Phytomedicine 19 (2012) 160–168

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

A new approach to enhance oral bioavailability of Silybum Marianum dry extract: Association of mechanochemical activation and spray congealing

N. Passerini [a], B. Perissutti [b], B. Albertini [a], E. Franceschinis [c], D. Lenaz [d], D. Hasa [b], I. Locatelli [e], D. Voinovich [b,*]

[a] Department of Pharmaceutical Sciences, University of Bologna, Via S. Donato 19/2, 40127 Bologna, Italy
[b] Department of Chemical and Pharmaceutical Sciences, University of Trieste, P.le Europa 1, I-34127 Trieste, Italy
[c] Department of Pharmaceutical Sciences, University of Padova Via F. Marzolo, 5 I- 35131 Padova, Italy
[d] Department of Geosciences, University of Trieste, Via Weiss 8, I- 34127 Trieste, Italy
[e] Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, SI-1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 2 December 2010
Received in revised form 16 May 2011
Accepted 25 June 2011

Keywords:
Silybum Marianum dry extract
Silybin
Spray congealing
Mechanochemical activation
Solid lipid microparticles
Oral bioavailability

ABSTRACT

The aim of the work was to produce a delivery system for Silybum Marianum dry extract with enhanced oral bioavailability by combining two technologies (mechanochemical activation and spray congealing). Initially, the active was coground with sodium croscarmellose in a planetary mill in order to reach an activated state more prone to dissolution. DSC, XRD, FT-IR and LD analyses showed the formation of nanosized particles of dry extract, with reduced degree of crystallinity of the main crystalline flavolignans (silybine A and B). Then, microparticles containing the activated coground and, as comparison, the corresponding physical mixture of extract and polymer and the dry extract alone were produced by spray congealing technology using Gelucire® 50/13 as a hydrophilic low m.p. carrier. Microparticles containing the activated coground were produced spherical in shape, achieved satisfactory yield and high encapsulation efficiency. These microparticles, in addition to a favourable *in vitro* solubilisation kinetic, in a preliminary *in vivo* study in five rats demonstrated their ability to improve very significantly the oral bioavailability of the main flavolignans of Silybum Marianum dry extract (silybin A and B). These results suggested that the association of mechanochemical activation and spray congealing could be considered an innovative and very useful approach to the oral delivery of Silybum Marianum. Furthermore, for the first time the possibility of successfully applying the spray congealing technology for the preparation of a herbal drug delivery system was shown.

Introduction

Standardized extracts from the fruit seeds of *Silybum marianum* (L.) Gaertn. (Milk thistle, Asteraceae) are used in humans for the treatment of a variety of acute and chronic liver diseases (Morazzoni and Bombardelli 1995). The main components of the dry extract are flavolignans, such as silybine, isosilybin, silydianin and silychristin, amongst which silybine is the main biologically active component (Kim et al. 2003a). The therapeutic use of these flavolignans is partly restricted by their insolubility in water. In particular, silybin, the main constituent, is sparingly soluble in water and spontaneously forms non-absorbable microcrystals, resulting in a limited oral bioavailability (Kim et al. 2003b). In order to overcome the biopharmaceutical challenges associated with this phytotherapeutic drug, two preparation technologies were investigated both alone and in combination: mechanochemical activation and production of microparticles by spray congealing.

First of all, the mechanochemical activation process was applied to the production in a high energy mill of coground systems composed of the herbal medicine and a common inert hydrophilic pharmaceutical excipient (crosslinked sodium carboxymethylcellulose, Ac-Di-Sol®). The mechanical stress generated by the process induced physical and/or chemical changes in the active principles, that was transformed to an activated state, more prone to dissolution (Boldyrev 2004). The polymer acts as a processing aid and helps the stabilization of the activated status of the drug (Magarotto et al. 2001).

Then, microparticles containing the coground system were produced by the spray congealing technique, using Gelucire® 50/13 as a hydrophilic low melting point carrier. The spray congealing technology consists in the atomization of a fluid into an environment maintained at a temperature below the carrier melting point (Passerini et al. 2010). The coground system is present in the fluid

* Corresponding author. Tel.: +39 040 5583106; fax: +39 040 52572.
E-mail address: vojnovic@units.it (D. Voinovich).

0944-7113/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2011.06.027

to be atomized in the form of a suspension. The atomization leads to the formation of molten droplets which then rapidly solidify upon cooling, producing the final microparticles (Killeen 1993). This approach allowed the production of non-aggregated highly spherical particles with good flowability and homogeneous dimensions (Ilić et al. 2009). Through appropriate selection of the low melting carrier, microparticles with enhanced drug release can be produced (Passerini et al. 2002).

The work presented in this paper investigates the possibility of applying, for the first time, the spray congealing technology to the preparation of microparticles for the delivery of a herbal drug.

