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Albumin micro/nanoparticles entrapping liposomes for itraconazole green formulation

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ABSTRACT

Itraconazole-loaded micro/nanoparticles containing albumin and liposomes were prepared by a technological process that avoids the use of organic solvents and crosslinker agents. The particles were characterized, lyophilized and formulated as tablets. Dynamic light scattering was used to determine the hydrodynamic diameter and zeta potential of the particles; optical and scanning-electron microscopy was used to evaluate their morphology. Spherical shaped particles of different sizes and zeta potential were obtained. An exponential relationship between the zeta potential and the albumin/cationic lipid molar ratio was established. Drug entrapment efficiency values were in the range of 51–68%, with no statistical differences among albumin feeding concentrations. Mannitol was used as lyophilization additive and the freeze-dried cake was directly compressed into tablets, suitable for vaginal administration. The results from the *in vitro* drug delivery assay show the influence of albumin on the itraconazole delivery profile; a rapid release was observed for particles with higher albumin amount compared to those with lower protein content. According to the results of this study, albumin particles entrapping liposomes prove to be a green pharmaceutical vehicle with a high potential for delivery of hydrophobic and highly albumin-bound drugs.

1. Introduction

Pharmaceutical vehicles aimed at controlled drug delivery are of particular interest, not only in the area of new active ingredients, but also in that of old generation drugs whose clinical use is restricted by unfavourable biopharmaceutical characteristics. Albumin nanoparticles (ANP) have gained considerable attention because of albumin transport capacity and physiological functions, among which is colloidal osmotic pressure regulation. Besides, albumin is thought to facilitate endothelial transcytosis of plasma constituents into the extravascular space by binding to the cell surface receptor albondin to form transcytotic vesicles referred to as caveolae (Vogel et al., 2001). Several drugs are delivered to the target organs/tissues by binding to human serum albumin, which not only protects the bound drug against oxidation, but also alters their pharmacokinetic and pharmacodynamic behavior (Mita et al., 2015; Yang et al., 2014). The relevant role of albumin in intracellular drug uptake has been recently considered and the impact of protein concentration on the uptake of drugs in cells has been proved (Poulin and Haddad, 2015). The mentioned properties, together with its preferential uptake by tumors and inflamed tissue (Elzoghby et al., 2012; Kolluru et al., 2013) make albumin an appropriate ingredient for pharmaceutical drug delivery systems. Antineoplastic drugs, immunosuppressant, antibiotics, antifungals and theranostic agents are all candidates to be formulated using albumin as the drug carrier. The first albumin-based commercial product was 130-nm human serum albumin (HSA)-paclitaxel nanoparticles obtained using nab™ technology (Fu et al., 2009; Hawkins et al., 2008), which was also used to prepare curcumin-loaded HSA nanoparticles (Kim et al., 2011) and bovine serum albumin (BSA) nanoparticles containing quercetin (Antonio et al., 2016). Drug entrapment within albumin particles has also been studied for doxorubicin, and recent formulations aimed at drug and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) co-delivery have shown improved antitumor efficacy (Choi et al., 2015; Thao le et al., 2016b). In the case of tacrolimus, the results observed for drug-loaded ANP involved a reduction of nephrotoxicity and an increase in its antiarthritic activity (Thao le et al., 2016a; Zhao et al., 2015). Moreover, albumin nanoparticles have been proposed for the development of multifunctional theranostic agents (Sahu et al., 2016).

Apart from its relevant role in drug transport and targeting, albumin is also used in the pharmaceutical field to formulate hydrophobic drugs.

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Organic solvents, emulsifiers and amphiphilic molecules, most of which are not innocuous, are frequently used to deal with poorly water-soluble drugs. Cremophor® is present in several formulations but solutions containing less amounts of this product are being proposed to reduce vehicle-related side effects. To pursue this aim, formulations based on nontoxic, biocompatible and biodegradable molecules such as albumin are being developed and already successfully applied to poorly water-soluble drugs. Amphotericin-B (AmB) and triazole antifungals are being investigated as ANP for this reason. When compared with the commercial lipid-associated formulation, BSA nanoparticles appear as potential carriers of AmB, reducing its molecular aggregation and prolonging its release while maintaining its antifungal activity (Casa et al., 2015). The fact that triazole antifungals marketed as intravenous solutions contain cyclodextrins (CD), which is contraindicated in patients with impaired renal function, offers great potential to the development of formulations where this type of component is excluded (Luke et al., 2012; Oude Lashof et al., 2012). Itraconazole and voriconazole have been incorporated into HSA nanoparticles, the former showing pharmacokinetic parameters similar to those corresponding to the CD formulation in studies using mice (Chen et al., 2008), and the latter showing a water solubility over 2 times greater than the API itself (Furedi et al., 2016). Specialized nanotechnological techniques such as desolvation, emulsification, thermal gelation, nano-spray drying or self-assembly are applied for the fabrication of albumin nanoparticles (Elzoghby et al., 2012; Lee and Youn, 2016). Technological strategies avoiding crosslinking agents are of particular interest not only to exclude these agents in the final formulation but also because albumin crosslinking seems to affect the protein's behavior, since the accumulation of paclitaxel-bound-non-crosslinked ANP in tumors significantly increased compared to crosslinked ANP (Li et al., 2014). One of the advantages provided by nab™ technology and self-assembly procedures is that they do not use crosslinkers; nevertheless, the former still uses chloroform or methylene chloride and ethanol (Fu et al., 2009) and the latter requires hydrophobic modification of albumin for this to form micelle-like structures that trap hydrophobic drugs in their inner core (Kratz, 2008). Formulations above commented are intended for parenteral drug administration, nevertheless drug injection is considered troublesome due to strict regulations and the involvement of health care professionals. Delivery of drugs *via* the mucosal surfaces, including the airways and the genital tract, represents a desirable alternative to invasive delivery since these have less regulatory requirements and circumvent the liver, avoiding hepatic first-pass metabolism. For these administration routes, liposomes and other nanoparticles have been proposed (Li et al., 2012), but albumin particles have not been considered yet, despite this protein appearing as a suitable drug carrier for both, pulmonary and vaginal drug delivery (Dhand et al., 2014; Mirza et al., 2016).

