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Research Paper

Evaluation of topical hesperetin matrix film for back-of-the-eye delivery

Goutham R. Adelli [a], Tushar Hingorani [a], Nagendra Punyamurthula [a], Sai Prachetan Balguri [a], Soumyajit Majumdar [a,b,c,*]

[a] Department of Pharmaceutics and Drug Delivery, School of Pharmacy, The University of Mississippi, MS, USA
[b] Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS, USA
[c] National Center for Natural Product Research, The University of Mississippi, MS, USA

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ABSTRACT

Purpose: The goal of the present study was to develop a poly (ethylene oxide) N10 (PEO N10) based melt-cast matrix system for efficient and prolonged delivery of hesperetin (HT), a promising bioflavonoid, to the posterior segment of the eye through the topical route.
Methods: HT film was prepared by melt-cast method using PEO N10 and cut into 4 mm × 2 mm segments, each weighing 8 mg. This film was evaluated with respect to *in vitro* release rates and also transmembrane delivery across Spectra/Por® membrane (MWCO: 10,000 Daltons) and isolated rabbit corneas. Ocular tissue concentrations were also determined postapplication of the film in *ex vivo* and *in vivo* models.
Results: HT release from the film was determined to be about 95.3% within 2 h. *In vitro* transcorneal flux was observed to be 0.58 ± 0.05 μg/min/cm^2 across the isolated rabbit cornea. High levels of HT were detected in the retina-choroid (RC) and vitreous humor (VH) in the *ex vivo* model following topical application of the film. Significant levels of HT were observed in both anterior and posterior segment ocular tissues 1 h post topical application of the 10 and 20%w/w HT films on the rabbit eye. Moreover, HT was detected in the VH and RC even after 6 h following topical application of the film *in vivo*.
Conclusion: The results from this study suggest that the melt-cast films can serve as a viable platform for sustained topical delivery of bioflavonoids, and other therapeutic agents, into the back-of-the eye tissues.

1. Introduction

Diseases affecting the posterior segment of the eye such as diabetic retinopathy (DR), age related macular degeneration (AMD), diabetic macular edema (DME) and proliferative vitreo-retinopathy (PVR) are some of the major causes of blindness in the United States [1–3]. According to the World Health Organization (WHO) approximately 39 million people are affected by vision loss and 246 million people suffer from moderate to severe vision impairment. Based on the 2010 U.S census, out of the 142.6 million above 40 years of age, 7.6 million people suffer from DR and 2.1 million are affected by AMD.

In case of DR, hyperglycemia and tissue hypoxia cause thickening of the capillary basement membrane and death of pericytes. Damage to the pericytes causes microaneurysms, vascular leakage and blockage of retinal capillaries leading to oxidative stress. High amounts of polyunsaturated fatty acids (primary targets of peroxidation), generation of free radicals through frequent photoexcitation and adequate oxygen supply are the three major causes of oxidative damage to the retina. Many studies have also reported the relationship between high blood glucose and oxidative stress and initiation of DR [4–7].

In early stages of AMD, accumulation of drusen (sub-RPE deposits) under the retinal pigmented epithelium (RPE) affects the macula and leads to the loss of central vision. This is followed by choroidal neovascularization (CNV) in the subretinal spaces.

Abbreviations: HD, Hesperidin; HT, hesperetin; PEO N10, polyethylene oxide N10; DR, diabetic retinopathy; AMD, age related macular degeneration; DME, diabetic macular edema; PVR, proliferative vitreo-retinopathy; CNV, choroidal neovascularization; WHO, World Health Organization; RPE, retinal pigmented epithelium; ROS, reactive oxidative species; ERKs, extracellular-signal-regulated kinases; COX, cyclooxygenase; PGE_2, prostaglandin E_2; IPBS, isotonic phosphate buffered saline ; HBSS, Hank's balanced salt solution; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; ATR, attenuated total reflectance; HPLC-UV, high performance liquid chromatography-Ultra violet; mM, millimolar; μg, microgram; DMSO, dimethyl sulfoxide; ACN, acetonitrile; AH, aqueous humor ; VH, vitreous humor ; IC, iris ciliary bodies; RC, retina-choroid.

* Corresponding author. 111 Faser Hall, Department of Pharmaceutics and Drug Delivery, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. Tel.: +1 (662) 915 3793; fax: +1 (662) 915 1177.
E-mail address: majumso@olemiss.edu (S. Majumdar).

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Progressive accumulation of abnormal chemicals in Bruch's membrane and formation of drusen aggregates lead to neovascular leakage and degeneration of the RPE cells [8].

Bioflavonoids, a group of plant polyphenols, are reported to exhibit antioxidant, anti-angiogenic and anti-inflammatory properties along with fluid retention reduction and capillary wall strengthening activities [9,10]. Hesperidin (HD), and its aglycone hesperetin (HT), a plant based flavanone (Fig. 1A and B) obtained from *Citrus sinensis*, possesses antioxidant [11,12], and neuroprotectant properties [11], and reduces vascular permeability. HT is recognized to be more potent than HD in scavenging reactive oxidative species (ROS) [13–15]. HT also prevents the cytotoxic effect of peroxynitrites by converting them to non-toxic mono-nitrated products and increasing phosphorylation of extracellular-signal-regulated kinases (ERKs) [16]. Anti-inflammatory activity of HT is thought to be achieved by inhibition of the COX-2 pathway and synthesis of PGE_2 [17], and inhibition of nitric oxide production by blocking nitric oxide synthase [18,19]. Additionally, HT was observed to increase ocular blood flow and promote recovery of retinal function following ischemic insult of retina [20]. Currently HT is available as an oral dietary supplement to improve blood flow and as a vasoprotectant. Cumulative urinary recovery of HT suggests a bioavailability of less than 25% after oral administration of HD and HT [21]. Poor oral bioavailability of HT can be attributed to its rapid metabolism into hydrophilic glucuronide metabolites [22] and short half-life (Plasma half-life: 6.7 h and vitreous humor half-life: 110 min) [23,24].