The main goal of this research project was to evaluate the potential of combining the mechanochemical activation and the production of microparticles by spray congealing to obtain a delivery system with enhanced oral bioavailability of the main flavolignans (silybine A and B) of Silybum Marianum. In the first part of the work, the dry extract was coground with sodium croscarmellose in a planetary mill in order to reach an activated state of the dry extract more prone to dissolution. The coground was characterised by means of Differential Scanning Calorimetry, Fourier Transform Infrared spectroscopy, X-ray Powder Diffraction, Scanning electron microscopy and Laser diffraction. Subsequently, microparticles containing the pure dry extract, the physical mixture of the extract and the polymer or the activated coground were produced by the spray congealing technology using Gelucire® 50/13 as a hydrophilic low melting point carrier. Then the *in vitro* solubility was assayed. Finally the best performing systems were tested *in vivo* in a pilot study in five rats.

The combination of the two technologies appears very promising. In fact on the one hand, the very fine dimensions of the coground particles permit an easy suspension in the atomizing fluid. On the other hand, these very fine particles can suffer for scarce wettability and flowability, due electrostatic and cohesive forces. These drawbacks can be possibly overcome by conversion into spherical particles through spray congealing process. Furthermore, the presence of the surface active-polymer, used as a melting binder, could give a positive contribution to the dissolution of the activated solid particles.

Experimental

Materials

Silybum Marianum dry extract (containing 83% by wt of silybin: 39%, w/w silybin A and 44%, w/w silybin B) was kindly donated by Indena S.p.A. (Milano, Italy), while Gelucire® 50/13 was kindly supplied by Gattefossé Italy (Milano, Italy), and crosslinked sodium carboxymethylcellulose (Ac-Di-Sol®) was provided by Shilling (Milan, Italy). Quercetin pure standard was from Extrasynthese (Genay, France).

All other chemicals, of analytical grade, and solvents, HPLC grade, were provided by Carlo Erba (Milan, Italy).

Preparation of coground mixtures

The planetary mill used for the mechanochemical process was a Fritsch P5 (Pulverisette, Contardi Fritsch s.r.l., Milan, Italy). The planetary mill was equipped by four agate cylindrical grinding chambers (internal height H_v = 2.6 cm, internal radius R_v = 3.2 cm, internal volume = 27.5 cm^3) adopting agate balls (diameter 2.2 cm) as grinding media.

Applying a previously published mathematical model (Voinovich et al. 2009a), and taking into account the mill geometrical characteristics, the number of vial was fixed at 4, while the mill was loaded with 5 g per vial, and the number of grinding media (agate balls) was 6. In order to maximize the grinding effect, the maximum mill plate angular velocity was chosen (corresponding to about 10,000 rpm).

By employing these process conditions, the dry extract and Ac-Di-Sol® were previously blended in 1:1 wt proportions with a stainless steel spatula, then simultaneously coground for 1 h, stopping every 15 min to homogeneously mix the mass with a stainless steel spatula. The coground system (CG) was then stored in a desiccator at room temperature for further characterisation and processing.

Preparation of the microparticles

Microparticles were produced using the wide pneumatic nozzle (WPN), an external-mixing two fluid atomizer, already described in details in a previous paper (Albertini et al. 2008). Two operating parameters could be set: the pressure of the air and the temperature of the device. In this case, the microparticles were obtained by setting the air pressure at 2 atm and the nozzle temperature at 70 °C. The atomization led to the formation of molten droplets which then solidified within the collecting chamber.

Gelucire® 50/13 was heated at a temperature 10 °C above its melting point. The pure dry extract or the 1:1 (wt) dry extract: Ac-Di-Sol® PM or the 1:1 (wt) coground system was then added to the molten carrier and magnetically stirred to obtain a uniform suspension, which was then loaded into the thermostated feeding chamber placed above the wide pneumatic nozzle. To favour the rapid solidification of the molten droplets, the collecting chamber was cooled using a liquid nitrogen flux.

The microparticles containing the pure drug, the 1:1 (wt) dry extract: Ac-Di-Sol® PM and the 1:1 (wt) coground system were named M1, M2 and M3, respectively.

The microparticles were then sieved and the 355–500 μm size fraction, representing the 85% by wt of the samples, was stored in a desiccator at room temperature for further characterisation and analyses.

Preparation of physical mixtures

For comparison purposes, physical mixtures (PM) were prepared by manually mixing dry extract and Ac-Di-Sol® and or Gelucire® 50/13 using the same weight ratios as the coground system or the microparticles. Their composition and acronyms are listed in Table 1.

Particle morphology

The shape and surface characteristics of the starting dry extract, PM, coground system and 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 microscope (Philips XL-40, Eindhoven, NL) at 10 KV accelerating voltage using the secondary electron technique.

Particle size measurements

Particle size measurements of the starting dry extract, coground and corresponding PM were carried out using a laser diffractometer (Malvern Mastersizer 2000, Malvern, UK). Before analysis, about 10 mg of each sample were dispersed by sonication in 100 ml of silicon oil (Silico DC 245 DOW Corning).