Toxicology 319 (2014) 53–62

Contents lists available at ScienceDirect

Toxicology

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

A nanoparticle formulation reduces the corneal toxicity of indomethacin eye drops and enhances its corneal permeability

Noriaki Nagai [a], Yoshimasa Ito [a,*], Norio Okamoto [b], Yoshikazu Shimomura [b]

[a] Faculty of Pharmacy, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan
[b] Department of Ophthalmology, Kinki University, Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan

ARTICLE INFO

Article history:
Received 16 January 2014
Received in revised form 17 February 2014
Accepted 20 February 2014
Available online 2 March 2014

Keywords:
Nanoparticle
Indomethacin
Corneal toxicity
Transcorneal penetration
Eye drops

ABSTRACT

Indomethacin (IMC) has been shown to reduce post-operative inflammation and to decrease intraocular irritation after cataract extraction and in cystoid macular edema; however, the clinical use of its most commonly used eye drops is limited due to topical side-effects that include burning sensation, irritation and epithelial keratitis. It is known that decreasing direct cell stimulation and reducing the amount applied *via* increasing bioavailability are useful for improving these issues. In this study, we designed ophthalmic formulations containing 0.5% IMC nanoparticles using zirconia beads and Bead Smash 12 (IMC_{nano} eye drops; particle size 76 ± 59 nm, mean $\pm$ S.D.), and investigated the corneal toxicity of these IMC_{nano} eye drops. IMC_{nano} eye drops are tolerated better by a human cornea epithelial cell line (HCE-T) than commercially available NDSAIDs preparations (IMC, pranoprofen, diclofenac, bromfenac and nepafenac eye drops), and corneal wound healing in rat eyes with debrided corneal epithelium instilled with IMC_{nano} eye drops is significantly better than that of eyes instilled with commercially available IMC eye drops. In addition, the accumulation of IMC in HCE-T cells treated with the IMC_{nano} eye drops for 30 min was 19.9% that of the accumulation from commercially available IMC eye drops. On the other hand, the corneal penetration of IMC from IMC_{nano} eye drops was significantly greater than in the case of the commercially available IMC eye drops in both *in vivo* and *in vitro* studies using rabbit corneas. Taken together, we hypothesize that a nanoparticle formulation reduces the corneal toxicity of IMC eye drops, probably because the accumulation of IMC from IMC_{nano} eye drops in the eye is lower than that from commercially available IMC eye drops. In addition, the nanoparticle formulation may allow a decrease in the amount of IMC used due to the increase in bioavailability, resulting in reduced drug toxicity. These findings provide significant information that can be used to design further studies aimed at developing less toxic eye drops.

1. Introduction

In cataract surgery, advanced surgical techniques such as phacoemulsification, capsulorhexis, small clear corneal incisions, improved viscoelastics and foldable implants have helped optimize postoperative results and reduce surgical trauma; however, postoperative inflammation may still occur (Alió et al., 1996; Pande et al., 1996; Laurell et al., 1998). The extraction of cataracts increases the concentration of prostaglandins (PGs) E and F in the aqueous humor, resulting in hyperemia, miosis and breakdown of the blood-aqueous barrier (Camras and Miranda, 1989). It is known that complications from cataract surgery increase when miosis occurs (Nichols and Snyder, 1998), and although steroidal agents are often used to control postoperative ocular inflammation (Korenfeld et al., 2008; Lorenz et al., 2008; Comstock et al., 2011), they are associated with adverse side-effects such as increased intraocular pressure (IOP), increased susceptibility to microbial infections, as well as delayed corneal epithelial and stromal wound healing (McGhee et al., 2002). Therefore, the use of non-steroidal anti-inflammatory drugs (NSAIDs) has increased over the past two decades. The main advantage of using topical NSAIDs is the avoidance of the undesirable effects of steroidal agents, which include a decreased immunological response to infection, cataract formation, steroid-induced increases in IOP, and the inhibition of re-epithelialization following epithelial denudation (Araújo et al.,

Abbreviations: Abs, absorbance; BAC, benzalkonium chloride; CA-IMC, commercially available indomethacin; CFU, colony-forming units; Eq, equation; HCE-T, human cornea epithelial cell line; HPβCD, 2-hydroxypropyl-β-cyclodextrin; IMC, indomethacin; IOP, intraocular pressure; mannitol, D-mannitol; MC, methylcellulose; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin.

* Corresponding author. Tel.: +81 6 4307 3640; fax: +81 6 6730 1394.
E-mail address: itoyoshi@phar.kindai.ac.jp (Y. Ito).

http://dx.doi.org/10.1016/j.tox.2014.02.012
0300-483X/

2009). Furthermore, preoperative treatment with NSAIDs has been shown to be effective in maintaining mydriasis during cataract surgery (Nichols and Snyder, 1998). The mechanism of the action of NSAIDs is dependent on their ability to inhibit cyclooxygenase and thereby inhibit the production of PGs in response to surgical trauma (Podos, 1976).

