European Journal of Pharmaceutics and Biopharmaceutics 83 (2013) 174–183

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Influence of polymeric microcarriers on the *in vivo* intranasal uptake of an anti-migraine drug for brain targeting

Elisabetta Gavini [a], Giovanna Rassu [a], Luca Ferraro [b], Sarah Beggiato [b], Amjad Alhalaweh [c], Sitaram Velaga [c], Nicola Marchetti [d], Pasquale Bandiera [e], Paolo Giunchedi [a,*], Alessandro Dalpiaz [f]

[a] Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy
[b] Department of Experimental and Clinical Medicine, University of Ferrara, Ferrara, Italy
[c] Department of Health Sciences, Luleå University of Technology, Luleå, Sweden
[d] Department of Chemistry, University of Ferrara, Ferrara, Italy
[e] Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[f] Department of Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy

ARTICLE INFO

Article history:
Received 4 June 2012
Accepted in revised form 10 October 2012
Available online 12 November 2012

Keywords:
Microspheres
Zolmitriptan
Chitosan
Nasal administration
Brain targeting
In vivo studies

ABSTRACT

The objective of this study was to investigate the effect of polymeric microcarriers on the *in vivo* intranasal uptake of an anti-migraine drug for brain targeting. Mucoadhesive powder formulations consisted of antimigraine drug, zolmitriptan, and chitosans (various molecular weights and types) or hydroxypropyl methylcellulose (HPMC). Their suitability for nasal administration was evaluated by *in vitro* and *ex vivo* mucoadhesion and permeation tests. The formulations based on chitosan glutamate (CG) or HPMC were tested *in vivo* because they showed good mucoadhesive properties and altered the permeation rate of the drug. The *in vivo* results from intravenous infusion and nasal aqueous suspension of the drug or nasal particulate powders were compared. The plasmatic AUC values obtained within 8 h following intravenous administration appeared about three times higher than those obtained by nasal administration, independent of the formulations. Zolmitriptan concentrations in the cerebrospinal fluid obtained from nasal and intravenous administrations were, respectively, 30 and 90 times lower than the concentrations of the drug in the blood. Thus, nasal administration potentiated the central zolmitriptan activity, allowing a reduction in the drug peripheral levels, with respect to the intravenous administration. Among nasally administered formulations, CG microparticles showed the highest efficacy in promoting the central uptake of zolmitriptan within 1 h.

1. Introduction

Intranasal delivery of antimigraine drugs has several advantages over oral, injectable or rectal routes of administration [1]. A drug that is administered intranasally is absorbed by the highly vascular mucous membranes of the nose, which allows for rapid delivery of un-metabolised drug to the central nervous system [2–4]. The onset of action is thus considerably faster than in the case of oral administration, requiring gastrointestinal absorption. The intranasal route also provides several practical advantages, such as greater acceptability to patients because of the noninvasive mode of delivery, the ability to take medication when severe nausea or vomiting is present, and a better adverse event profile [5].

Among the intranasal medications approved by the Food and Drug Administration (FDA) for the treatment of migraine headache, dihydroergotamine, sumatriptan and zolmitriptan constitute the first line treatments. Intranasal dihydroergotamine, approved by the FDA in 1997, was originally marketed in Canada and Europe. The triptan nasal spray formulations of sumatriptan and zolmitriptan were approved by the FDA in 1997 and 2003, respectively. Clinical trial data indeed clearly demonstrate that the intranasally administered triptans provide more rapid relief than their oral counterparts. This, along with their greater acceptability to patients with concomitant nausea and vomiting, provides compelling reasons for considering their routine use [5,6].

Although absorption of zolmitriptan is initially more rapid after intranasal administration than oral administration, clinical evidences show pharmacokinetic parameters such as half-life, bioavailability and therapeutic gain for zolmitriptan were similar for nasal spray and oral formulations. An absolute bioavailability up

* Corresponding author. Department of Chemistry and Pharmacy, University of Sassari, via Muroni 23, 07100 Sassari, Italy. Tel.: +39 079 228754; fax: +39 079 228715.
E-mail addresses: eligav@uniss.it (E. Gavini), grassu@ uniss.it (G. Rassu), frl@unife.it (L. Ferraro), sarah.beggiato@unife.it (S. Beggiato), amjalh@ltu.se (A. Alhalaweh), sitvel@ltu.se (S. Velaga), mrcncl@unife.it (N. Marchetti), bandiera@uniss.it (P. Bandiera), pgiunc@uniss.it (P. Giunchedi), dla@unife.it (A. Dalpiaz).

0939-6411/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejpb.2012.10.010

to 40% was reported for both oral and nasal dosage forms [1,7–9], but the amount of drug reaching the central nervous system (CNS) is currently unknown. Therefore, there is a growing interest in developing new, reliable and effective nasal dosage forms with the aim to deliver the drug to CNS and produce therapeutic effects. The nasal spray of zolmitriptan currently employed is a simple buffer solution. Thus, current research is focused on developing novel drug delivery systems for the nose-to-brain delivery of zolmitriptan, which can offer benefits in terms of low dosage, high effective and rapid relief from migraine for patients. Several strategies have been employed to improve brain delivery of drug by intranasal administration such as addition of penetration enhancers, mucoadhesive materials or preparation of micro- and nano-particulate formulations can be counted [10,11]. Mucoadhesive microemulsion formulations for zolmitriptan were studied [7,12]. Vyas and co-workers demonstrated that intranasal administration of mucoadhesive microemulsion are more effective in drug transport as compared to the intravenously administered microemulsions, and that the rapid and larger extent of transport into the rat brain was observed following intranasal administration [12]. Recently, submicron emulsions for intranasal application has been proposed as safe, non-irritating formulations to enhance zolmitriptan absorption, as they can exhibit higher brain targeting efficiency than a simple nasal solution formulation. It was presumed that the nano-sized oil droplets in the submicron emulsion might enhance the action of the surfactants on the nasal mucosa [13,14]. In a recent work, micellar nanocarriers were developed to rapidly deliver zolmitriptan directly to the brain via the olfactory and trigeminal systems [15].