Treating the posterior segment eye diseases has always been a great challenge because of the unique physiological and anatomical barriers of the eye. Our earlier studies demonstrate that systemic application fails to deliver HT to the ocular tissues [22]. This makes topical or intraocular/periocular application the most effective. Less than 5–10% of topically applied drug, however, permeates into the intraocular tissues [25–27]. Various ocular delivery systems are being investigated to increase drug contact time and site specific delivery to the posterior segment of the eye using liposomal formulations and other sustained release and controlled release systems such as ocular inserts, collagen shields, matrix systems, and hydrogel lenses [28–31].

The goal of the present study was to evaluate the effectiveness of topical melt-cast polymeric matrix systems with respect to the delivery of HT to the back-of-the-eye tissues, especially to the retina-choroid (RC) and vitreous humor (VH). The matrix film was evaluated *in vivo* for dose dependent and time dependent drug delivery.

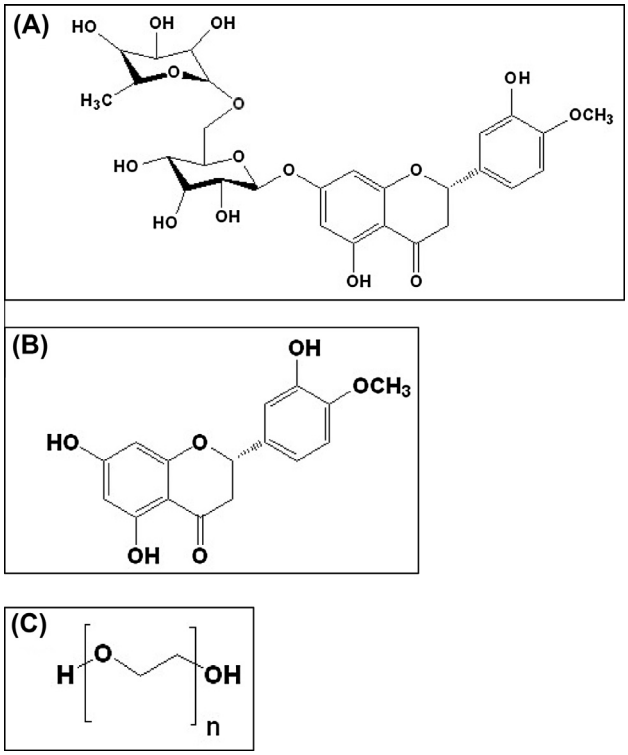

Fig. 1. Chemical structure of (A) Hesperidin, (B) Hesperetin and (C) PEO N10.

2. Materials and methods

2.1. Chemicals

PEO (PubChem CID: 5327147) [PolyOx® WSR N-10 (PEO N-10), MW: 100,000 Daltons] was kindly donated by Dow Chemical Company (Midland, MI). HT (PubChem CID: 72281) (Type HP-2, from *Helix pomatia*) was purchased from Sigma Aldrich (St. Louis, MO). All other chemicals were purchased from Fisher Scientific (St. Louis, MO).

2.2. Animal tissues

Whole eye globes of New Zealand albino rabbits were purchased from Pel-Freez Biologicals® (Rogers, AK), shipped overnight in Hanks Balanced Salt Solution (HBSS) over wet ice [32]. Corneas and whole eye globes were used on the day of receipt.

2.3. Animals

Male New Zealand albino rabbits (2.0–2.5 kg) procured from Harlan Laboratories® (Indianapolis, IN) were used in all the studies. All animal experiments conformed to the tenets of the Association for Research in Vision and Ophthalmology statement on the Use of Animals in Ophthalmic and Vision Research and followed the University of Mississippi Institutional Animal Care and Use committee approved protocols.

2.4. Preparation of polymeric matrix film

Melt-cast method was employed in the preparation of the polymeric matrix film. PEO N10 (Fig. 1C) was selected as the matrix forming polymer. A physical mixture of HT and PEO N10 was prepared by geometric dilution. Drug load in the film was 10% or 20% of the total weight of film. A 13 mm die was placed over a brass plate and heated to 70 °C using a hot plate. The physical mixture of HT and polymer was added in the center of the die and compressed to form a flat matrix surface. The mixture was further heated for 2–3 min. After cooling, 4 mm × 2 mm sections each weighing approximately 8 mg and with a drug load of 0.8 mg or 1.6 mg for the 10%w/w and 20%w/w films, respectively, were cut out from the film.

2.5. Assay and content uniformity

To determine HT content, the film was placed in a mixture of methanol and dimethyl sulfoxide (DMSO) 50:50 and sonicated for 15 min until the film was completely dissolved in the solvent mixture. Content uniformity was determined using four separate sections of 8 mg each, randomly cut from a single 13 mm film, and analyzed as described under analytical procedure using an HPLC-UV method.