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Bioadhesive emulsions for control release of progesterone resistant to vaginal fluids clearance

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ABSTRACT

The aim of this study is to propose that mucoadhesive vaginal emulsions can be able to resist the clearance effect of vaginal fluid and to have an effective control release of progesterone. With this goal, silicon derivative, cyclomethicone pentamer, was selected as the bioadhesive and water resistant material. In order to obtain a system which is insensitive to the dilution of aqueous fluids, water in silicone (W/S) emulsions were prepared and different proportions of cyclomethicone as well as 8% or 15% w/w of progesterone were employed. The rheological, mechanical and mucoadhesive properties of emulsions were characterized and the drug release was measured for each formulation. Mucoadhesive behavior was determined and the influence of simulated vaginal fluid (SVF) at bioadhesion was assessed using three commercial mucoadhesive vaginal gels (Crinone®, K-Y jelly® and Zidoval®) as the bioadhesive references. All assayed emulsions have good rheological and mechanical properties and their consistence and viscosity increase when the proportion of the internal phase increases. Related to mucoadhesion, in the absence of SVF, W/S emulsions showed similar bioadhesive levels like the commercial formulations. However, in the presence of SVF, W/S emulsions are able to keep their mucoadhesive properties while the marketed references drastically lose their consistency and adherence to the vaginal mucosa. Drug release profiles from W/S emulsion show that progesterone is released with pseudo-order zero kinetics and a constant release rate is maintained for at least two weeks. The results of the in vivo studies developed in rats show that after a single vaginal administration, bioadhesive W/S emulsions increase the uterine tissue progesterone levels in young and postmenopausal rats. Moreover in postmenopausal rats, they provide high uterine levels of progesterone compared to the bioadhesive-marketed gel used as a reference. Therefore, W/S emulsions have an interesting potential as bioadhesive vaginal delivery systems for drug administration.

1. Introduction

The vagina is an interesting route for drug delivery due to its anatomical position, the rich blood supply and the large surface area. Despite the fact that vaginal delivery is only available for females, there are a big number of advantages for the vaginal route of administration such as the avoidance of hepatic first-pass metabolism, the reduction in the incidence and severity of gastrointestinal side effects, the reduction in hepatic side effects and the possible self-insertion and removal of the dosage form (das Neves and Bahia, 2006; Valenta, 2005; Woolfson et al., 2000).

Conventional vaginal dosage forms (solutions, suspensions, gels, ovules, creams) are low cost and easy to use but show problems derived from their poor vaginal retention time. Due the effect of gravity and the clearance of the vaginal fluids, it causes deficient dosages and loss of the formulation and so, leads to a low patient acceptability (Woolfson et al., 2000). For this reason, researches have focused their attention on the development of new systems that can increase the time of permanence of formulations in the vaginal area, basically by using mucoadhesive formulations (Woolfson et al., 2000; Justin-Temu et al., 2004; Valenta, 2005; Merabet et al., 2005; das Neves ans Bahia, 2006; Acartürk, 2009). Those formulations are elaborated with hydrophilic bioadhesive polymers, such us poly-acrylic acids, cellulose or polysaccharides derivatives that are able to improve their effectiveness by increasing, significantly, the retention time on the vaginal mucosa.

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In particular, the bioadhesive formulations based on polycarbophil (such as Crinone® and Repelens®) can be retained at the vaginal cavity for 3 or 4 days Robinson and Bologna (1994). Nevertheless, some contradictory reports were obtained for the in vivo vaginal retention behavior of those formulations in humans (Valenta, 2005). Brown et al., (1997), studied the vaginal spreading and clearance of Replens® in menopausal women, showed a great variability in the retention time for this formulation, finding cases between 2% and 80% of retention time (Owen et al., 2003). The main problem of these mucoadhesive formulations is that they are highly sensitive to the dilution effect of vaginal fluids which can lead to a significant decrease in their effectiveness over time. Some alternatives were proposed to avoid these problems based on the use of thermosensitive hydrogels (Aka-Any-Grah et al., 2010), non-aqueous silicone elastomer gels (Forbes et al., 2011) and the use of mucoadhesive water in oil emulsions (Merabet et al., 2005).

In this work, an alternative based in the use of water in silicone W/S emulsions is proposed. These emulsions are also called "third generation" emulsions or oil free emulsions, and are formed by a polar internal phase (generally constituted by saline aqueous solutions) and a "non-polar and non lipidic" external phase formed by silicone polymers, concretely by cyclomethicones. These low molecular weight silicones have a low vaporization temperature and once the W/S emulsions are applied, the cyclomethicones volatilized slowly and formed an adhesive and a water resistant layer. One additional advantage is that silicones are an inert material with low toxicity (Scientific Committee on Consumer Safety, SCCS/1241/10), and also semisolid W/S emulsions, in contrast to the non-aqueous silicone elastomer gels, are formed mainly by water (more than 70–80%) conferring them great biocompatibility. Moreover as silicones are not lipids, they exhibit a very good cosmetic behavior, an ability to improve the aesthetic performance of formulations and to prevent the formulation from staining during application.

