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Calcium alginate microspheres containing metformin hydrochloride niosomes and chitosomes aimed for oral therapy of type 2 diabetes mellitus

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ABSTRACT

Metformin is an oral hypoglycemic agent used in the type 2 diabetes, whose poor bioavailability and short half-life make the development of effective extended-release formulations highly desirable. Different metformin-loaded chitosomal and niosomal formulations were developed and suitably characterized, but were unable to provide the desired sustained release. The entrapment of both kinds of colloidal dispersions in calcium alginate beads enabled to strongly reduce the amount of drug released at gastric level (from 18 up to a maximum of 30%), and to obtain a sustained release in simulated intestinal fluid, which was properly tuned by varying the percentage of calcium alginate in the beads. In vivo studies on rats revealed a significant improvement of metformin hypoglycemic effect when orally administered as chitosomal and even more as niosomal dispersion entrapped in alginate beads, not only with respect to the drug as such, but also to the alginate beads loaded with the plain drug. The more intense and sustained therapeutic effect with time provided by the drug-in niosomes-in alginate bead formulation could be very profitable for maintaining tight blood glucose levels over prolonged period of time after oral administration, allowing a reduction of its dose and related collateral effects, and improving patient compliance.

1. Introduction

Oral administration of metformin is a first choice therapeutic treatment for patients with type 2 diabetes, due to its good glucose-lowering power and low risk of hypoglycemia. The drug presents few side effects, even though problems of gastrointestinal intolerance (nausea, vomiting, diarrhea, abdominal pain, etc.) may limit its use; moreover chronic administration may cause hyperlactatemia, due to drug accumulation, with resultant lactic acidosis (Page, 2011). The poor bioavailability and short half-life of this drug (Dunn and Peters, 1995; Scheen, 1996), make the development of extended-release formulations desirable, in order to improve patient compliance and reduce the dosing frequency, resulting in better glycemic control and less side effects appearance (Di Colo et al., 2002; Hu et al., 2006; Corti et al., 2007, 2008; Momoh et al., 2013, 2014; Nayak et al., 2013; Li et al., 2014; Kim and Park, 2015).

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The extended release tablets of the drug currently on the market consist in swellable matrix system based on a combination of sodium carboxymethylcellulose and hydroxypropylmethylcellulose, (USP Extended Release metformin hydrochloride tablets, Slowmet®, Glucophage SR®) which cannot provide a constant release profile and could be broken up by normal peristalsis in GI tract, giving rise to unpredictable and uncontrollable variations in the release profile. Moreover this extended-release matrix tablet formulation showed no advantages with respect to the traditional ones either in terms of increased drug bioavailability or decreased within-subject variability of plasmatic levels (https://www.drugs.com/pro/metformin-extended-release-tablets.html). The only benefit observed was the improved gastrointestinal tolerability (Davidson and Howlett, 2004). These unsatisfying results make the development of more effective extended-release formulations of metformin a current challenge.

Different kinds of colloidal carriers, such as lipid and polymeric nanoparticles, and different kinds of vesicular systems, have been investigated as a strategy to improve drug bioavailability, control its release rate and/or obtain site-specific drug targeting (Barratt 2003; Mishra et al., 2010; Gonçalves et al., 2016). Among these, vesicular carriers like liposomes are of particular interest, due to their ability to easily encapsulate both hydrophobic and hydrophilic drugs, together with their biocompatibility, biodegradability and very low toxicity (Jain et al., 2014; Maestrelli et al., 2016). However, the practical use of liposomes is often restricted, mainly by problems related to leakage of encapsulated molecules, short half-life and, in particular, limited physical and chemical stability in solution and in biological environment, being destabilized by low pH, lipases and bile salts (Bozzuto and Molinari, 2015; McClements, 2015).

Liposomes coated with chitosan (chitosomes) have been recently proposed as an interesting alternative to conventional liposomes (Gonçalves et al., 2012; Yang et al., 2015). Chitosan is a polymer well known for its attractive properties, such as biocompatibility, biodegradability and bioadhesivity (Gulbake and Jain, 2012). Chitosomes maintained the positive properties of liposomes, but showed greater stability and provided longer residence times in the gastrointestinal tract, in virtue of their mucoadhesion properties (Takeuchi et al., 2003, 2005; Werle and Takeuchi, 2009; Manconi et al., 2010, 2013; Shao et al., 2015; Caddeo et al., 2016).

