International Journal of Pharmaceutics 512 (2016) 126–136

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Development of microparticles for oral administration of the non-conventional radical scavenger IAC and testing in an inflammatory rat model

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ARTICLE INFO

Article history:
Received 24 June 2016
Received in revised form 2 August 2016
Accepted 11 August 2016
Available online 12 August 2016

Keywords:
Solid lipid microparticles
Spray congealing
Radical scavenger
Iac
Oral administration
Inflammation
Oedema
Pain

ABSTRACT

The bis (1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decandioate (IAC), is an innovative non- radical scavenger used with success in numerous disease models such as inflammation, neurological disorders, hepatitis and diabetes. The pharmacological treatments have been performed by the intraperitoneal route of administration, representing to date, the main limit for the drug use. The aim of this study was to develop a delivery system that allows the oral administration of IAC while maintaining its therapeutic efficacy. Solid Lipid Microparticles (SLMs) containing a theoretical 18% (w/w) of IAC have been produced by the spray congealing technology; three formulations have been tested (A, B and C) using different low melting point carriers (stearic acid, Compritol® HD5ATO and carnauba wax) alone or in combination. All IAC loaded SLMs exhibited a spherical shape, encapsulation efficiency higher than 94% and particle size suitable for the oral route. Administered *per os* at different dosages in an inflammation rat model, all SLMs demonstrated their efficacy in reducing oedema and alleviating pain, compared to the gold standards Indomethacin and Paracetamol. These results suggested that the SLMs are an efficacious delivery system for the oral administration of IAC, potentially useful for the treatment of others diseases related to an over production of free radicals.

1. Introduction

The bis (1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decandioate (IAC) is an original synthetic compound which has been synthesized for the first time in the laboratories of the University of Bologna (Paolini et al., 1996; Valgimigli et al., 2001). IAC is an innovative non-peptidyl low-molecular weight radical scavenger which is characterized by its unique activity in rapidly reacting with the majority of radical species involved in the oxidative stress. IAC is highly water-soluble in its protonated chlorhydrate form, membrane permeant and acts as an effective and self-regenerating intracellular scavenger of most oxygen, nitrogen and carbon-centered radicals at an early stage, *i.e.* prior to the generation of ROS-, RNS and RCS-derived toxic products (Valgimigli et al., 2001). This property has been shown to be of interest in several pathological conditions and disease mechanisms, such as neurological disorders, hepatitis, irritable bowel disease and diabetes, all related to an over production of free radicals (Canistro et al., 2010; Carnevale et al., 2011; D'Aleo et al., 2009; Mancarella et al., 2008; Novelli et al., 2007, 2010, 2014; Nurmi et al., 2008; Puoliväli et al., 2011; Vasina et al., 2009, 2010). In particular, the beneficial effect of IAC against inflammation, with a reduction of oedema diameter and cytokines production, likely dependent on significant decrease of oxidative stress in cells, as well as against ulceration induced by Indomethacin, was also reported (Corsi, 2010; Corsi et al., 2011; Zavatti et al., 2009).

However, due to the breakage of the IAC aliphatic chain by gastric acidity, all the pharmacological treatments have been performed by the intraperitoneal route of administration, representing to date, the main limit for the drug use. The oral availability

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http://dx.doi.org/10.1016/j.ijpharm.2016.08.024
0378-5173/

of IAC could be very useful as this route of delivery is by far most popular, with over 80% of medicines being given by mouth, mainly because it is the simplest, most convenient and the safest means of drug administration.

The aim of this study was therefore, to develop a successful delivery system which allows the oral administration of IAC while maintaining its antioxidant properties. In particular the pharmaceutical form should both protect the IAC in the gastric fluid and control the IAC release in the gastrointestinal tract in order to reduce the number of administrations. The selected approach involved the investigation of a micro-scale multiparticulate dosage form which provides many advantages over single-unit systems: dosing flexibility, transport in the gastrointestinal tract independent of gastric empting, less absorption variability (Vervaeck et al., 2013). Among the range of microparticulate systems, we focus our efforts on developing solid lipid microparticles (SLMs). SLMs containing IAC have been produced by the spray congealing technology using tree low melting point carriers having different chemical composition and properties: stearic acid (a C18 fatty acid), Compritol® HD5 ATO (a polyoxylglycerides) and carnauba wax (a mixture of esters of fatty acids and long chain alcohols). Stearic acid has been selected as a pH-dependent excipient able to resist in acid environment, while Compritol® HD5 ATO and carnauba wax have been utilised to obtain a sustained release (Albertini et. al., 2014). IAC loaded microparticles were produced and characterized with regard to morphology, particle size and actual drug loading.

