Journal of Pharmaceutical Sciences xxx (2016) 1–8

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Research Article

Zolpidem Mucoadhesive Formulations for Intranasal Delivery: Characterization, *In Vitro* Permeability, Pharmacokinetics, and Nasal Ciliotoxicity in Rats

Yanfeng Wang [1], Mi Li [1], Shuai Qian [2], Qizhi Zhang [3], Limin Zhou [2], Zhong Zuo [2], Benjamin Lee [1], Melvin Toh [1], Tony Ho [1,*]

[1] *Renascence Therapeutics Limited, 2 Dai Fu Street, Tai Po Industrial Estate, New Territories, Hong Kong*
[2] *School of Pharmacy, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong*
[3] *School of Pharmacy, Fudan University, Shanghai, China*

ARTICLE INFO

Article history:
Received 27 December 2015
Revised 7 March 2016
Accepted 22 March 2016

Keywords:
bioavailability
excipients
formulation
nasal absorption
nasal drug delivery
permeability
pharmacokinetics
physicochemical properties
solubility

ABSTRACT

Zolpidem is a non-benzodiazepine hypnotic for the treatment of insomnia characterized by difficulties with sleep initiation. Our study aimed at developing a zolpidem mucoadhesive formulation with minimal local toxicity, prolonged nasal residence time, and enhanced absorption after intranasal delivery. *In vitro* permeability studies using artificial membrane and Calu-3 cell culture model indicated efficient permeability of zolpidem. Aqueous solubility of zolpidem was found to be significantly improved by hydroxypropyl-β-cyclodextrin. Various mucoadhesive formulations were then prepared comprising zolpidem, hydroxypropyl-β-cyclodextrin, and mucoadhesive polymers such as hydroxypropyl methylcellulose, sodium carboxymethylcellulose, and sodium alginate. Pharmacokinetic studies in rats demonstrated that intranasally administered zolpidem could achieve significantly faster absorption rate and higher plasma concentration than that from oral route. In comparison with solution formulation (ZLP-S03), the optimized mucoadhesive formulation (ZLP-B01) containing 0.25% hydroxypropyl methylcellulose was found to improve C_{max} from 352.6 ± 86.0 to 555.7 ± 175.8 ng/mL, and $AUC_{0\text{-inf}}$ from 32,890 ± 7547 to 65,447 ± 36,996 ng·min/mL with mild nasal ciliotoxicity in rats.

Introduction

Insomnia has a prevalence of 20%-50% in the general western population and is characterized by difficulties in initiating sleep, disorders of maintaining sleep (frequent or long awakening), premature awakening, and feelings of non-restorative sleep.[1] The inability to have restful sleep can result in impaired daytime functioning.[2] Management of insomnia includes sleep hygiene education, cognitive behavioral therapy, and pharmacological therapy. Of these, hypnotic agent is the most commonly used treatment for insomnia.[3]

Zolpidem is a non-benzodiazepine hypnotic for the treatment of insomnia characterized by difficulties with sleep initiation. Due to the delayed sleep onset and relative long-acting period, which is associated with next-day residual effects (e.g., dizziness, drowsiness), the conventional oral tablets, including Ambien® and Ambien® CR, have to be taken daily before bedtime with at least 7-8 h remaining sleep.[4] In order to achieve a rapid sleep onset, new dosage forms by oral mucosal route, including sublingual tablets (Edluar® and Intermezzo®) and lingual spray (Zolpimist®), have been developed for management of difficulty in sleep initiation or awakenings in the middle of the night.[5-7] The onset of pharmacological action of these products, however, are not significantly superior to Ambien tablet.[8] Moreover, sublingual tablets have to be placed under the tongue for about 10 min until completely dissolved in saliva, while the bioavailability of Zolpimist oral spray is markedly affected by food.