Niosomes, self-assembled colloidal vesicles mainly composed by non-ionic surfactants and cholesterol, represent another attractive alternative to classic liposomes as potential drug delivery systems, due to the numerous advantages they can offer, including higher chemical stability, greater ease of production, lower cost and wider flexibility in design and structure (Mahale et al., 2012; Marianecci et al., 2014; Moghassemi and Hadjizadeh, 2014). Niosomal formulations resulted suitable also for oral delivery of hypoglycemic agents (Pardakhty et al., 2007), including metformin (Sankhyan and Pawar, 2013), and demonstrated their effectiveness in prolonging drug release and improving its therapeutic effect (Hasan et al., 2013).

Calcium alginate is a polyanionic polymer widely investigated for its potential to control drug delivery in the intestinal tract, due to its ability to shrink at acidic pH and swell at neutral or basic pH, joined to excellent mucoadhesion properties, biodegradability, biocompatibility and absence of toxicity (González-Rodríguez et al., 2002; George and Abraham, 2006; Patel et al., 2008, 2017; Agarwal et al., 2015). Alginate beads have been successfully used as a vehicle for liposomes (Xing et al., 2003; Bansal et al., 2016) to protect the entrapped hydrophilic drugs and release them to the lower bowel, after specific local biodegradation of the polymer. Such an effect could be particularly beneficial in the case of metformin, since it has been recently proved the important role of the lowel bowel-mediated mechanism in the primary glucose-lowering effect of the drug (Buse et al., 2016).

Taking in mind all the above considerations, in the present work we developed metformin-loaded chitosomes and niosomes, using different components and preparation procedures. The best formulations, included in mucoadhesive calcium alginate beads, to avoid problems of uncontrolled premature leakage of the hydrophilic drug and further control and sustain its release in the intestinal tract, were evaluated in vivo on rats for their therapeutic efficacy in improving and prolonging glycemic control.

2. Materials and methods

2.1. Materials

Metformin hydrochloride (MTF) was kindly provided by Menarini S.P.A. (Florence, Italy). Cholesterol (CH), phosphatidylcholine (PC), dicetylphosphate (DCP), cholate (SC), deoxycholate (SDC) and taurocholate (ST) sodium salts were from Sigma-Aldrich (Barcelona, Spain). Chitosan (CS, viscosity 50 cP; MW 70,000 Da) was a gift from PADETEC Enterprise (Fortaleza-Ceará, Brazil). Dipotassium glycyrrhizinate (DG) was donated from Carlo Erba (Italy). Span® 60 was from Acofarma (Barcelona, Spain). Sodium alginate and other reagents were obtained from Panreac Química (Barcelona, Spain). All solvents were of HPLC quality.

2.2. Preparation of colloidal dispersions

Chitosomes were prepared by coating multilamellar vesicles (MLV) obtained according to the thin-layer evaporation (TLE) method. In brief, 90 mg of a PC-CH lipid mixtureat two different molar ratios (1:1 and 1:0.66) and a fixed amount (5 mM) of charged surfactant (DCP, SC, SDC or ST) were dissolved in chloroform, and the solution placed in a rotary evaporator at 58 °C under vacuum until the formation of a thin lipid film; this was then hydrated with 10 mL of pH 6.8 phosphate buffer solution (PBS) containing MTF. Samples were submitted to five vortex cycles (each consisting in 2 min stirring and 5 min heating at 58 °C) until vesicle formation. The temperature was maintained at 58 °C (above the gel-liquid transition temperature of the amphiphilic and lipid substances) until the end of the process.For vesicle coating with CS, the liposomal sample was added drop wise (0.67 mL/min) at room temperature, under constant stirring rate (100 rpm), into 10 mL of a CS solution (0.1–0.4–0.7% w/v in 0.5% v/v glacial acetic acid solution) followed by incubation, under stirring, for 1 h at 10 °C (González-Rodríguez et al., 2007). The final MTF concentration in the chitosomes was 3 mg/mL.

Niosomes were prepared by thin-layer evaporation (TLE) by dissolving 10 mM of non-ionic surfactant (Span® 60), 5 mM CH and 1 mM of charged surfactant (DCP) in chloroform. This solution was deposited as a thin film in a round-bottom flask, after chloroform was removed by rotary evaporator under vacuum; the vacuum was applied for 1 h to ensure the total removal of solvent traces. The film was then hydrated by adding 10 mL of the hydrophilic phase, consisting of a pH 6.8 PBS containing the drug (3 mg/mL). Five cycles of vortex were then performed, as previously described for liposomes. The obtained niosomes were submitted to sonication at 60 °C for 2 or 3 h at 100 or 400 W.

2.3. Characterization of the colloidal dispersions

Particle size, polydispersity Index (PI) and surface charge (Zeta potential) of the vesicles were determined at 25 °C, using a Zetasizer Nanoseries ZS90 (Malvern Instruments, Malvern, UK).

To measure the size of vesicles, colloidal dispersions were opportunely diluted with purified water to avoid multi-scattering