Nevertheless, liquid formulations show drawbacks such as risk of chemical and physical instability, and microbiological growth, low drug loading and limited choices of dosage forms. Also, the residence time of the liquid formulation in the nasal cavity is short as the liquid is often rinsed into the GIT or out [16]. Many of these disadvantages can be overcome by using powder-based formulations as they are ease of handling compared with liquid formulations [17]. Further, powders can also be used as carrier for proteins or other drugs, which are unstable in liquid media because of hydrolysis reactions or pH [18]. Powder-based formulations can be obtained in the desiderated size and morphology that determine the deposition site of formulation inside the nasal cavity and thereby, the site of absorption of drugs, after nasal administration [19–23]. Furthermore, there is evidence that powders are cleared more slowly than liquids from the nose in human volunteers, offering long resident time of the drug in nasal cavity [16,24]. Mucodhesive powder formulations can be characterised by longevity and higher retention effects in the desired areas by withstanding mucociliar clearance, in turn, provide an enhanced drug action and improve intranasal absorption of both systemic or brain targeted drugs [23,25–29]. In particular, Charlton and co-workers studied the use of bioadhesive polymers such as pectin and chitosan to achieve the delivery to the olfactory region and increase the residence time of the formulation on the olfactory epithelium [26,27].

The aim of this work is to investigate the effect of polymeric microcarriers on the intranasal uptake of an anti-migraine drug, zolmitriptan, for brain targeting by testing the mucoadhesive and the permeation performances of formulations both *in vitro* and *ex vivo* and studying *in vivo* pharmacokinetic. Microspheres based on chitosan and HPMC are tested to exploit their mucoadhesive and/or penetration enhancement properties for delivering the drug to the CNS. The distribution of zolmitriptan in blood and cerebrospinal fluid (CSF) after *in vivo* intranasal administration is determined for powder-based formulations compared to aqueous suspension and intravenous infusion of a zolmitriptan solution, with the aim to assess the effect of polymers in improving the brain targeting of zolmitriptan and eventually increase the bioavailability of the drug by nasal administration.

2. Materials and methods

2.1. Materials

Zolmitriptan (Zol; batch number 070701) was purchased from Haorui Pharma-Chem Inc. (New Jersey, USA). Chitosan glutamate Protasan UP G 113 (CG113; Mw, <200 kDa; deacetylation degree, 75–90%) and chitosan glutamate Protasan UP G 213 (CG213; Mw, 200–600 kDa; deacetylation degree, 75–90%) were purchased from NovaMatrix/FMC Biopolymer (Sandvika, Norway). Chitosan base (CB; Mw, 150 kDa; deacetylation degree, >85%) was purchased from SeeLab (Wesselburenerkoog, Germany). Hydroxypropyl methylcellulose (HPMC; Methocel K4 M Premium CR; 19–24% methoxyl content, 7–12% hydroxypropyl content, and 300–5,600 cPs apparent viscosity as a 2% aqueous solution) was obtained from Dow Chemical (Auburn Hills MI, USA). Mucin from porcine stomach Type II (bound sialic acid 1%) was purchased from Sigma–Aldrich (Steinheim, Germany).

Acetonitrile (LC-MS grade) and N6-cyclopentyladenosine (CPA) were purchased from Sigma Aldrich (St-Louis, MO, USA). Formic Acid (LC-MS grade) was purchased from Fluka (St. Louis, MO, USA). Ultra-pure grade water was obtained by Millipore filtration system (Billerica, MA, USA). All used solvents were of analytical grade.

2.2. Preparation of nasal powders by spray drying

The formulations employed in this work are prepared by Spray Drying [30,31]. Table 1 summarises the composition of all formulations used.

Briefly, drug and polymers to a total weight of 750 mg, in ratios described in Table 1, were dissolved in 250 ml of water adjusted to pH 5.0 using 0.25% acetic acid to aid the dissolution of the components. These solutions were spray-dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik AG, Switzerland) with an inlet temperature of 160 °C, an aspiration rate of 100%, air flow of 357 l/h and a solution feed rate of 5 ml/min.

2.3. In vitro mucoadhesion study

The mucoadhesive properties of all drug-loaded microspheres were tested by determination of the quantity of microspheres sticking to a filter paper saturated with mucin after exposure to air stream following the method set by Gavini and co-workers [32]. Briefly, a filter paper (d = 2.5 cm, A = 4.9 cm^2) was soaked with a mucin solution (2% w/v in distilled water) in a chamber with controlled humidity (90–100%) and temperature (25 °C), for 10 min. The wet filter was then transferred to a support, then 10 mg of microparticles were spread by a suitable sieve onto the disc and a stream of air (flow rate 6.37 m/s) was blown over the microparticles for 15 s. Microparticles sticking to disc surface were recovered by washing the filter with water; the volume was then adjusted to 25 mL and the amount of drug was determined by HPLC analysis. The mucoadhesion was calculated as a percentage of the Zol content in the microspheres attached to mucosa after applied air load, with respect to the amount of Zol in the microparticles used for the experiment.

The mucoadhesive property of zolmitriptan crystalline as received was evaluated for a comparison. Moreover, the *in vitro* mucoadhesion behaviour of Zol as function of mucus pHs was studied as zolmitriptan solubility is dependent on the pH of the medium as it became lower when pH value increases [11]; in this