Nasal cavity is covered by a thin and highly vascularized mucosa, with total area over 180 cm^2.[9] Drug molecules can quickly penetrate across the epithelial cell layer and directly to the systemic blood circulation without first-pass hepatic and intestinal metabolism.[10] Nasal administration can therefore be an alternative to oral administration for fast effect. In comparison to the conventional oral dosage forms, intranasal formulation can offer several advantages, such as rapid absorption, high bioavailability, and

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2016.03.035.

* *Correspondence to:* Tony Ho (Telephone: +852-2126-1102; Fax: +852-2126-1399).
E-mail address: tho@rns-therapeutics.com (T. Ho).

http://dx.doi.org/10.1016/j.xphs.2016.03.035
0022-3549/

easiness to use.[11] Such formulation is particularly suitable for as-needed or intermittent use for difficulties of sleep initialization or early awakening. However, due to nasal mucociliary clearance, substances administered intranasally are rapidly removed from the nasal cavity, with mean clearance half-life of approximately 21 min.[12] Such clearance may result in short nasal residence time, limited drug absorption, and insufficient pharmacologic effect, that is, the sleep maintenance capacity for zolpidem.[13] The mucoadhesive technology utilizes the bioadhesive properties of certain water-soluble polymers, which become adhesive on hydration, and hence can be used for targeting a drug to a particular region of the body (i.e., epithelial tissue) for extended periods of time.[14] In this study, zolpidem was formulated with various mucoadhesive polymers, in an attempt to achieve prolonged nasal retention time and enhanced absorption with minimized nasal irritation.

Experimental

Reagents and Materials

Zolpidem tartrate (EP grade) was ordered from Lu Nan Better Pharmaceutical Company, Ltd. (Shandong, China). Sodium dihydrogen phosphate and disodium hydrogen phosphate, 1-octanol, hexadecane, hexane, sodium alginate, carboxymethylcellulose sodium (0 Na), sodium chloride, and benzyl alcohol were products of Sigma-Aldrich Company (St. Louis, MO, USA). Methanol (HPLC grade) was manufactured by VWR International Ltd. (Radnor, PA). Hydroxypropyl-β-cyclodextrin (HP-β-CD, ChP grade) was ordered from Li De Chemical Company (Xi'an, China). Hydroxypropyl methylcellulose METHOCEL K100 LV (HPMC) was a gift from Shanghai Colorcon Coating Technology Limited (Shanghai, China). Sodium deoxycholate was purchased from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). MultiScreen Permeability Plate Assembly (MAPB MN3) was manufactured by Millipore Company (Billerica, MA). Pfeiffer classic nasal spray pump (0.10 mL) was obtained from Aptar Suzhou Dispensing Systems Company, Ltd. (Suzhou, China).

Calu-3 cell line was provided by American Type Culture Collection. Dulbecco's modified Eagle medium (with L-glutamine), Ham's F12 nutrient mixture (with L-glutamine), non-essential amino acid solution (100×), Hank's balanced salt solution (HBSS), trypsin-ethylenediaminetetraacetate (EDTA) (0.25%, 1 mM EDTA), penicillin-streptomycin (100 IU/mL), non-essential ammonites fetal bovine serum, and other cell culture media were purchased from Invitrogen Life Sciences (Hong Kong, China). Tissue culture flasks (75 cm^2, TC-treated) and 96-well cell culture plate were supplied by IWAKI (Tokyo, Japan). CellTiter96® AQ_{ueous} one solution cell proliferation assay ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt/phenazine ethosulfate [MTS/PES] assay) was provided by Promega Company (Madison, WI).

