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Preparation and characterization of intravaginal vardenafil suppositories targeting a complementary treatment to boost *in vitro* fertilization process

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ABSTRACT

Vaginal route has been recently considered as a potential route for systemic delivery of drugs with poor oral bioavailability. Vardenafil (VDF) is a relatively new phosphodiesterase-5 inhibitor that exhibits a limited oral bioavailability (≈15%) due to extensive first-pass metabolism. In this study, we attempted to enhance the systemic bioavailability of VDF *via* its formulation within vaginal suppositories. Witepsol H15 and Suppocire NA50 were adopted as lipophilic suppository bases while polyethylene glycol 4000/400 and glycerogelatin were used as hydrophilic suppository bases. The effect of different base types and/or the incorporation of bioadhesive polymer on *in vitro* release of VDF were evaluated. The *in vivo* fate and organ biodistribution of VDF following intravaginal (IVG) administration were also investigated. VDF release from water-soluble bases was higher than that from lipophilic bases. The incorporation of bioadhesive polymers, such as Na alginate, remarkably sustained drug release from suppository base. The organ biodistribution study showed a higher C_{max} (32 times) and $AUC_{0-4\ h}$ (20 times) of VDF in uterus following IVG administration of conventional suppositories, compared to oral administration of VDF suspension. In addition, cyclic guanosine monophosphate (cGMP) serum levels, used as an indicator of the *in vivo* activity of VDF, in animals were higher following IVG administration rather than oral administration. This study suggests that IVG administration of VDF might represent a potential alternative to oral route with superior therapeutic benefits especially when targeting the uterus.

1. Introduction

In vitro fertilization (IVF) is a procedure used for treatment of infertility in which an egg (ovum) from a woman is removed and fertilized by a sperm in a laboratory dish. One or more embryos after being fertilized are selected and then transferred to the woman's uterus (Boltz et al., 2017; Qin et al., 2016). Many reports have emphasized a relationship between the morphological characteristics of the endometrium and the rate of pregnancy in assisted reproduction therapy (Child et al., 2002; Momeni et al., 2011; Zhao et al., 2012). An endometrial thickness of ≥ 8 mm has been verified to be correlated with a high chance of pregnancy in patients being treated with IVF (Gonen and Casper, 1990; Sher et al., 1991). Endometrial growth is dependent on the uterine blood flow and the vascular uterine muscle relaxation which is thought to be a cyclic guanosine monophosphate (cGMP)-mediated process (Agarwal et al., 2005; Khan et al., 2010).

Phosphodiesterase (PDE5) inhibitors are a relatively new class of drugs that induce vascular smooth muscle relaxation and vasodilatation *via* preventing cGMP degradation and potentiating the effects of nitric oxide (NO) on vascular smooth muscle (Huang and Lie, 2013; Rosen and Kostis, 2003; Sandner et al., 2007). Currently, PDE5 inhibitors are considered an effective therapy for erectile dysfunction (Corbin, 2004; Huang and Lie, 2013). Nonetheless, recent reports have emphasized the potential use of PDE5 inhibitors, particularly sildenafil citrate, for the treatment of recurrent spontaneous abortion, intrauterine growth retardation and other pregnancy-related complications (Shanmugam et al., 2014; von Dadelszen et al., 2011).

Vardenafil (VDF), a highly selective PDE5 inhibitor, is an on-demand treatment for erectile dysfunction and it displays the highest potency compared with its competitors (Markou et al., 2004; Mazo et al., 2004). However, VDF shows low oral bioavailability (15%) due to extensive metabolism and low solubility (Fahmy, 2015). This elicited the need to develop other formulations, as alternatives, to improve its *in vivo* fate.

The vaginal route has been accounted for its efficacy in granting the efficient delivery of some drugs such as progesterone, preferentially to the uterus while alleviating the systemic side effects (Ferguson and Rohan, 2011; Norman et al., 2016). The vagina represents a promising

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site for local effect as well as systemic drug delivery because of its large surface area, reduced chance of drug degradation, avoidance of the first-pass metabolism, rich blood supply, relatively high permeability to many drugs and ease of administration (Bassi and Kaur, 2015; de Oliveira et al., 2015; Shanmugam et al., 2014).

Unlike sildenafil citrate, there have been no reports on the clinical adoption of VDF for the treatment of recurrent spontaneous abortion, intrauterine growth retardation or other pregnancy-related complications. Therefore, in the current study, we aimed at investigating the potential of the intravaginal (IVG) administration of VDF, compared to oral route of administration, for enhancing drug exposure in the uterus and investigating whether IVG delivery of the drug could have an impact on implantation and/or pregnancy rates. For such purposes, VDF was formulated in the form of either conventional vaginal suppositories or bioadhesive suppositories. The formulated suppositories were then characterized for content uniformity, weight variation, hardness and disintegration time. The *in vitro* release studies were also investigated. In addition, organ biodistribution of VDF after its IVG application into female rats was evaluated. Furthermore, serum cGMP level, used as an indicator for VDF effect on endometrial development, was measured after different time intervals following IVG administration.