Indomethacin (IMC), [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetic acid, molecular weight 357.8, pK_a 4.5, is practically insoluble in water. Topically applied indomethacin, an NSAIDs, is used in the management and prevention of ocular inflammation (Mochizuki et al., 1977; Sanders et al., 1982), cystoid macular edema related to cataract surgery (Miyake, 2008) and the maintenance of mydriasis during cataract surgery (Dubé et al., 1990). Other common uses are for reducing discomfort after refractive surgery or for allergic conjunctivitis. On the other hand, the clinical use of its most commonly marketed eye drop formulation is limited due to topical side-effects that include burning sensation, irritation, and epithelial keratitis (Calvo et al., 1996). It is known that decreasing direct cellular stimulation and reducing the amount used by increasing bioavailability are usefulness for improving these issues (Ammar et al., 2009). In order to overcome these side-effects and increase ocular drug bioavailability, several strategies, including the preparation of viscous solutions, micro/nanoparticles and hydrogels, have been developed and investigated (El-Kamel, 2002; Sultana et al., 2006; Diebold et al., 2007; Asasutjarit et al., 2011; Gupta et al., 2011; Casolaro et al., 2012; Li et al., 2012). In the case of viscous solutions, numerous studies have demonstrated that they do not possess sufficient mechanical strength to resist the ocular clearance mechanism, and offer only a transient improvement in ocular residence time (Davies et al., 1991). Recently, it has been reported that the drug penetration capability across the cornea can be significantly improved by decreasing the particle size of the drug using nanoparticles (Rafie et al., 2010; Gupta et al., 2011; Li et al., 2012). Implants fabricated using the biodegradable polymer PLGA [poly(DL-lactide-co-glycolide)] with mean particle diameters of 50–200 nm have been widely utilized as carriers for bioactive molecules and present a possible solution to the limitations surrounding ocular drug penetration (Cohen et al., 1991; Tomoda et al., 2011, 2012a,b). It is expected that ophthalmic drug systems using nanoparticles may provide an alternative strategy for decreasing corneal stimulation and increasing ocular drug penetration (Cohen et al., 1991; Tomoda et al., 2011, 2012a,b). Our previous report showed that dispersions containing tranilast nanoparticles prepared by a bead mill method caused less corneal damage to human corneal epithelium cells, and enhanced corneal penetration than commercially available tranilast eye drops (RIZABEN® eye drops) (Nagai and Ito, 2014; Nagai et al., 2014). It is possible that decreasing corneal damage and enhancing the transcorneal penetration of IMC will increase its effectiveness against ocular inflammation (as can occur in uveitis and after cataract surgery), and lead to an expansion of its usage for therapy in the ophthalmologic field.

In this study, we designed new ophthalmic formulations containing IMC solid nanoparticles, and investigated the effect of these ophthalmic formulations on corneal toxicity. In addition, we demonstrated the corneal permeability of ophthalmic formulations containing IMC solid nanoparticles.

2. Materials and methods

2.1. Animals and materials

Male Wistar rats, 7 weeks of age, and rabbits, 2.5–3.0 kg, were housed under standard conditions (12 h/d fluorescent light (07:00–19:00), 25 °C room temperature), and allowed free access to a commercial diet (CE-2 and CR-3, Clea Japan Inc., Tokyo, Japan) and water. All procedures were performed in accordance with the Kinki University Faculty of Pharmacy Committee Guidelines for the Care and Use of Laboratory Animals and the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. 2-Hydroxypropyl-β-cyclodextrin (HPβCD, average molar substitution, 0.6; average MW, 1380) was purchased from Nihon Shokuhin Kako Co. Ltd. (Tokyo, Japan). Low-substituted methylcellulose (MC, METOLOSE SM-4, average viscosity, 4 Pa s at 20 °C) was provided by Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan). Commercially available 0.5% IMC eye drops (INDOMELOL® ophthalmic solution 0.5%, CA-IMC eye drops), 0.1% pranoprofen eye drops (NIFLAN® ophthalmic solution 0.1%) and 0.1% bromfenac sodium hydrate (BRONUK® ophthalmic solution 0.1%) were obtained from Senju Pharmaceutical Co., Ltd.; benzalkonium chloride (BAC) was provide by Kanto Chemical Co. Inc. (Tokyo, Japan). IMC and mannitol (D-mannitol) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Commercially available 0.1% diclofenac eye drops (DICLOD® ophthalmic solution 0.1%) and 0.1% nepafenac eye drops (NEVANAC® ophthalmic suspension 0.1%) were obtained from WAKAMOTO Co. Ltd. (Tokyo, Japan) and ALCON Japan Ltd. (Tokyo, Japan), respectively. All other chemicals used were of the highest purity commercially available.

