59 ± 59</td>
<td>0</td>
</tr>
<tr>
<td>Device 2</td>
<td>0.5</td>
<td>10</td>
<td>65 ± 83</td>
<td>0</td>
</tr>
<tr>
<td>Device 3</td>
<td>0.5</td>
<td>20</td>
<td>405 ± 25a</td>
<td>6.7 ± 0.8a</td>
</tr>
<tr>
<td>Device 4</td>
<td>0.5</td>
<td>30</td>
<td>425 ± 53</td>
<td>9.2 ± 1.9</td>
</tr>
<tr>
<td>Device 5</td>
<td>0.5</td>
<td>50</td>
<td>Hypoglycemia</td>
<td></td>
</tr>
<tr>
<td>Eyedrop 2</td>
<td>1</td>
<td>20</td>
<td>81 ± 10b</td>
<td>0.9 ± 0.3b</td>
</tr>
<tr>
<td>Device 6</td>
<td>1</td>
<td>0</td>
<td>218 ± 92</td>
<td>0</td>
</tr>
<tr>
<td>Device 7</td>
<td>1</td>
<td>10</td>
<td>162 ± 90</td>
<td>0</td>
</tr>
<tr>
<td>Device 8</td>
<td>1</td>
<td>20</td>
<td>552 ± 93a</td>
<td>10.2 ± 0.4a</td>
</tr>
<tr>
<td>Device 9</td>
<td>1</td>
<td>30</td>
<td>Hypoglycemia</td>
<td></td>
</tr>
<tr>
<td>Device 10</td>
<td>1</td>
<td>50</td>
<td>Hypoglycemia</td>
<td></td>
</tr>
<tr>
<td>Device 11</td>
<td>2</td>
<td>0</td>
<td>182 ± 109</td>
<td>0</td>
</tr>
<tr>
<td>Device 12</td>
<td>2</td>
<td>10</td>
<td>174 ± 139</td>
<td>3.8</td>
</tr>
<tr>
<td>Device 13</td>
<td>2</td>
<td>20</td>
<td>Hypoglycemia</td>
<td></td>
</tr>
</tbody>
</table>

*a* Significantly different from the corresponding eyedrop formulations (*p* < 0.05).

*b* Significantly different from eyedrop 1 (*p* < 0.05).

*c* Significantly different from device 3 (*p* < 0.05).

Figure 2—Mean blood glucose concentrations after ocular administration of 1-mg insulin delivery systems: eyedrop 2 (●), device with no Brij-78 (▲), device with 10 μg of Brij-78 (●), device with 20 μg of Brij-78 (●), device with 30 μg of Brij-78 (■), and device with 50 μg of Brij-78 (▲). Each value represents the average ± SD of three rabbits, except the last formulation, that was carried out with two rabbits.

OCULAR ADMINISTRATION FOR SYSTEMIC DELIVERY

- **Glucagon**
  - Used in treatment of hypoglycemia
  - Can be delivered by the ocular route and has been reported to increase blood glucose
  - Mol wt. is lower than insulin; may not need absorption enhancers

- **Calcitonin**
  - Long term administration required for treatment of hypercalcemia
  - Besides the ocular route, other alternative routes like nasal, rectal, transdermal have also been explored
TRANS-SCLERAL DELIVERY OF IgG TO THE RETINA

Delivery of Bioactive Protein to the Choroid and Retina, Ambati J, Gragoudas ES, Miller JW, You TT, Miyamoto K, Delori FC, Adamis AP, Invest Ophthalmol Vis Sci. 2000 Apr;41(}
STRATEGIES FOR THE OCULAR DELIVERY OF PROTEINS/PEPTIDES

Prodrugs

Change physicochemical properties of a drug to improve permeation across cornea and enhance bioavailability

First prodrug for ocular delivery: Dipivefrin, prodrug of epinephrine used to treat glaucoma