2. Materials and methods

2.1. Materials

Vardenafil was kindly supplied from GNP Company (6th of October City, Giza, Egypt). Polyethylene glycol (PEG) 400 and 4000, Witepsol H15 and Suppocire NA50, were purchased from (Hoechest Chemikalien, Werk Gendort, Germany). Gelatin, glycerin, Na alginate, tween 80 and acetone were kindly supplied by Egyptian International Pharmaceuticals Industries Company (EPICO, 10th of Ramadan City, Egypt).

2.2. Preparation of VDF vaginal suppositories

2.2.1. Preparation of conventional suppositories

Suppositories of 20 mg VDF were prepared by a cream melt method (Ghorab et al., 2011). Witepsol H15 (WH-15) or Suppocire NA50 (SPNA) were used as oleaginous bases, while polyethylene glycol (PEG) or glycerogelatin (G.G.) were used as water soluble ones. The calculated amount of base was melted in a water bath maintained at a temperature of 40 ± 2 °C, then allowed to cool down and mixed with 20 mg VDF. The mixture was then heated again and poured into moulds previously lubricated with a suitable lubricant and stored at 4 ± 2 °C until further investigation.

2.2.2. Preparation of VDF bioadhesive suppositories

Na alginate, at a concentration of 0.5, 1 or 1.5% w/w, was used to prepare bioadhesive suppositories from PEG and WH-15 formulae. The polymeric phase was prepared by its sprinkle onto the calculated amount of distilled water with continuous stirring. Then being added to the melted base gradually with stirring to ensure homogenous mixing. Finally the drug was incorporated (Yahagi et al., 1999). The composition of either conventional or bioadhesive suppositories was represented in Table 1.

2.3. Characterization of vaginal suppositories

2.3.1. Weight variation

The weight variation test was performed according to the British Pharmacopoeia (2011). In brief, twenty suppositories were weighed and the average weight was determined. Not > 2 suppositories differ from the average weight by > 5%, and no suppository differs from the average weight by > 10%.

2.3.2. Measurement of pH value

The suppository was digested in warm water then filtered and the pH of the filtrate was measured at 37 ± 0.5 °C by suitable pH meter.

2.3.3. Hardness

The hardness test which refers to the resistance of suppositories to rupture was carried out on 10 suppositories using Erweka hardness tester (PharmaTest, Germany) at room temperature (25 ± 0.5 °C).

2.3.4. Disintegration time

The disintegration time of VDF suppositories was determined at 37 ± 0.5 °C using tablet disintegrator by the method suggested by Kale et al. (2005). The mean time taken for six different suppositories to completely melt in the medium was determined.

2.3.5. Measurement of melting zone

The test was conducted according to Kauss et al. (2013). The suppository was completely immersed in the dissolution medium at a constant temperature water bath, and the time for the entire suppository to melt or disperse in the surrounding medium was recorded.

2.3.6. Measurement of deformation time

The deformation time was determined using Erweka suppository deformation tester according to the British Pharmacopoeia (2011). Each suppository was put in a tester glass tube in a water bath maintained at 37 ± 0.5 °C and the rod was introduced until it touched the flat end of suppository. The time required for the rod to sink down the glass tube was recorded.

2.4. In vitro studies

2.4.1. In vitro release study

The *in vitro* release of VDF from the suppositories was determined using USP XXIII, dissolution apparatus II. Each suppository was immersed in a glass diffusion cell containing 100 ml of phosphate buffer (pH 4.5) as a dissolution medium, maintained at a temperature of 37 °C and paddle speed was set at 50 rpm. 3 ml sample was withdrawn at different time intervals (10, 20, 30, 40, 50, 60, 90 and 120 min), and an equal volume of fresh medium was replaced into the dissolution medium after each sampling to maintain constant volume throughout the study. The drug concentration in each sample was measured at λ_{max} 270 nm using UV spectrophotometer. Dissolution studies were conducted in triplicate.

2.4.2. Kinetic analysis of the release data

In order to describe the release model, the *in vitro* release data from VDF vaginal suppositories were analyzed according to zero, first and Higuchi diffusion models. The model that produced the highest correlation was used for the assessment of the drug release rates (Abou el Ela Ael et al., 2016).

2.5. In vivo study

2.5.1. Animals

Adult albino female rats, 200–250 g each, were supplied by the animal center, (Faculty of Pharmacy, Zagazig University, Egypt) and handled according to Ethical Committee of Animal Handling in Zagazig University "ECAHZU". Rats were maintained in a light- and temperature-controlled animal facility. The animals were fasted for 24 h prior to dosage administration with free access to water.

2.5.2. In vivo organ distribution studies

For organ biodistribution study, the rats were randomly divided into four groups each containing 12 rats: Group 1, negative control (non-treated rats), Group 2, rats treated with an oral VDF suspension, Group 3, rats treated with VDF containing WH-15 vaginal suppositories and