To test the effectiveness of the IAC loaded microparticles, an inflammation rat model was used as a tool and oedema and pain as final endpoints in comparison to Indomethacin and Paracetamol as gold standards. It is indeed widely recognised that, when a tissue-injury occurs, a series of chained events are trigged, such as an increase in microvascular permeability, that in turn leads to oedema formation, inflammatory response, and hyperalgesia. A leading role in these responses is mainly covered by inflammatory lipid metabolites, ROS, and cytokines (Loram et al., 2007). It is also well known that ROS production affects the modulation of inflammatory reactions where a superoxide anion released by leukocytes represents a defence mechanism against pathogens and regulates the recruitment of macrophages, PMNs, and mast cells in the inflammation sites. ROS themselves could act as a second messenger, since they are able to activate the inflammatory signal transduction mediated by MAPK (Haddad and Land, 2002). The tight link between ROS and inflammation, coupled with the association between ROS overproduction and the depletion of endogenous glutathione (GSH), encouraged to consider IAC as a possible challenge for a novel anti-inflammatory compound (Corsi et al., 2011).

Finally, the SLMs having the best *in vivo* performance were evaluated as concern the stability of IAC both during the manufacturing of the microparticles and during the storage at different conditions over 6 months.

2. Materials and methods

2.1. Materials

IAC and the nitroxide II (called Prostab) which is produced by oxidation of IAC were kindly supplied from Sigma-Aldrich (St. Louis, MO, USA), behenoyl polyoxyl-8 glycerides (Compritol® HD5 ATO, melting point of ca. 64 °C), generously donated from Gattefossè (Milan, Italy), stearic acid (melting point of ca. 69 °C) and carnauba wax (melting point 82–86 °C), purchased from ACEF Spa (Piacenza, Italy), were used as low melting point excipients in the manufacturing of IAC loaded solid lipid microparticles. Colloidal silicon dioxide (Aerosil 200) was kindly supplied by Evonik Industries (Germany). Freund's complete adjuvant was purchased by Sigma-Aldrich (St. Louis, MO, USA). All other materials and reagents were of analytical grade.

2.2. HPLC method for the analysis of IAC and Prostab

A reverse phase HPLC method was developed for the quantification of the active ingredient and the relative oxidation product Prostab. The HPLC system consisted of two mobile phase delivery pumps (LC-10ADvp, Shimadzu, Japan) and a UV–vis detector (SPD-10Avp, Shimadzu, Japan). An autosampler (SIL-20A, Shimadzu, Japan) was used to inject samples (20 μl) onto a Kinetex 5 μm C18 column (150 mm × 4.60 mm; Phenomenex, Bologna, Italy). The mobile phase comprised of 0.02 M of potassium dihydrogen phosphate buffer with a pH adjusted at 6.6 by adding drop by drop a 1 M KOH solution and methanol (20:80, V/V). The flow rate was 1 ml/min and the detection wavelength was set at 215 nm. Retention times of Prostab and IAC and were 8 and 10 min, respectively. Standard solutions were prepared by dissolving 15 mg of IAC or Prostab in 20 ml of methanol. Quantitation of both molecules was carried out by integration of the peak areas using the external standardization method. The following method validation characteristics were addressed for both IAC and Prostab: linearity, range, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The level amount for both molecules was analysed at least in triplicate.

2.3. Preparation of solid lipid microparticles

SLMs were produced by the spray congealing process. The compositions of the formulations are reported in Table 1. The carrier or the mixtures of carries were heated at a temperature of 10 °C above their melting point. Then, IAC (18% w/w) was added to the molten carrier and magnetically stirred for five minutes to obtain a suspension, which was then loaded into a thermostated feeding chamber placed above the wide pneumatic nozzle (WPN) of the spray congealing apparatus. WPN is an innovative external-mix two fluid atomizer already described in details in a previous paper (Albertini et al., 2008). Briefly, the main differences of WPN respect to commercial two fluid atomizers are the following: the internal diameter of the orifice is bigger than usual, being 4.5 mm, and it works in an unusual configuration. Another important aspect of WPN is that all the device is heated by two resistors connected to an inverter; in this way the atomization air is heated inside the nozzle and inlet air at an ambient temperature can be employed. Two operating parameters can be set: pressure of the air and temperature of the device. The IAC loaded microparticles were obtained setting the air pressure at 3.0 bar and the nozzle temperature at 80 °C. The atomization leads to the formation of melted droplets which then solidify during the fall in a chamber at room temperature, producing the final SLMs. The microparticles were characterized as reported in the following paragraphs, then stored at different conditions for the stability tests. The microparticles utilised for the *in vivo* experiments were then mixed with 2% w/w of colloidal silicon dioxide to increase the viscosity of the liquid vehicle (mixture of light yogurt and water, see 2.5.2) in which the SLMs were dispersed, thus avoiding their sedimentation.

2.4. Characterization of the microparticles

2.4.1. Morphology

The shape and surface characteristics of the microparticles were observed using scanning electron microscopy (SEM). Samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards, Milano, Italy) and examined using a scanning electron