Desirable properties
- Good stability
- High enzyme lability

Most common barriers that can be overcome are

- A low aqueous solubility, which prevents the development of aqueous eyedrops
- A low lipid solubility, which results in low corneal permeation and low ophthalmic bioavailability
- A short duration of action due to rapid drug elimination from site of action
- Systemic side-effects, due to low corneal and high systemic absorption
STRATEGIES FOR THE OCULAR DELIVERY OF PROTEINS/PEPTIDES

Mucoadhesive Particulate Carriers

- Cornea and conjunctiva have a net negative charge
- Cationic polymers help to increase the concentration and residence time of polymer-associated drug
- Chitosan – biocompatible, biodegradable, enhances the paracellular transport of drugs

Effect of Chitosan on Zeta Potential of Microparticles

Conventional eye drops

Anionic or poly-anionic

Cationic poly-cation

washout

Electrostatic Repulsion

Electrostatic Attraction
DELIVERY MECHANISM OF CATIONIC NANOPARTICLES

Electrostatic interaction leading to

- Retention at the surface
- Reservoir effect in:
  - Cornea
  - Conjunctiva
- Transcorneal Route
- Diffusion via the scleral route
  - Sustained release to the retina
CHITOSAN NANOPARTICLES FOR CYCLOSPORIN A DELIVERY

Hydrogel Delivery Systems

- Allows slow release of drug from a hydrogel inserted beneath the eyelid
- Ocusert: First such device
  - Non-erodible ocular insert
  - Pilocarpine alginate core sandwiched between two transparent, rate controlling membranes
PLGA MICROSPHERES FOR DELIVERY OF VANCOMYCIN

**Figure 4.** VA concentration profiles in the aqueous humor of rabbits after administration of VA_micr and VA_micr-HPC microsphere suspensions and of the reference solutions (VA_sol and VA_sol-HPC) (Mean ± SE, n = 6; *significantly different from the VA_micr and the VA_micr-HPC formulations, P < 0.05, #significantly different from the VA_micr-HPC formulation, P < 0.05).

**Table 2.** Pharmacokinetic parameters in aqueous humor after in vivo administration in rabbits of the preparations under study.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>C_{max} (µg/ml ± SE)</th>
<th>T_{max} (min)</th>
<th>AUC (min µg/ml ± SE)</th>
<th>AUC relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA_micr</td>
<td>2.47 ± 0.49</td>
<td>15</td>
<td>248.2 ± 35.2</td>
<td>2.31</td>
</tr>
<tr>
<td>VA_micr-HPC</td>
<td>1.80 ± 0.54</td>
<td>30</td>
<td>206.6 ± 52.8</td>
<td>1.92</td>
</tr>
<tr>
<td>VA_sol</td>
<td>0.94 ± 0.05</td>
<td>30</td>
<td>107.4 ± 17.3</td>
<td>1.00</td>
</tr>
<tr>
<td>VA_sol-HPC</td>
<td>1.26 ± 0.21</td>
<td>15</td>
<td>122.9 ± 26.7</td>
<td>1.14</td>
</tr>
</tbody>
</table>
STRATEGIES FOR THE OCULAR DELIVERY OF PROTEINS/PEPTIDES

Absorption Enhancers
- Promote penetration of drugs through corneal barrier by changing integrity of epithelial cell layer
- Examples: EDTA, sodium glycocholate and related cholates, tween-20, saponin

Miscellaneous Approaches
- Cell penetrating peptides: TAT (Trans-activating transcription factor from human immunodeficiency virus) exhibit efficient penetration to the retina after topical delivery
- Intravitreal injections
  - Can cause several complications like hemorrhage and retinal displacement
  - Bevacizumab (Avastin): Used for the treatment of ocular vascularization
**TABLE 5.1** List of disorders/indications where therapeutic peptides could be delivered through ocular route