2.2. Preparation of ophthalmic dispersions containing IMC nanoparticles

IMC nanoparticles were prepared using zirconia beads and Bead Smash 12 (a bead mill, Wakenyaku Co. Ltd., Kyoto, Japan). The zirconia beads (diameter: 2 mm) were added to the IMC microparticles (solid, original IMC) containing BAC, mannitol or MC, and the mixture was crushed with the Bead Smash 12 for 30 s (3000 rpm, 4 °C). The mixture was dispersed in saline with or without 5% HPβCD, and crushed with the Bead Smash 12 (5.500 rpm, 30 s × 15 times, 4 °C) using zirconia beads (diameter: 0.1 mm). The compositions of the dispersions containing IMC are shown in Table 1. 0.5% IMC is equivalent to 14.0 mM IMC; the pH was 6.5 for both ophthalmic dispersions containing IMC micro- or nanoparticles. The particle size and image were obtained using a nanoparticle size analyzer SALD-7100 (Shimadzu Corp., Kyoto, Japan; refractive index 1.60-0.10i) and scanning probe microscope SPM-9700 (Shimadzu Corp., Kyoto, Japan), respectively. The image of IMC_{nano} was created by combination of a phase and height image using image analysis software connected to the SPM-9700. The solubility of IMC in saline containing BAC, mannitol, MC and 5% HPβCD was 0.013% (the solubility of IMC in saline was 0.003%).

2.3. Stability of ophthalmic dispersions containing IMC

Three milliliters of ophthalmic dispersions containing IMC as described in Table 1 was incubated in 5 mL test tubes in the dark at 20 °C for 7 days, after which 50 μl of sample solutions was withdrawn from 5 mm under the surface at the indicated time intervals (total height of liquid, 4 cm). The IMC concentrations in the samples were determined by the following HPLC method. Fifty microliters of filtrate was added to 100 μl methanol containing 0.1 μg propyl p-hydroxybenzoate (internal standard), and the mixture was filtered through a Chromatodisk 4A (pore size 0.45 μm, Kurabo Industries Ltd., Osaka, Japan). The solution (10 μl) was injected into an Inertsil® ODS-3 (3 μm, column size: 2.1 mm × 50 mm) column (GL Science Co. Inc., Tokyo, Japan) on a Shimadzu LC-20AT system equipped with a column oven CTO-20A (Shimadzu Corp., Kyoto, Japan). The mobile phase consisted of acetonitrile/50 mM acetic acid (40/60, v/v) at a flow rate of 0.25 mL/min; the column temperature was 35 °C, and the wavelength for detection was 254 nm.

2.4. Antimicrobial activity of dispersions containing IMC nanoparticles

Dispersions containing IMC nanoparticles (IMC_{nano}) as described in Table 1 were tested for antimicrobial activity against *Escherichia coli* (ATCC 8739). The organism was selected based on Japanese Pharmacopoeia (JP) test protocols (Yakuji Nippo Ltd., 2011). According to the standard methodology, the bulk dilution was split into 10 mL aliquots, each of which was inoculated with between 10^5 and 10^6 colony-forming units (CFU)/mL of *E. coli* (ATCC 8739) (1 organism per aliquot) and incubated in the presence of vehicle (solution containing 0.001% BAC, 0.5% mannitol, 5% HPβCD and 0.5% MC) or IMC-containing dispersions at 20–25 °C. The inoculated samples were sampled and counted on days 2, 7, 14 and 28. One milliliter aliquots were serially diluted in phosphate buffer, plated in duplicate on soybean-casein digest agar (casein soya bean digest agar for JP general test, Wako, Osaka, Japan), and incubated at 31 °C for 3 days. Raw data counts were converted to log (CFU) values. Since the samples were diluted at least 1:10 at the time of testing, 10 CFU reduction is the lowest sensitivity allowed by the test.

2.5. Image analysis of corneal wound healing in rats instilled with dispersions containing IMC nanoparticles

Rats were anesthetized with isoflurane, and a patch of corneal epithelium was removed with a BD Micro-Sharp™ (blade 3.5 mm, 30°, Becton Dickinson, Fukushima, Japan), as described previously (Nagai et al., 2009). The areas of debrided corneal epithelium were as follows: vehicle, 12.19 ± 0.51 mm^2; IMC_{micro}, 12.30 ± 0.76 mm^2; IMC_{nano}, 12.37 ± 0.63 mm^2; CA-IMC eye drops, 12.54 ± 0.59 mm^2 (mean ± S.E. for 5 independent rat corneas). Forty microliters of eye drops was instilled into the eyes of rats subjected to corneal abrasion five times per day (9:00, 12:00, 15:00, 18:00 and 21:00). The eyes were kept open for approximately 1 min