European Journal of Pharmaceutical Sciences 97 (2017) 1–8

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Mesoporous silica nanoparticles as a new carrier methodology in the controlled release of the active components in a polypill

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ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form 17 September 2016
Accepted 1 November 2016
Available online 3 November 2016

Chemical compounds studied in this article:
Hydrochlorothiazide (PubChem CID: 3639)
Amlodipine besylate (PubChem CID: 60496)
Losartan potassium (PubChem CID: 11751549)
Simvastatin (PubChem CID: 54454)

Keywords:
Polypill
MCM-41 nanoparticles
Antihypertensive drugs
Cholesterol lowering
Diuretic

ABSTRACT

Polypill is a medication designed for preventing heart attacks through a combination of drugs. Current formulations contain blood pressure-lowering drugs and others, such statins or acetylsalicylic acid. These drugs exhibit different physical chemical features, and consequently different release kinetics. Therefore, the concentration in plasma of some of them after the release process can be out of the therapeutic range. This paper investigates a new methodology for the control dosage of a polypill recently reported containing hydrochlorothiazide, amlodipine, losartan and simvastatin in a 12.5/2.5/25/40 weight ratio. The procedure is based on mesoporous silica nanoparticles (MSN) with MCM-41 structure (MSN-41) used as carrier, aimed to control release of the four drugs included in the polypill. In vitro release data were obtained by HPLC and the curves adjusted with a kinetic model. To explain the release results, a molecular model was built to determine the drug-matrix interactions, and quantum mechanical calculations were performed to obtain the electrostatic properties of each drug. Amlodipine, losartan and simvastatin were released from the polypill-MSN-41 system in a controlled way. This would be a favourable behavior when used clinically because avoid too quick pressure decrease. However, the diuretic hydrochlorothiazide was quickly released from our system in the first minutes, as is needed in hypertensive urgencies. In addition, an increase in the stability of amlodipine and hydrochlorothiazide occurred in the polypill-MSN-41 system. Therefore, the new way of polypill dosage proposed can result in a safer and effective treatment.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide (Castelli et al., 1986; Gordon et al., 1977; Kannel and McGee, 1979; Mitchell et al., 2010; Wilson et al., 1998). Hypertension is an important risk factor for CVD. Thus, in those patients already having a cardiovascular problem, hypertension can intensify the damage (Collins et al., 1990; Ford et al., 2007; MacMahon et al., 1990). Current hypertension therapies display two key problems. First, the majority of hypertensive treatments need to take daily several drugs (Chobanian et al., 2003; D'Amico et al., 1998; James et al., 2014). Therefore, if the patient could forget to take some of the doses, decreasing the safety and increasing the side effects of hypertension. Second, too quick or too slow decrease in blood pressure produce is undesirable. Thus, an excessively rapid decrease may result in hypoperfusion of central nervous system with can yield to stroke, paraplegia, blindness or death. On the other hand, a very slow decrease is ineffective in hypertensive emergencies. Due to these facts, the maximum recommended blood pressure drop by 25% within the first 2 h of treatment to reach 160/100 mm Hg after 6 h and then to the normal blood pressure levels in the following hours or days.

To avoid possible inadvertences of the patient in taking medication doses, the multi-target drug design proposes a systemic solution, safer and with lower side effects. Thus, medications containing several active components in a single dose (Bender et al., 2006; Bolognesi, 2013; Petrelli and Valabrega, 2009) that for hypertension treatment is called polypill (Lonn et al., 2010; Sleight et al., 2006; TIP Study, 2009). For instance, Laboratorios Ferrer (Spain) commercialized Sincronium® and Trinomia® polypill containing acetylsalicylic acid to prevent heart attacks, ramipril, an angiotensin-converting enzyme inhibitor, and

Abbreviations: MSN, mesoporous silica nanoparticles; MSN-41, mesoporous silica nanoparticles with MCM-41 structure; MCM-41, Mobil composition of matter #41; MCM-41s, Mobil composition of matter #41 simplified molecular model; CVDs, cardiovascular diseases; ESC, European Society of Cardiology; HTZ, hydrochlorothiazide; AML, amlodipine; LS, losartan; SV, simvastatin; EtOH, ethanol; BET, Brunauer, Emmett and Teller method; BJH, Barrett-Joyner-Halenda equation; PSA, polar surface area; DM, dipole moment; ADME, absorption, distribution, metabolism, and excretion.