<table>
<thead>
<tr>
<th>Disorder/Indication</th>
<th>Therapeutic peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiallergic, antiinflammatory</td>
<td>ACTH</td>
</tr>
<tr>
<td>Analgesic</td>
<td>β-Endorphin, Leu-enkephalin</td>
</tr>
<tr>
<td>Antiscarring agent in glaucoma filtration surgery</td>
<td>Integrin-binding peptide</td>
</tr>
<tr>
<td>Attenuate miotic response</td>
<td>Somatostatin</td>
</tr>
<tr>
<td>Choroidal or retinal neovascularization</td>
<td>Octreotide, Urokinase derived peptide, Cyclic integrin-binding peptide</td>
</tr>
<tr>
<td>Corneal epithelial wound</td>
<td>Insulin-like growth factor derived peptide, Substance P derived peptide</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Insulin</td>
</tr>
<tr>
<td>Diabetes insipidus</td>
<td>Vasopressin</td>
</tr>
<tr>
<td>Diagnosis of thyroid cancer</td>
<td>TSH</td>
</tr>
<tr>
<td>Dry eye disease</td>
<td>Cyclosporine A</td>
</tr>
<tr>
<td>Hypoglycemic crisis</td>
<td>Glucagon</td>
</tr>
<tr>
<td>Immunostimulant</td>
<td>Met-enkephalin</td>
</tr>
<tr>
<td>Induction of uterine contractions</td>
<td>Oxytocin</td>
</tr>
<tr>
<td>Induction of vitreous detachment in vitrectomy</td>
<td>Integrin-binding peptide</td>
</tr>
<tr>
<td>Paget's disease</td>
<td>Calcitonin</td>
</tr>
<tr>
<td>Secretion of insulin</td>
<td>VIP</td>
</tr>
<tr>
<td>Uveal melanoma and retinal blastoma</td>
<td>Apoptosis inducing peptide</td>
</tr>
</tbody>
</table>

**TABLE 5.2** Reported literature related to ocular delivery of proteins and peptides

<table>
<thead>
<tr>
<th>Protein/peptide</th>
<th>Delivery strategies</th>
<th>Concluding remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Penetration enhancer</td>
<td>The insulin bioavailability was 5.7 to 12.6% with polyoxyethylene-9-lauryl ether, 4.9 to 7.9% with GC, 3.6 to 7.3% with Na taurocholate and 8.2 to 8.3% with Na deoxycholate, as compared to 0.7 to 1.3% in the absence of absorption promoters.</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>Azone penetration enhancer</td>
<td>Cyclosporine-treated grafts contained significantly fewer infiltrating T-lymphocytes than did the drug/solvent-treated allografts, indicating that the topical application of cyclosporine actively inhibited the entry of T-cells into the grafts.</td>
</tr>
<tr>
<td>IgG protein</td>
<td>Transscleral delivery</td>
<td>IgG protein delivered to the retina and choroid in an optimum concentration for the treatment of chorio-retinal disorders with negligible systemic absorption.</td>
</tr>
<tr>
<td>Vancomycin (peptide)</td>
<td>PLGA microparticles</td>
<td>PLGA microparticles loaded with peptide drug showed high and prolonged concentration of vancomycin and increased level of AUC (2-fold) as compared to aqueous solutions.</td>
</tr>
<tr>
<td>Cangiclovir (CCV)</td>
<td>Prodrug</td>
<td>Glycine-valine CCV is the effective and lead candidate for the treatment of Human Cytomegalovirus (HCMV).</td>
</tr>
<tr>
<td>Vasoactive intestinal peptide (VIP)</td>
<td>Liposome</td>
<td>Treatment of ocular inflammation by modulation of macrophage and T-cell activation of the immune system.</td>
</tr>
<tr>
<td>VIP</td>
<td>Liposomes</td>
<td>For the treatment of endotoxin induced uveitis (EU), liposomal delivery increased VIP efficiency and bioavailability.</td>
</tr>
<tr>
<td>Cangiclovir (CCV)</td>
<td>Prodrug</td>
<td>Diester CCV produgs demonstrated excellent chemical stability, high aqueous solubility and marked enhanced antiviral potency against the herpes viruses without any increase in cytotoxicity.</td>
</tr>
</tbody>
</table>