Albumin microspheres containing liposomes (named albusomes) have been proposed as a green pharmaceutical vehicle for drug delivery (de Jesús Valle et al., 2016) and this was first applied to the hydrophilic and moderately albumin-bound vancomycin, although the vehicle may be applied to other drugs and/or diagnostic agents, including hydrophobic and poorly water soluble products. Itraconazole (ITZ) is currently formulated as a beta-CD inclusion complex due to its extremely low water solubility. Since this antifungal azole shows a plasma protein binding > 99% (Schafer-Korting et al., 1991; Thiry et al., 2016), the above mentioned vehicle might provide an alternative for ITZ formulation devoted to parenteral or transmucosal routes. ITZ is gaining prominence in the treatment of vulvovaginal candidiasis (VVC), since isolates from patients with recurrent VVC remain susceptible to azole-based antifungal drugs and do not show increased resistance to them despite long-term exposure (Nagashima et al., 2016). According to this, the aim of this study was the investigation of albumin particles entrapping liposomes (albusomes) as a green pharmaceutical vehicle for ITZ, as representative of poorly water-soluble drugs, and its suitability for parenteral and non-parenteral formulations.

2. Materials and methods

Egg L-α-phosphatidylcholine (EPC), lanolin cholesterol (Ch), diethyldodecylammonium bromide (DDA) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Quimica S.A. Itraconazole was purchased from Fagron Ibérica SAU and mannitol from Guinama. Acetonitrile and methanol HPLC reagents, Karl Fischer Aqualine Complete 5 and dry methanol were purchased from Fisher Chemical. Ultra-pure water was obtained using a Milli-Q A10 system (Millipore). The Chromafil® PET-45/25 (0.45 µm) syringe filters were purchased from Nacherey-Nagel.

2.1. Itraconazole solubility in the presence of albumin

The influence of albumin on ITZ solubility was studied prior to the preparation of albusomes. 7.5 mg of drug was weighed in a plastic disk and placed in a vessel containing 500 ml of medium prepared by adding albumin at different protein concentrations (0.1–4% w/v) to HCl 0.1 M. The assay was also carried out in absence of albumin as control. The USP apparatus II set at 37.0 ± 0.5 °C and a rotation speed of 100 rpm was used in a Hanson Research SR6 Validata SRII dissolution test equipment. After 24 h, a sample was extracted and split into two aliquots, one for direct quantification of ITZ and the other to be filtered by means of a Chromafil® PET-45/25 (0.45 µm, 25 mm diameter), both being analyzed using HPLC. The mixtures were then stored at room temperature (20–24 °C) for 1 week, after which they were analyzed again for ITZ quantification.

2.2. Preparation of liposomes

Cationic lipid vesicles were prepared by direct sonication of components (at a concentration of 1.73% w/w), following the previously published organic solvent-free procedure (de Jesús Valle and Sanchez Navarro, 2015). Briefly, phosphatidylcholine (EPC), diethyldodeccyiamonium (DDA) and cholesterol (Ch) were dispersed directly in Milli–Q water pre-warmed at 60 ± 2 °C, gently mixed and then placed in a Fisher Scientific FB 15061 ultrasonic bath (50 Hz) for 20 min at 60 ± 2 °C. Sonicated samples were kept at room temperature for 60 min, centrifuged (4000 rpm, 10 °C) for 10 min in a Centrikon T-24 Kontron centrifuge to confirm 100% lipid recovery and then filtered through a 0.45 µm filter. An aliquot of the filtrate was used for liposome characterization (morphology, size and zeta potential) and the rest was used to prepare drug-loaded particles.

2.3. ITZ-loaded particles

ITZ was added to HCl 0.1 M in sufficient amount to reach 15 mg/l, then albumin was incorporated at different protein concentrations (0.1–4% w/v) and kept under stirring conditions for 24 h at 37 °C. The resulting mixtures were mixed with an equal volume of the above described liposome suspension and slowly stirred for 2 h at 37 °C in a Nahita model 632/6 drying oven. After this, pH was adjusted to 7.4 with KOH, maintained under agitation for 1 h at 37 °C and finally placed for 20 h at 4 °C in a shaking water bath (Unitronic OR Selecta P). Opposite charges of albumin and liposomes combined with low temperature enable the protein and vesicles to flocculate forming particles entrapping the liposomes as well as the ITZ-bound and unbound albumin. Aliquots were taken for zeta potential and size determination, for microscopic observation and for estimation of the entrapment efficacy. The remaining sample was freeze-dried to obtain a bulk used for tablet fabrication.

2.4. Lyophilization and tablet fabrication

Mannitol was added to the above samples until a total solute concentration of 3.0% w/w. The mixtures were frozen at − 80 °C (Nuaire