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http://dx.doi.org/10.1016/j.ejps.2016.11.002
0928-0987/

simvastatin or atorvastatin, as cholesterol lowering drugs. To reach the suitable blood pressure reduction rate, an effective controlled release of the drugs is required, which seems unlikely in the polypill currently commercialized with a conventional formulation. Thus, new research approaches are required in this field. In this sense, MSN-41was investigated as effective carrier for the controlled local release of individual active ingredients (Colilla et al., 2013; Knežević et al., 2013; Ruiz-Hernandez et al., 2011; Slowing et al., 2008; Tang et al., 2012; Vallet-Regí et al., 2001, 2007) but not for a polypill.

On the other hand, several hypertension guidelines recommend the inclusion in the treatment of a diuretic like hydrochlorothiazide (HTZ) e.g. European Society of Cardiology (2016) (ESC) guidelines. In this regards, the combination of HZT and amlodipine (AML), a calcium channel blocker, with an angiotensin inhibitor, such as benazepril, ramipril or losartan (LS), is very effective to decrease the rate of cardiovascular events (Jamerson et al., 2008). Moreover, if the polypill containing a cholesterol lowering drug, it can be reduced by 80% the risk of CVD in patients with vascular disease (Chrysant and Chrysant, 2014; Exaire-Murad et al., 2015; Gadepalli et al., 2014; Katsiki et al., 2013; Wald and Law, 2003; Yusuf, 2002). Likewise, in a prevention trial of a polypill composed by AML, LS, HTZ, and simvastatin (SV), the mean systolic blood pressure was reduced by 12%, the diastolic blood pressure by 11%, and low-density lipoprotein (LDL) cholesterol by 39% (Wald et al., 2012).

In the present work, we investigated, for the first time, the capability of a nanocarrier, based on MSN-41 silica nanoparticles, to host and release the four active components: HTZ, AML, LS and SV in 12.5/2.5/25/40 wt-ratio, contained in a polypill previously reported (Wald et al., 2012) according with the National Center of Biotechnology Information (NCBI). The objective was to reach a controlled release and a higher stability of the drugs to improve the traditional pharmaceutical formulation. For this purpose, analytical methods, molecular modelling and docking analysis were used as described elsewhere (Doadrio et al., 2010, 2014).

2. Experimental

2.1. Synthesis of MSN-41 mesoporous material

Synthesis of MSN-41 with hexagonal pore arrangement, was performed by sol-gel in the presence of structure directing agents and following a modified Stöber et al. (1968) method. Hence, to a 1 L round-bottom flask, 1 g of cetyltrimethyl-ammonium bromide (CTAB, Sigma-Aldrich, Spain) as a structure-directing agent, 480 mL of H_2O (Milli-Q) and 3.5 mL of NaOH (2 M) were added. The mixture was heated at 80 °C and stirred at 600 rpm. When the reaction mixture was stabilized at 80 °C, 5 mL of tetraethyl-orthosilicate (TEOS, Sigma-Aldrich, Spain) were added dropwise at 0.33 mL/min. The suspension obtained was stirred further 2 h at 80 °C. After filtration and washing with water and ethanol, the surfactant was removed by extraction with a NH_4NO_3 (Sigma-Aldrich, Spain) solution (10 mg/mL) in ethanol (EtOH) 95%. Finally, the product was filtered and washed 3 times with 100 mL of H_2O and with 50 mL of EtOH and dried under vacuum at 40 °C.