Also, local tolerance testing results using the Slug Mucosal Irritation Assay (Campaña Seoane et al., 2013) showed that W/S emulsions, elaborated with the same components as proposed in this work, are non-irritating and do not generate tissue damage. Slug Mucosal Irritation Assay is as a local tolerance testing alternative to the use of laboratory mammal species developed at the University of Ghent (Belgium) that can be used to predict the mucosal tolerance of pharmaceuticals in different tissues as vaginal, ophthalmic or oral mucosa (Adriaens and Remon, 1999, 2008; Dhondta et al., 2005).

To evaluate the potential of W/S emulsions as a vaginal bioadhesive drug delivery system, progesterone was selected as the drug model. Progesterone is used in fertility treatment via different route of administration. Oral progesterone formulations exist, but they are not effective due to its very low oral bioavailability (less than 10%), which is caused by rapid intestinal and hepatic metabolism. Daily intramuscular injections are effective but injections are painful and are not conceivable for long-term treatments (Cicinelli et al., 2000). Currently, vaginal route is considered as a better choice for progesterone administration because it shows a direct vagina-to-uterus transport which is also known as "first uterine pass effect". This route increases the accumulation of the drug in this area by avoiding the hepatic first pass effect (Cicinelli et al., 2000). There is an evidence that at equal doses (100 mg), vaginal administration allows to obtain a concentration of 10 times higher than those obtained via intramuscular administration (Toner, 2000). Due to these considerations, some vaginal formulations of progesterone have been developed in the past years. Crinone® is a bioadhesive gel, with the dose of 8% micronized progesterone, which is made of a combination of the two bioadhesive polymers, polycarbophil and Carbomer 974P, shows synergistic properties regarding its mucoadhesion, allowing less frequent administration. The development of a vaginal gel Crinone® with sustained release was a breakthrough in vaginal application systems (Owen et al., 2003).

The aim of this work is to study the potential use of silicone emulsions as a possible alternative for the elaboration of bioadhesive vaginal system which is insensitive to the dilution with vaginal fluids, with extended drug control release capacity.

2. Materials and methods

2.1. Materials

Progesterone (Lot 08K0766, Fagron Iberica), cyclomethicone pentamer (Fagron Iberica), Abil WE 09 (mixture of polyglyceryl-4 isostearate, PEG/PPG-10/1cetyl dimethicone and hexyl laurate, Lot: 801,267, Fragon Iberica), sodium chloride (Lot: 139,866,850. Pancreac Chemical, SA), glycerol (Lot: K13884591, Merck) Kleptose Crysmeb (methyl-β-cyclodextrin, degree of substitution 0.47, Lot: 789,874, was a gift from Roquette Laisa, Spain).

In the rheology, syringeability, extensibility and bioadhesion test, commercial formulations Crinone® (Wyeth-Ayerst Laboratories, Radnor, PA, USA) KY Jelly® (Johnson & Johnson) and Zidoval® (MEDA PHARMA S.A.U.) were used as references. KYJelly® is a personal lubricant based in hydroxyethyl-cellulose polymers while Zidoval® and Crinone® are formulations based in poly-acrylic acid polymers (Cabopol® 974P or their mixtures with polycarbophil, respectively) containing metronidazole and progesterone as the drug respectively.

Crinone® was also used as a reference in the progesterone release assays.

2.2. Solubility studies

20 mg of progesterone was added to 1 ml of solvent in Eppendorf tubes and after gently shaking, the Eppendorf tubes were introduced in a thermostatic bath with horizontal shaking (Unitronic 320 OR) at 25 °C for a week. Samples were centrifuged at 10,000 rpm in a Sigma® centrifuge and the supernatants were filtered through nylon membrane filters of 0.45 microns to eliminate the undissolved progesterone. The progesterone concentration was determined spectrophotometrically at a wavelength of 323 nm. Each sample was made in six replicates. Solvents used for this study were: water, cyclomethicone, an aqueous solution of 2% NaCl and 3% glycerol (W/V) and an aqueous solution containing 2% NaCl, 3% glycerol and 1.5% of metil-β-CD.

2.3. Determination of water/cyclomethicone partition coefficient

A solution of 5 μg/ml progesterone in water was prepared. 0.5 ml of the solution and 0.5 ml of cyclomethicone was added in the Eppendorf tubes and were placed on an orbital shaker for 24 h at 30 °C at 200 rpm. Samples were centrifuged at 12,000 rpm for 1 h at 30 °C in order to separate the aqueous phase from the organic phase. The progesterone was determined by LC–MS/MS using UPLC QSM Waters Acquity fitted to a mass tandem quadrupole spectrometer Xevo TQD and the application ProfileLynx (MassLynx, Waters Inc., 2012). The MRM transitions, cone voltages, and collision cell energies optimized for progesterone was 315 > 97 m/z and 20 v, 20 v, respectively. UPLC conditions was: column ACQUITY BEH C_{18} 2.1 mm × 100 mm (1.7 μm) at 40 °C; mobile phase water: methanol at a flow rate of 0.4 ml/min. A linear gradient from 30% to 97% methanol in 5 min was used.

2.4. Preparation of W/S emulsions

Silicone emulsions were prepared by direct emulsification using a homogenizer Unguator 2 (Microcaya). The composition of