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Intracorneal melatonin delivery using 2-hydroxypropyl -β-cyclodextrin ophthalmic solution for granular corneal dystrophy type 2

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ABSTRACT

Melatonin (MT), an effective antioxidant, has therapeutic implications for granular corneal dystrophy type 2 (GCD2) treatment. Eye drop formulations containing cyclodextrins (CDs) were studied with the objective of improving MT solubility, stability, and ocular absorption, while decreasing eye irritation. MT complexes with αCD, βCD, γCD, and 2-hydroxypropyl-βCD (HPβCD) were characterized by phase solubility studies, which demonstrated Higuchi's A_L-type phase solubility profiles. The MT/HPβCD complex showed the highest MT solubility (2.75 mg/mL). Ocular irritation experiments showed HPβCD inclusion alleviated irritation of the eye. After administration of MT formulations to rabbit corneas, each harvested cornea was separated into corneal epithelium, stroma, and endothelium. MT concentrations in the corneal epithelium, stroma, and endothelium for the F1-treated group were 55.5 ± 9.24, 26.7 ± 2.66, and 21.1 ± 1.77 μM while those for the F2-treated group were 127.2 ± 21.01, 43.7 ± 16.93, and 51.0 ± 13.91 μM, respectively. Stability studies for 60 days showed no significant change in pH, osmolarity, and MT content. In conclusion, MT/HPβCD formulations can lower irritation, enhance MT stability, and improve therapeutic efficacy.

1. Introduction

Granular corneal dystrophy type 2 (GCD2), also known as Avellino corneal dystrophy, is an autosomal dominant disorder caused by a point mutation, changing arginine-124 to histidine in the transforming growth factor-beta-induced (TGFβI) gene located on chromosome 5q31 (Skonier et al., 1992). Consequently, substitution of histidine for arginine-124 generates abnormal kerato-epithelin, which accumulates in the cornea with hyaline (Korvatska et al., 2000). Owing to the accumulation of kerato-epithelin, the cornea loses transparency progressively and the symptom intensifies with aging. Early in the progression of the disease, granular deposits with clear boundaries are found at the posterior corneal epithelium and anterior corneal stroma. As the

Abbreviations: MT, melatonin; GCD-2, granular corneal dystrophy type 2; CDs, cyclodextrins; HPβCD, 2-hydroxypropyl βCD; αCD, α-cyclodextrin; βCD, β-cyclodextrin; γCD, γ-cyclodextrin; TGFβIp, transforming growth factor beta-induced protein; mTOR, mechanistic target of rapamycin; MWCO, molecular weight cut off; PVDF, polyvinylidene difluoride; HPLC, high-performance liquid chromatography; PBS, phosphate-buffered saline; LOD, limit of detection; CE, complexation efficiency; S_0, intrinsic solubility; STF, simulated tear fluid.

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disease progresses, granular or lattice-like deposits are observed in the midsection or posterior corneal stroma (Konishi et al., 1997). Although surgical treatment is a potential option for patients with elevated anterior stromal opacities, corneal dystrophies may reappear after surgery (Kim et al., 2006). Recent studies demonstrated that primary cultured GCD2 corneal fibroblasts are highly susceptible to oxidative stress-induced cell death compared to primary cultured normal corneal fibroblasts, and oxidative stress is involved in the corneal pathogenesis of GCD2 (Choi et al., 2009).

Melatonin (MT) is chemically known as *N*-Acetyl-5-methoxy-tryptamine which, is a hormone produced mainly by the pineal gland from the amino acid precursor, L-tryptophan. It plays a major role in regulating the internal clock that sets daily events and in controlling diverse physiological functions, such as regulation of the circadian rhythm of sleep and waking (Reiter, 1991), anti-inflammatory actions (Esposito and Cuzzocrea, 2010), anticancer effects (Mediavilla et al., 2010), antioxidant activity (Reiter et al., 2003), and reduction of intraocular pressure (IOP) (Samples et al., 1988). There are three membrane MT receptor subtypes (MT1, MT2, and MT3) in the ocular tissues comprising the retina, ciliary body, lens, lachrymal glands, and cornea (Rada and Wiechmann, 2006). Based on the results of numerous studies, several clinical trials have investigated the usefulness of MT in treating ocular diseases. MT is an effective antioxidant, which may be involved in defense mechanisms against oxidative stress in GCD2 corneal fibroblasts. MT induces autophagy to clear TGFβI protein (TGFβIp) via a mechanistic target of rapamycin (mTOR)-dependent pathway, thereby reducing the retention of cytoplasmic TGFβIp in patients with GCD2 (Choi et al., 2013). In addition, MT protects against apoptotic cell death of GCD2 corneal fibroblasts induced by oxidative stress (Choi et al., 2011). Therefore, MT may have potential therapeutic implications for GCD2 treatment (Choi et al., 2011).