Solubility Measurement

Solubility of zolpidem tartrate in phosphate buffered saline (PBS) with pH ranging from 4.0 to 7.0 and HP-β-CD solution was measured at room temperature (25°C) using the "Shake Flask" method. Briefly, excess amount of zolpidem tartrate was added into a glass tube containing 3 mL of the aforementioned solvent, sonicated for 10 min, and then agitated in a rotating mixer for 48 h. The suspensions were filtered through 0.45 μm syringe filter to obtain clear and saturated solutions. Exact 80 μL of the saturated solution was immediately diluted to 100 mL using deionized water. Zolpidem content in the diluted solution was measured using the HPLC method as described in zolpidem tartrate monograph of British Pharmacopoeia (2009).[15]

Determination of Distribution Coefficient (Log D)

1-Octanol and deionized water were pre-equilibrated at room temperature (20°C) for 24 h. After separation of the 2 phases, exact 3 mL of the octanol was transferred into a screw-capped glass tube and then mixed with 3 mL of zolpidem tartrate solution (1 mg/mL in 0.05 M phosphate buffer, solution pH was adjusted by either HCl to 4.0 and 5.5 or NaOH to 7.0) at room temperature. The 2 phases were allowed to continuously equilibrate in a shaking water bath. After 24 h, the phases were left to separate and the zolpidem concentration in aqueous phase and octanol phase was measured using HPLC method as described previously. The distribution coefficient is determined according to

$$\mathrm{Log}\, D = \mathrm{Log}\left(\frac{[\mathrm{solute}]_{\mathrm{octanol}} \times V_{\mathrm{aqueous}}}{[\mathrm{solute}]_{\mathrm{aqueous}} \times V_{\mathrm{octanol}}}\right) \quad (1)$$

where $[\mathrm{solute}]_{\mathrm{octanol}}$ and $[\mathrm{solute}]_{\mathrm{aqueous}}$ are the drug concentration in octanol and aqueous phases after partition experiment, respectively. V_{aqueous} and V_{octanol} are the volume of aqueous phase and octanol phase, respectively.

Parallel Artificial Membrane Permeability Assay

As described in the previous literature, the membrane solution was prepared by dissolving hexadecane in hexane to a final concentration of 5% (vol/vol).[16] Fifteen microliters of the membrane solution was added into each donor well, and the plate stood in fume hood for 1 h until an artificial membrane layer formed. Sample solution (containing zolpidem tartrate 1 mg/mL, total 100 μL) and PBS (pH 4.5, 300 μL) were added into the donor well and acceptor well, respectively. The plate assembly was incubated at 25°C for 5 h. Zolpidem concentration in acceptor solution was quantified by the HPLC method as described previously. The permeability coefficient ($\log P_e$) was calculated by the following equation:

$$\log P_e = \log\left\{\left(\frac{V_d \cdot V_a}{(V_d + V_a)\cdot S \cdot t}\right)\cdot\left(-\ln\left(1 - \frac{C_a}{C_e}\right)\right)\right\} \quad (2)$$

where V_d and V_a are the volumes in the donor and acceptor wells, respectively; S is the "effective area" of the membrane (0.3 cm^2); t is the incubation period; C_a is the drug concentration in acceptor well at the end of permeability experiment; C_e is the theoretical equilibrium drug concentration after incubation of infinite period, and can be obtained from the initial amount of drug added in the donor well and the total volume of donor and acceptor wells.

In Vitro *Cytotoxicity and Permeability Using Calu-3 Cell Culture Model*

Cell Culture

Calu-3 cells were cultured in 75 cm^2 flasks using 15 mL culture medium and maintained in a humidified, 5% CO_2 atmospheric air incubator at 37°C. The culture medium consisted of 500 mL Dulbecco's modified Eagle medium/F-12 (1:1, containing L-glutamine and 2.438 g/L sodium bicarbonate), 50 mL fetal calf serum, 5 mL non-essential amino acid solution (×100), and 5 mL penicillin/streptomycin solution. The culture medium was changed every other day. Cells reaching 70%-80% confluence were sub-cultured by 2-3 ml, 0.25% Trypsin-EDTA solution.

MTS/PMS Assay

Cell number and viability were determined by Trypan blue exclusion. Cell suspension was diluted to 5×10^5 cells/mL and