Colloids and Surfaces B: Biointerfaces 143 (2016) 336–341

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Development of intranasal nanovehicles of itraconazole and their immunological activities for the therapy of rhinovirus infection

Jeong-Jun Lee[a,1], Aeri Shim[b,1], Jae Young Jeong[a], Song Yi Lee[a], Hyun-Jeong Ko[b,*], Hyun-Jong Cho[a,*]

[a] College of Pharmacy, Kangwon National University, Chuncheon 200-701, Republic of Korea
[b] Laboratory of Microbiology and Immunology, College of Pharmacy, Kangwon National University, Chuncheon, Republic of Korea

ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form 11 March 2016
Accepted 17 March 2016
Available online 18 March 2016

Keywords:
Intranasal delivery
Itraconazole
Microemulsion
Rhinovirus
Therapy

ABSTRACT

Itraconazole (ITZ)-loaded microemulsion (ME) systems for intranasal (IN) delivery were developed for the treatment of human rhinovirus serotype 1B (HRV1B) infection. ITZ was incorporated into the oil-in-water (o/w) ME formulation composed of benzyl alcohol (oil), Cremophor EL (surfactant), Solutol HS15 (cosurfactant), and water. The optimized composition of ME was determined by constructing pseudo-ternary phase diagram. ITZ ME formulation with about 150 nm mean diameter and spherical shape was prepared and the solubility of ITZ in blank ME was markedly improved (up to 13.9 mg/mL). The initial value of droplet size was maintained with four times dilution in the aqueous buffer and 72 h incubation. Released amounts of drug from ME formulation were significantly enhanced compared to drug suspension group ($p < 0.05$). Particularly, ITZ ME group displayed lower levels of inflammatory markers in the lung compared to ITZ suspension group after their IN administration in the HRV1B-infected mouse model ($p < 0.05$). Developed ITZ ME formulation via IN route can be a promising candidate for the treatment of rhinovirus infection.

1. Introduction

An intranasal (IN) route has gained many attentions as a local drug administration route for the therapy of rhinitis, sinusitis, and other diseases in the nasal cavity and its adjacent areas [1]. As the nasal mucosa has relatively large absorption area (approximately 150–160 cm^2) and high vascularization [2], IN route can be regarded as an alternative administration route for systemic application. Feasibility of direct nose-to-central nervous system delivery and non-invasiveness are also regarded as the merits of IN delivery [3]. Particularly, drugs administered via IN route can avoid the first-pass effect in the intestine and liver, thus it can improve the bioavailability of those drugs [3]. However, limited volume of the nasal cavity, drug elimination by mucociliary clearance, and anatomical structure of nasal cavity can restrict the administration of drugs with high clinical dose and chronical use [2]. To overcome these drawbacks, various IN formulations have been investigated [4]. Among them, colloidal drug carriers, such as microemulsion (ME), have been used for the enhancement of solubilization and mucosal absorption of drugs [5,6]. Oil and surfactants included in the ME formulation can improve the solubility of poorly water-soluble drug and its penetration across the nasal mucosa. The liquid state of ME formulation provides several advantages, such as a rapid onset time, precise dosing by specialized devices, and low irritation to the mucosa, compared to solid formulations.

Itraconazole (ITZ) is a triazole antifungal agent with a broad spectrum of activity for blastomycosis, sporotrichosis, histoplasmosis, and onychomycosis. That is due to the inhibition of fungal-mediated synthesis of ergosterol. Recently, it is known that ITZ can inhibit the hedgehog signaling pathway and angiogenesis, therefore it can be used as an anticancer agent [7–9]. In addition, its antiviral activities against several virus types were also demonstrated [10,11]. In spite of its diverse pharmacological efficacies, the physicochemical properties of ITZ have confined its administration with a clinical dose. It is known that its solubility is less than 1 μg/mL at neutral pH and approximately 4 μg/mL at pH 1, respectively [12–15]. Though its commercial formulation (Sporanox) has been marketed, a variety of formulations, such as emulsion, nanostructured lipid carrier, spray-dried powder, and polymeric micelle, have been developed to improve its solubility and its efficient applications [13,15–18].

* Corresponding authors.
E-mail addresses: hjko@kangwon.ac.kr (H.-J. Ko), hjcho@kangwon.ac.kr (H.-J. Cho).
[1] These authors have equally contributed to this work.

http://dx.doi.org/10.1016/j.colsurfb.2016.03.050
0927-7765/

Herein, we report about the development and evaluations of ITZ ME for the treatment of rhinovirus infection. As the volume of nasal cavity is limited, the solubility of ITZ was obviously elevated to meet the clinical dose using ME formulation. ME system based on benzyl alcohol (oil), Cremophor EL (surfactant), Solutol HS15 (cosurfactant), and water was prepared for IN delivery of ITZ. The properties of ITZ ME and its antiviral activities were also assessed in *in vitro* and *in vivo* models.

2. Materials and methods

2.1. Materials

ITZ and sodium dodecyl sulfate (SDS) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Solutol HS15, Cremophor EL, and benzyl alcohol were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). The human rhinovirus serotype 1B (HRV1B) was obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). All other reagents were of analytical grade.

2.2. Preparation and characterization of ITZ-loaded ME

The solubility of ITZ in benzyl alcohol (oil phase), Cremophor EL and Solutol HS15 (surfactant and cosurfactant; 5:1, w/w), and distilled water (DW) was determined using high-performance liquid chromatography (HPLC) system equipped with a pump (PU-2089 Plus; Jasco, Tokyo, Japan), an automatic injector (AS-2050 Plus; Jasco), and a fluorescence detector (FP-2020; Jasco). A reverse phase C18 column (Gemini, 250 mm × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA) was used and the mobile phase was composed of acetonitrile, water, and triethylamine (75:25:0.05, v/v/v; pH 7.0). The eluent was monitored at 260 nm (excitation) and 365 nm (emission) wavelengths, respectively. The flow rate was 1 mL/min and the injection volume was 20 μL. The inter- and intra-day variances of the measurements were within the acceptable range.