2.2. Drug-MSN-41 samples loading

We prepared five different drug-MSN-41 samples. Samples 1 to 4 are the result of loading each drug in MSN-41. Sample 1 (HZT-MSN-41) was hydrochlorothiazide (99.25% purity)-MSN-41; sample 2 (AML-MSN-41) was amlodipine besylate (100% purity)-MSN-41; sample 3 (LS-MSN-41) was losartan potassium (99.80% purity)–MSN-41 and sample 4 (SV-MSN-41) was simvastatin (100% purity)-MSN-41. Sample 5 was the result of the polypill containing the four component simultaneously loaded in MSN-41 (polypill-MSN-41). Polypill was prepared using a mixture of HTZ, AML besylate, potassium LS and SV in a 12.5/2.5/25/40 weight ratio. Normon Laboratories, Madrid, Spain, supplied all these products.

Samples were prepared dissolving: HTZ, 50 mg; AML, 10 mg; LS 100 mg and SV 160 mg (separately or together in the polypill) in 20 mL EtOH. Thereafter, 400 mg of MSN-41 were added with stirring for 24 h at room temperature for loading. After filtration, samples were dried in a vacuum oven at 20 °C for 24 h.

The loading process was always performed following the protocol described, which guaranteed its reproducibility. Furthermore, measurements were performed in triplicate and the indicated values correspond to the average of three measurements.

The concentration of drug adsorbed in each MSN-41 sample (C_a) was obtained by the equation:

$$C_a = C_i - C_r \quad (1)$$

where C_i is the initial concentration before loading i.e. HTZ, 2.5; AML, 0.5; LS 5 and SV 8 in mg/mL and C_r is the concentration analysed by HPLC in the residue of the drug adsorption on MSN-41. From these data, was estimated the % of adsorption for each drug.

2.3. HPLC method

RP-HPLC (Reversed Phase High Performance Liquid Chromatography) measurements were performed with a liquid chromatographic system equipped with a Waters Alliance 2695 separation module (Waters, Milford, Massachusetts, USA), a variable-wavelength diode array detector Waters 2996 and controlled by Millennium 32 software. A Zorbax Eclipse XDB-C-18 reversed-phase column (5 μm, 4.6 × 150 mm), supplied by Agilent Technologies, USA, was employed operating at 40 °C. The mobile phase was acetonitrile/Sörensen buffer at pH 3.5 (v/v) in a concentration gradient showed in Table 1. The flow rate was 1 mL/min. The effluent was monitored at 254 nm for HTZ and at 234 nm for LS, AML and SV. The injection volume was 10 μL. In these conditions, the retention time (t_r) for HTZ, AML, LS and SV was 1.79, 2.61, 4.45 and 15.8 min, respectively. Four-calibration curves using the standard drug loaded in MSN-41 were plotted using concentrations in methanol for each drug of 0.1, 0.5, 1 and 1.5 mg/mL.

2.4. Characterization

Samples were characterized by powder X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and N_2 adsorption-desorption. The XRD patterns were obtained in a Philips X'Pert MPD (Cu Kα radiation) diffractometer. The diffractograms were collected in the 2θ range of 0.6–10° with a step size of 0.02°. FTIR spectra were recorded with a Nicolet Nexus spectrophotometer in the range of 4000–400 cm^{-1} by using an ATR golden gate accessory. The surface area and pore size of the materials were determined by N_2 adsorption using a Micromeritics ASAP 2020 porosimeter. Previously, loaded samples were degassed at 100 °C for 24 h under vacuum (1.3 Pa). The pore size distribution was calculated from adsorption branches of the nitrogen isotherms using the Barrett-Joyner-Halenda (BJH) equation (Barrett et al., 1951) and the BET surface area was calculated by Brunauer, Emmett and Teller method (Brunauer et al., 1938). Furthermore, the particle morphology was analysed by scanning electron

Table 1
HPLC concentration gradient in acetonitrile (A)/Sörensen buffer at pH 3.5 (B).

Step	Time (min)	%A	%B
1	0	40	60
2	5	40	60
3	5.1	60	40
4	25	60	40
5	25.1	40	60