Cyclodextrins (CDs) are cyclic oligosaccharides and were first described more than 100 years ago. For practical purposes, CDs can be classified as follows: carriers as solubilizers or stabilizers (Aloisio et al., 2016); functional excipients (Antoniuk and Amiel, 2016); taste masking agent (Dungarwal and Patil, 2016); and additives as detergents, viscosity modifiers, etc. (Szejtli, 1998). In ophthalmic formulations, CDs not only improve solubility and stability, but also reduce ocular irritation and discomfort (Loftsson and Stefansson, 1997). Non-irritating to the eye is an essential prerequisite for ophthalmic formulations as irritation induces tears and eye blinking response, which can cause wash out of the administered drugs (Loftsson and Stefansson, 2007). Inclusion of CDs with drugs at the molecular level avoids direct contact with the ocular membrane, thereby decreasing local irritation without altering the therapeutic effects (Uekama et al., 1998). CD complexes are also more stable in light and showed highest photo-stability of isradipine than pure isradipine after 4 days radiation (Park et al., 2013). Additionally, some researchers proposed that CDs can increase drug permeability through the cornea by incorporating into the mucin layer (Loftsson et al., 2006). CDs increase the corneal permeability of the drug in contrast to other surfactants that disturb the corneal cell layer (Loftssona and Jarvinen, 1999).

At most stability of MT in aqueous solution observed, when stored in sterile vials under vacuum at 4 °C, without loss of activity (Cavallo and Hassan, 1995). In addition, gradual decline of MT concentration at variable pH was observed above 20 °C over a period of 21 days (Daya et al., 2001). MT is known to be photodegradable and its stability declines gradually in both acidic and alkaline conditions at room temperature (Andrisano et al., 2000). Additionally, in a preliminary experiment, we found that eye drop formulations containing MT above a certain concentration irritated eyes. Therefore, the aims of this study are to reduce eye irritation caused by MT, improve its stability for commercial use, and increase its delivery to the cornea for improved therapeutic effect.

2. Material and methods

2.1. Materials

MT was purchased from Tokyo Chemical Industry Co., Ltd (Chuo-ku, Japan). 2-hydroxypropyl-β-CD (HPβCD) was obtained from Alfa Aesar (Haverhill, MA, USA). α-Cyclodextrin (αCD), β-cyclodextrin (βCD), and γ-cyclodextrin (γCD) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride and potassium chloride were purchased from Samchun Pure Chemicals Co. Ltd (Seoul, Korea). Sodium phosphate monobasic dihydrate, sodium phosphate dibasic anhydrous, and calcium chloride dihydrate were purchased from Duksan Pure Chemicals (Seoul, Korea). Solvable® was purchased from PerkinElmer (Waltham, MA, USA). Dialysis tubing cellulose membrane with molecular weight cut off (MWCO) of 12,400 Da was purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinylidene difluoride (PVDF) syringe filters (0.2 μm) were purchased from Whatman International Ltd. (Maidstone, England). Female albino rabbits were purchased from Samtako Biokorea (Osan, Korea). All other chemicals were of reagent grade, and purified water of Milli-Q® quality (Millipore®, Molsheim, France) was used throughout the study.

2.2. MT assays

MT content was determined by high-performance liquid chromatography (HPLC) using the Agilent-HPLC system 1200 infinity series (Agilent Technologies, Germany). For the phase solubility study, MT content was determined at 223 nm using an HPLC-UV spectrometer (Agilent 1290 infinity) with a C_{18} column (CAPCELL, 120 Å pore size, 5 μm, 4.6 mm inside diameter × 250 mm; SHISEIDO, JAPAN). The mobile phase, a mixture of 0.1 M triethylammonium acetate (TEAA) (70%; pH 7.0) and acetonitrile (30%), was pumped at 0.6 mL/min (Yerlikaya et al., 2010). In the stoichiometry and *in vivo* experiments, MT was determined using an HPLC-fluorescence detector (Model 1260 FLD) at excitation and emission wavelengths of 286 and 352 nm, respectively. The samples were diluted with distilled water or phosphate-buffered saline (PBS) before injecting 20 μL onto a C_8 column (KROMACIL, 100 Å pore size, 5 μm, 4.6 mm inside diameter × 250 mm; AkzoNobel, SWEDEN) at 25 °C using an auto sampler (Model 1260 ALS). A mixture of methanol (60%) and water (40%) was used as mobile phase at a flow rate of 0.6 mL/min (Model 1260 Quat Pump VL). The methods were linear up to 500 μg/mL with a limit of detection (LOD) <3 ng/mL.

2.3. Phase solubility studies of MT complexes with αCD, βCD, HPβCD, and γCD

The phase solubility of MT with various CDs was analyzed using the phase-solubility method (Higuchi and Connors, 1965). An excess amount of MT (20 mg) was added to centrifuge tubes (Effendorf®) containing increasing concentrations of CDs (1–16 mM) in 2 mL water. All samples were screw-capped and sealed with Parafilm for 48 h and placed on an agitator at 25 °C at a speed of 200 rpm. Excess MT precipitated to the bottom of the tube and no degradation of the drug was observed. Preliminary experiments showed that equilibrium was reached during the stirring period. Each sample was centrifuged at 30,000*g* for 15 min at 4 °C, and the supernatant was diluted to an adequate concentration with water before spectrophotometric determination.