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Research Paper

Enhanced solubility and bioavailability of simvastatin by mechanochemically obtained complexes

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ABSTRACT

In the present work, complexes of simvastatin (SIM) with polysaccharide arabinogalactan (AG) or disodium salt of glycyrrhizin acid (Na_2GA) have been prepared using mechanochemical technique to improve the solubility of SIM and enhance its oral bioavailability. The interactions of SIM with AG or Na_2GA were investigated by FTIR, DSC, XRD and SEM. Self-association of SIM in various solvents was investigated by UV/Vis and NMR techniques. The molecular masses of supramolecular systems–inclusion complexes and micelles, which are the "hosts" for SIM molecules were measured. The parallel artificial membrane permeability assay (PAMPA) revealed a strong increasing of SIM permeability in the presence of Na_2GA in comparison with pure SIM used as a control. On the other hand, the rapid storage assay (+40 °C for 3 months) showed that the chemical stability of SIM/AG complexes was similar to pure SIM, but SIM/Na_2GA complexes had an enhanced stability. Pharmacokinetic tests *in vivo* on laboratory animals showed a significant increase in SIM's bioavailability after its introduction as a complex with Na_2GA or AG. Moreover, SIM/AG inclusion complex performed better than SIM in reducing total cholesterol level. Therefore, the mechanochemically synthesized complexes of SIM with AG or Na_2GA might have a promising future as novel formulations for hyper-cholesterolemia treatment.

1. Introduction

SIM (chemically referred to as (1S,3R,7S,8S,8aR)-8-[2-[(2R, 4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2yl]ethyl] 3,7-dimethyl-1,2,3,7,8, 8a-hexahydronaphthalen-1-yl2,2-dimethylbutanoate), an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and derived synthetically from a fermentation product of *Aspergillsterreus* (Subhan et al., 2016), is widely used to control hyper-cholesterolemia and prevent cardiovascular diseases. Additionally, this compound also has beneficial actions in many other diseases, such as osteoporotic fractures and cancers (Jadhav and Jain, 2006; Kheirallah and Almeshaly, 2016; Li et al., 2017a). Nonetheless, it is reported that SIM has low oral bioavailability (< 5%), which may be attributed to its slow dissolution rate in the gastrointestinal tract (low intestinal uptake) coupled with extensive first pass metabolism (Geboers et al., 2016). To obtain the desired therapeutic effect, large oral doses of SIM are used. SIM therapy can be accompanied by different side effects but most common of them are myopathy and rhabdomyolysis which rate is rising with dose elevation (Ucar et al., 2000; Silva et al., 2006; Magni et al., 2015). Thus, several approaches for new safer long-acting SIM with lower daily dose efficiently improving the pharmacokinetic properties have been reported, such as preparation of solid dispersions (Kim et al., 2011; Javeer et al., 2013), cyclodextrins inclusion complexes (Jun et al., 2007; Octavia et al., 2015; Terauchi et al., 2016), self-emulsifying drug delivery system (Franceschinis et al., 2015), nanoparticles (Chavhan et al., 2013), or preparation of amorphous form (Graeser

Abbreviations: AG, Arabinogalactan; API, active pharmaceutical ingredient; DSC, differential scanning calorimetry; GA, glycyrrhizic acid; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; Na_2GA, disodium glycyrrhizate; PAMPA, parallel artificial membrane permeability assay; P-gp, P-glycoprotein; PM, physical mixture; PXRD, powder X-ray diffractometry; SEM, scanning electron microscopy; SIM, simvastatin

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et al., 2008), or eutectic mixture (Górniak et al., 2013). Although a definite progress has achieved in previous research, many formulations were normally prepared as liquids that is considered unfriendly to the environment and some formulations used large quantity of surfactants which can induce gastrointestinal irritation.

In this study, immediate-release complexes of SIM were prepared by employing the solid-state mechanochemistry, which induced by the input of mechanical energy that is transferred to the solid state compounds and results in local energy accumulation in submicroscopic zones (James et al., 2012). The strain generated by the high level of mechanical energy may cause plastic deformation and concurrent changes in the crystal structure, along with crystalline phase transitions and amorphization (Boldyrev, 2004; Dushkin, 2004). Mechanochemical processing of medicinal substances with auxiliary components usually is performed by grinding in mills of different types can be used both in laboratory and in industrial scale. Recently, the use of mechanochemistry has become very attractive in the field of pharmaceutical industry to enhance the dissolution rate of poorly water soluble active pharmaceutical ingredients (APIs) (Dushkin, 2004, 2010). In fact, compared with traditional "liquid phase" method, mechanochemical process offers significant advantages such as one-stage technological process, absence of solvents or melts and respective additional procedures, lowering of unwished admixtures, high strength of formed complexes and low operating cost (Barzegar-Jalali et al., 2010; Dushkin et al., 2010a). The more recent publications confirm prospectivity of mechanochemical approach on the examples of anthelmintic, aspirin and other drugs (Chistyachenko et al., 2015a,b; Zhi et al., 2015; Zhong et al., 2013; Borba et al., 2015; Nart et al., 2015; Prabhu and Patravale, 2016).

In our article, we attempted to synthetize intermolecular complexes of SIM with polysaccharide arabinogalactan (AG) and disodium salt of glycyrrhizic acid (GA).