The composition of ME (oil, surfactant mixture, and DW) was determined based on the pseudo-ternary phase diagram. It was constructed using a water titration method. Surfactant (Cremophor EL) and cosurfactant (Solutol HS15) were mixed in a 5:1 weight ratio. Oil (benzyl alcohol) and surfactant mixtures (S/CoS) were completely mixed in different weight ratios from 1:9 to 9:1. In each ratio of oil and S/CoS, DW was gradually added and the change of color, between transparency and opaqueness, was recorded. By plotting the points corresponding to the boundary between transparency and opaqueness, ME region (monophasic area) was assigned [19,20].

ITZ ME formulation was prepared according to the composition, which belongs to the ME region, as shown in Table S1. ITZ, at 10-fold higher concentration compared to final concentration, was dissolved in benzyl alcohol. The mixture of Solutol HS15 and Cremophor EL was added to benzyl alcohol containing ITZ. Then, DW was blended with that mixture of benzyl alcohol, surfactants, and drug.

The particle size, polydispersity index, and zeta potential of ME were measured by electrophoretic light scattering (ELS) method (ELS-Z1000; Otsuka Electronics, Tokyo, Japan) according to the manufacturer's protocol.

The morphology of ME was examined using energy filtering-transmission electron microscope (EF-TEM). Aliquots of ITZ ME placed on the copper grids with films were stained with 2% (w/v) phosphotungstic acid, washed out with DW, and dried under the gentle air stream. Samples were observed using EF-TEM (LEO 912AB Omega; Carl Zeiss, Oberkochen, Germany).

2.3. In vitro *stability of ITZ ME*

In vitro stability of developed ME was measured according to the dilution with the simulated fluids in the nasal cavity. ME formulation was diluted with phosphate buffered saline (PBS, pH 6.4 adjusted with phosphoric acid) and its particle size was measured by ELS method. Drug concentration-dependent stability of developed ME was evaluated by measuring particle size. For preparing determined final ITZ concentrations (1, 2.5, and 5 mg/mL), corresponded ITZ was dissolved in benzyl alcohol and ME was formulated by described method. The mean diameter of oil droplet in different ME samples was measured by ELS method.

2.4. In vitro *drug release*

The release patterns of ITZ from suspension and ME groups were evaluated in pH 1.2 buffer including 1% (w/v) SDS. ITZ ME (5 mg/mL, 0.15 mL) or ITZ (5 mg/mL) suspended in DW (0.15 mL) was loaded into the mini GeBA-flex dialysis tube (14 kDa molecular weight cut-off; Gene Bio-Application Ltd., Kfar Hanagide, Israel). Then, this dialysis tube was immersed in pH 1.2 buffer containing 1% (w/v) SDS (15 mL) and it was incubated in a shaking bath at 37 °C with 100 rpm agitation speed. Aliquots (200 μL) of dissolution media were collected at determined times (15, 30, 60, 90, 120, 240, and 360 min), and an equal volume of fresh media was supplemented at each time point. The released amounts of ITZ from ME formulation were quantitatively determined by described HPLC method in Section 2.2.

2.5. Animal studies

BALB/c mice (female, 4 weeks old) were purchased from Koatech Bio (Pyeongtaek, Korea). Mice were reared in experimental facility at the Kangwon National University and the experiments were approved by the Institutional Animal Care and Use Committees of the Kangwon National University (KW-140811-2). Mice were infected with 20 μL of 1×10^8 50% tissue culture infective dose ($TCID_{50}$)/mL HRV1B intranasally. ITZ suspension (susp) or ITZ ME, at a dose of 10 mg/kg, was administered intranasally to mice 1 h before HRV1B infection. After 8 h HRV1B infection, lungs were dissected from the mice and homogenized for obtaining supernatants of the lung. The levels of cytokines and chemokines in the supernatant of the lung were determined according to the manufacturer's instructions. The levels of cytokines and chemokines were measured by enzyme-linked immunosorbent assay (ELISA). ELISA kits for chemokine (CC motif) ligand 2 (CCL2) (monocyte chemotactic protein 1; MCP1), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were purchased from e-Bioscience (eBioscience, San Diego, CA, USA), and ELISA kit for keratinocyte chemoattractant (KC) [chemokine (C-X-C motif) ligand 1; CXCL1] was obtained from R&D Systems (Minneapolis, MN, USA). The absorbance was then read at 450 nm using a SPECTRA MAX 340 (Molecular Devices, Palo Alto, CA, USA).

Total RNA was isolated from each group using the QIAamp viral RNA Mini Kit (QIAGEN Inc, Valencia, CA, USA). Reverse transcription was performed using RNase Inhibitor, M-MLV Reverse Transcriptase 5 × Buffer, M-MLV Reverse Transcriptase, oligo(dT) 15 primer, and dNTP mixture (all from Promega, Madison, WI, USA). And quantitative real-time polymerase chain reaction (PCR) was conducted using THUNDERBIRD™ SYBR qPCR Mix (Toyobo Co., Ltd., Osaka, Japan). Real time PCR was performed with following sequences, HRV5′-NCR-up:5′-TCCTCCGGCCCCTGAATG-3′, HRV5′-NCR-down:5′-GAAACACGGACACCCAAAG-3′, using the CFX96 Touch™ Real-Time Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA).