International Journal of Pharmaceutics 478 (2015) 180–186

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Cationic solid lipid nanoparticles enhance ocular hypotensive effect of melatonin in rabbit

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ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form 11 November 2014
Accepted 13 November 2014
Available online 15 November 2014

Keywords:
Melatonin
Nanoparticles
Eye drops
Glaucoma
Intraocular pressure

ABSTRACT

The study was aimed at evaluating whether the ocular hypotensive effect of melatonin (MEL) was enhanced by its encapsulation in cationic solid lipid nanoparticles (cSLN), as well as at determining the tolerability of these formulations on the ocular surface. MEL was loaded in cSLN that had already been shown to be suitable for ophthalmic use. The formulations were prepared using Softisan® 100 as the main lipid matrix, with the presence of either stearic (SA) or palmitic acid (PA) as lipid modifiers. A fixed positive charge was provided by the addition of a cationic lipid (didecyldimethylammonium bromide). The ocular hypotensive effect was evaluated by measuring the intraocular pressure (IOP) during 24 h in albino rabbits. MEL elicited a significant ($p < 0.01$) IOP reduction in rabbit eye. All the formulations tested *in vivo* demonstrated a good tolerability. The nanocarrier containing SA was the most effective in terms of IOP reduction (maximum IOP reduction: −7 mmHg), and its effect lasted approximately 24 h.

The experimental data indicate that the new formulations based on cSLN loaded with MEL represent a potent anti-glaucoma treatment with a safe profile, warranting further clinical evaluation of the proposed nanotechnological strategy.

1. Introduction

Glaucoma is a progressive optic neuropathy and is one of the leading causes of blindness in industrialized countries. Elevated intraocular pressure (IOP) is the main risk factor for glaucoma; elevated IOP is caused by an impaired outflow of aqueous humor resulting from abnormalities within the drainage system of the anterior chamber angle (primary open-angle glaucoma, POAG) or impaired access of the aqueous humor to the drainage system (angle-closure glaucoma, ACG). Epidemiological studies have shown that the risk of glaucoma increases by 12% with every 1 mmHg increase in IOP (Nemesure et al., 2007). There are five main classes of approved drugs for topical treatment of glaucoma: prostaglandin derivatives, beta-blockers, carbonic anhydrase inhibitors, sympathomimetics and miotics. Despite several classes of drugs are available to treat glaucoma, clinicians need more potent medications to manage the disease. Recently, melatonin (MEL), a neurohormone whose synthesis takes place primarily in the pineal gland, received great attention as a new potential medication for glaucoma (Alcantara-Contreras et al., 2011). MEL is also synthesized in the eye of mammalians, including humans (Lundmark et al., 2007). As in the pineal gland, retinal MEL content significantly changes during the 24-hour cycle, with a peak concentration occurring at night (Wiechmann and Summers, 2008). It is well known (Ido et al., 1991) that intraocular pressure has rhythmicity during the day with the highest level at the daytime and the lowest at night, when MEL levels increase. This has been the basis to hypothesize that MEL influences IOP rhythm. MEL exerts its mechanism *via* a class of G-protein-coupled receptors (GPCRs) that are negatively coupled with adenylyl cyclase (Jockers et al., 2008), although cAMP-independent transduction pathways are also involved (Dubocovich, 1983). Two subtypes of MEL receptors have been identified in mammals, MT1 and MT2 receptors, which are encoded by the *MTNR1A* and *MTNR1B* genes, respectively.

MEL receptors have been detected in ciliary body (Alarma-Estrany and Pintor, 2007) and MEL and some MEL analogues are able to reduce the IOP in several species (Agorastos and Huber, 2011). Recently, Alcantara-Contreras et al. (2011) demonstrated that administration of MEL in wild-type mice significantly reduced IOP, on the contrary IOP did not change in MEL receptor type 1 (MT1) knockout mice after drug administration. It has been suggested that

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http://dx.doi.org/10.1016/j.ijpharm.2014.11.032
0378-5173/

MEL and its analogues can modulate the IOP by decreasing the amount of aqueous humor production (Bucolo et al., 2013).

Topical delivery into the conjunctival cul-de-sac is the most common route of ocular drug delivery. Despite its apparent accessibility, the eye is well protected from foreign materials, including therapeutic substances, by a number of very efficient mechanisms such as blinking, induced lacrimation, tear turnover, and nasolacrimal drainage. These mechanisms cause the rapid removal of substances from the eye's surface. The eye is also protected by the cornea, which represents an impressive barrier. These issues limit the ocular bioavailability of many drugs, justifying the development of innovative drug delivery systems (DDS) and, in particular, of different nanotechnology strategies (Bucolo et al., 2012, 2013; Pignatello and Puglisi, 2011). Also MEL can benefit from being delivered by nanocarriers.

To improve the effectiveness of MEL in reducing IOP, we prepared novel SLN formulations loaded with the drug. Optimization of the quasi-emulsion solvent diffusion (QESD) technique (Leonardi et al., 2014a; Pignatello et al., 2002a,b, 2006) for the production of ophthalmic lipid NP was also assessed in the study. QESD has been generally used to obtain polymeric NP; the method possesses a number of operative benefits, such as low or very-low working temperatures, need for low or no surfactant concentrations, and using highly biocompatible solvents (i.e., ICH class 3 for residual solvents). In particular, in the present study, Softisan® 100, a mixture of coconut hydrogenated triglycerides, was chosen as the main lipid matrix, combined with the cationic lipid didecyldimethylammonium bromide (DDAB) at different percentages, to achieve the desired nanoparticle surface charge, with the goal of increasing SLN mucoadhesiveness. Indeed, a fixed positive charge on the surface of cationic SLN (cSLN) would facilitate the electrostatic interaction with the negatively charged mucin on eye epithelium, prolonging the pre-corneal residence of the nanosystems and the corneal permeation of the loaded cargo, as demonstrated by many authors with other nanosized vectors (De Campos et al., 2003; Rabinovich-Guilatt et al., 2004). To further modulate the fluidity of the lipid nanomatrices, Softisan® 100 was mixed with a low percentage of either palmitic acid or stearic acid, as lipid modifiers (Stancampiano et al., 2006).