AG is a good water soluble polysaccharide isolated from the wood of larch Larix sibirica and Larix gmelinii, which is a natural polysaccharide polymer composed of galactose and arabinose fragments consisting of a highly substituted backbone of 1–3 linked β-D-galactopyranose units with side chains of galactose and arabinose with total molecular weight 14–20 kDa (Odonmažig et al., 1994; Trofimova et al., 2012). In this case, the branched structure of AG macromolecules is especially favorable for complexing drugs (Dushkin et al., 2008; Mikhailenko et al., 2015; Du et al., 2016). As a result of such complexation following positive changes in drugs' toxic-pharmacological properties were obtained: increase of water solubility, effective dose and toxic side effects reduction (Dushkin et al., 2012; Chistyachenko et al., 2015a,b; Khvostov et al., 2017).

GA is a good soluble natural saponin, extracted from licorice's roots. GA is a conjugate of two glucuronic acid molecules and a glycyrrhetic acid molecule and is capable of self-association in aqueous and water-alcohol solutions due to its amphiphilic properties. The structure of such aggregates depends on the GA concentration and the solution pH. GA exists mainly as a dimer at low concentrations (10^{-5}–10^{-3} M), while at high concentrations ($> 10^{-3}$ M) it forms large micelle-like aggregates with molecular weight 50–100 kDa (Dushkin et al., 2010b; Polyakov and Leshina., 2011). Indeed, GA has own spectrum of biological activity. It has long been used to treat and prevent various diseases from the common cold to stomach and duodenal ulcers. It has pronounced anti-inflammatory activity and according to recent studies it is able to induce apoptosis in cancer cells (Asl and Hosseinzadeh, 2008). Apart from its intrinsic biological activity GA forms non-covalent complexes with a variety of drugs due to its amphiphilicity. Such supramolecular complexes could increase the solubility of hydrophobic compounds up to dozens of times and, consequently, reduce the therapeutic doses of drugs (Tolstikova et al., 2009; Dushkin et al., 2010b, 2012; Polyakov and Leshina, 2011; Yang et al., 2015). In this form GA is not used as a therapeutic agent. Moreover we use small doses of GA that comparable with that used in food products and chewing tobacco where GA used as a sweetener. No examples of GA toxicity were published when it was used as a drug delivery system. In addition, GA can increase the permeability of cell membranes for small molecules (Selyutina et al., 2015, 2016a,b, 2017a,b).

Recently, the complex between SIM and GA was found to be effective to increase the bioavailability of SIM (Ragino et al., 2008; Vavilin et al., 2008). Unfortunately, the authors used liquid-phase synthesis of obtaining complexes. Our especial further analyses showed the formation of large quantities of impurities (about > 20%), which prevent using of this way for drug manufacturing. Nevertheless clean solid state mechanochemical technique for synthesis of complexes (Dushkin et al., 2012) could be a potential approach to create efficient complexation agent for drug delivery. Additionally, the advantage of Na_2GA is that it is very easily soluble in water but does not form gel in contrast with pure GA. Therefore, in this work, Na_2GA was used to enhance the solubility of SIM by clean solid state mechanochemical technique.

2. Materials and methods

2.1. Materials

SIM (Well Green Technology Co. Ltd., Xi'an, China) of pharmaceutical grade was used without further purification. AG from *Siberian Larch* wood (purity of AG > 99.5%, moisture–0.5%, the content of phenolic impurities–0.15%, MW–14,300 Da) was provided by company "Wood chemistry", Russia. Na_2GA (CFS, 98%) was purchased from Shaanxi Sciphar Biotechnology Co. Ltd., Xi'an, China. All other chemicals were of analytical grade and used without further purification.

2.2. SIM processed by planetary mill

Type of mill: AGO-2. Processing mode: acceleration of grinding media–20 *g* (free fall), mass of SIM–2.0 g, drum capacity–50 mL, grinding media-steel balls (diameter 6 mm, 75.0 g load), processing time were 5 and 10 min, respectively.

2.3. Preparation of SIM composition with excipients

Mechanical treatment of SIM compositions with AG and Na_2GA carried out in the roll mill VM-1 with cylindrical vessel which was coated with Teflon and possessed 300 mL volume. Acceleration of grinding bodies is 1 *g* (free fall). Rotational speed of cylindrical vessel is 157 rpm. Steel balls (diameter 22 mm, 675 g load) were used as grinding bodies. The total load of the treated powders mixture was 22 g. The duration of mechanical processing was from 2 to 24 h (2, 4, 8, 16, and 24 h). To prepare the complexes, we used SIM/AG mass ratio 1/10, and SIM/Na_2GA mass ratios 1/10 (molar ratio 1/5), 1/4 (molar ratio1/2) and 1/2 (molar ratio 1/1).

2.4. UV/Vis spectrophotometry

The absorption measurements was carried out on a UV/Vis spectrophotometer (SPEKS SSP-700, Spectroscopy Systems, Moscow, Russia) equipped with a conventional 1 cm path (1 cm × 1 cm × 4 cm) quartz cell. The scanned area ranges from 700 nm to 210 nm for each sample. Therein, the concentration of SIM in water was same with in alcohol and water/alcohol mixtures.

2.5. Fourier transform infrared spectrophotometry (FT-IR)

FT-IR spectra of samples were collected from 500 to 4000 cm^{-1} using Fourier spectrophotometer "Infralum FT-801" ("Simeks", Novosibirsk, Russia). All samples were taken in thin tablets with KBr.