The ocular tolerability and the pharmacological activity of selected cSLN formulations on IOP reduction was tested *in vivo*, in comparison with an aqueous solution of the drug.

2. Experimental procedures

2.1. Materials

Softisan® 100 (S100) was supplied by Sasol GmbH (Hamburg, Germany); melatonin, Tween® 80, palmitic acid (PA), stearic acid (SA), didecyldimethylammonium bromide, acetone (analytical grade), and phosphate buffered saline (PBS) were purchased from Sigma–Aldrich (Milan, Italy). Ethanol was purchased from VWR (Milan, Italy); HPLC-grade water was purchased from Merck (Darmstadt, Germany).

2.2. SLN production

The cationic SLN were prepared according to an adapted QESD method. The procedure includes dissolution of the required lipids (see Table 1) and MEL in 1 ml of an ethanol/acetone (1:1, v/v) mixture. This solution was slowly injected, by a thin teflon tube connected to a syringe, in 10 ml of an aqueous phase kept at 0 °C under constant agitation at 13,500 rpm (Ultraturrax T25 equipped with a G-8 accessory; IKA, Königswinter, Germany). The aqueous phase consisted of HPLC-grade water or PBS containing Tween® 80 (0.05% w/v). After 15 min, the milky suspension was sonicated with either a bath sonifier (Branson 5002, Danbury, CT, USA) for 25 min at room temperature, or a probe apparatus (S-250A analog Sonifier; Branson) in continuous processing time for 4 min, with 20% amplitude and at room temperature. To completely evaporate the organic solvents, the samples were then left to stir for 24 h at 500 rpm on a magnetic plate at room temperature.

2.3. Characterization

Mean particle size, polydispersity index (PDI), Zeta potential and drug encapsulation efficiency (EE) of the prepared SLN formulations were determined. Moreover, the physical stability under different storage temperatures was assessed.

The mean size and PDI were determined by photocorrelation spectroscopy (dynamic light scattering) with a NanoSizer ZS90 (Malvern Instruments, UK). Samples were ten-fold diluted with HPLC-grade water before the analysis. The reported values (Table 1) are the mean ± SD of 90 measurements (three sets of 10 measurements in triplicate).

The electrophoretic mobility and Zeta potential were determined by the technique of laser Doppler anemometer with the above apparatus. The instrumentation consists of a He–Ne laser with a power of 5 mW at a wavelength of 633 nm. A potential of ±150 mV was set. An appropriate amount of each sample (100 μl) was diluted with 20 ml of HPLC-grade water and submitted to the analysis. The Zeta potential value was calculated by the instrument software from the average values (up to 100 measurements per sample) of the electrophoretic mobility, using the Smoluchowski equation.

Table 1
Physico-chemical and technological properties of the various MEL-loaded SLN formulations.

Batch	Sonifier[a]	Softisan 100 (%p/v)	DDAB (%p/v)	SA (%p/v)	PA (%p/v)	MEL (%p/v)	TWEEN 80 (%p/v)	Mean size (nm) ± S.D.	PDI ± S.D.	Zeta potential (mV) ± S.D.	EE%	Drug content (μg/ml)
1	P	1	0.03	–	–	0.05	0.05	175.0 ± 2.3	0.315 ± 0.069	+61.20 ± 3.24	85.64	405.2
1	B	1	0.03	–	–	0.05	0.05	182.3 ± 3.0	0.285 ± 0.414	+59.18 ± 6.14	83.47	395.1
1-P[b]	P	1	0.03	–	–	0.05	0.05	232.3 ± 3.0	0.233 ± 0.009	+2.54 ± 0.43	87.61	460.9
2A	P	1	0.03	0.1	–	0.05	0.05	326.2 ± 10.5	0.348 ± 0.055	−1.60 ± 0.28	90.34	451.9
2B	B	1	0.07	0.1	–	0.05	0.05	168.7 ± 4.9	0.202 ± 0.006	+20.40 ± 1.06	89.96	453.8
2C	B	1	0.1	0.1	–	0.05	0.05	237.1 ± 3.9	0.309 ± 0.048	+58.30 ± 5.01	90.62	452.1
2C-P[b]	P	1	0.1	0.1	–	0.05	0.05	839.8 ± 60.0	0.341 ± 0.128	−1.92 ± 0.48	90.42	455.2
3A	P	1	0.03	–	0.1	0.05	0.05	206.5 ± 3.8	0.221 ± 0.010	+9.14 ± 0.51	96.50	454.5
3B	B	1	0.07	–	0.1	0.05	0.05	214.4 ± 4.6	0.267 ± 0.057	+49.90 ± 2.95	95.40	456.2
3C	B	1	0.1	–	0.1	0.05	0.05	222.9 ± 1.0	0.239 ± 0.003	+59.70 ± 0.52	95.89	451.1
3C-P[b]	P	1	0.1	–	0.1	0.05	0.05	303.2 ± 12.0	0.269 ± 0.021	−3.99 ± 0.45	94.11	467.2

[a] Probe sonifier (P); bath sonifier (B); didecyldimethylammonium bromide (DDAB); palmitic acid (PA); stearic acid (SA); polydispersity index (PDI); drug encapsulation efficiency (EE).
[b] Batch prepared in PBS, pH 7.4.