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Research paper

Self-emulsifying drug delivery systems: Design of a novel vaginal delivery system for curcumin

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ABSTRACT

Aim: The aim of this study was to develop a vaginal self-emulsifying delivery system for curcumin being capable of spreading, of permeating the mucus gel layer and of protecting the drug being incorporated in oily nanodroplets towards mucus interactions and immobilization.
Methods: The emulsifying properties of curcumin loaded SEDDS containing 30% Cremophor RH40, 20% Capmul PG-8, 30% Captex 300, 10% DMSO and 10% tetraglycol (SEDD formulation A) as well as 25% PEG 200, 35% Cremophor RH40, 20% Captex 355, 10% Caprylic acid and 10% Tween 80 (SEDD formulation B) after diluting 1 + 2 with artificial vaginal fluid were characterized regarding droplet size and zeta potential. Collagen swelling test was used to examine the irritation potential of SEDDS. Additionally to mucus binding studies, permeation studies in the mucus were performed. Furthermore, spreading potential of the novel developed formulations was compared with a commercial available o/w cream (non-ionic hydrophilic cream) on vaginal mucosa.
Results: SEDDS displayed a mean droplet size between 38 and 141 nm and a zeta potential of −0.3 to −1.6 mV. The collagen swelling test indicated no significant irritation potential of both formulations over 24 h. An immediate interaction of unformulated curcumin with the mucus was determined, whereas both SEDDS facilitated drug permeation through the mucus layer. Formulation B showed a 2.2-fold improved transport ratio of curcumin compared to SEDD formulation A. In comparison to the vaginal cream, SEDD formulation A and B were able to spread over the vaginal mucosa and cover the tissue to a 17.8- and 14.8-fold higher extent, respectively.
Conclusion: According to these results, SEDDS seems to be a promising tool for vaginal application.

1. Introduction

Curcumin, a phytopolyphenol pigment and the main bioactive component of turmeric, has already demonstrated anti-microbial and -viral properties in pre-clinical studies. As human papilloma virus (HPV) is the most common sexually transmitted infection, the treatment with curcumin via vaginal application seems to be a promising strategy as the drug inhibits the transcription of HPV 16 E6/E7 and recovers the expression of tumor suppressor protein p53 [1]. The potential of a vaginal curcumin cream to eliminate HPV infection from cervix was already demonstrated in a phase II randomized controlled study in humans [2]. As curcumin strongly interacts with mucus [3] being likely immobilized to the network of mucins where it is released from the delivery system, it will only to a minor extent spread over the mucosa reaching its target sites. According to these considerations, vaginal curcumin treatments have by far not reached their full potential yet. In order to overcome this shortcoming, novel vaginal carrier systems are of great interest. Self-emulsifying drug delivery systems so called SEDDS represent one of the most promising groups of lipophilic nanocarriers. They are isotropic mixtures of oil, surfactant and co-surfactant spontaneously forming nanodroplets when getting into contact with body fluids, encapsulating the drug and protecting it against mucus interactions. Moreover, the small size of the formed nanodroplets in combination with their shape deformation ability allows comparatively high permeation through the mucus layer [4]. So far, however, the high mucus permeating properties of SEDDS were not utilized for vaginal drug delivery. It was therefore the aim of this study to develop a vaginal carrier system based on SEDDS providing high mucus permeation and fast spreading of curcumin on the vaginal mucosa. Within this study, SEDDS

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were investigated regarding droplet size, zeta potential and stability over 24 h. In order to confirm curcumin-mucus interactions, binding studies of curcumin with vaginal mucus were carried out. The novel developed SEDDS containing curcumin were investigated regarding spreading potential over vaginal mucosa and permeation through the mucus. Furthermore, irritation potential of the vaginal carrier systems was evaluated by collagen swelling.

2. Materials and methods

2.1. Materials

Caprylic acid, Cremophor RH 40, Cremophor EL, Curcumin, tetraglycol were obtained from Sigma-Aldrich, Vienna, Austria. Tween 80 and PEG 200 were obtained from Roth, Graz, Austria. Non-ionic hydrophilic cream was purchased from Bombastus-Werke AG, Freital, Germany. Capmul PG-8 EP/NF, Captex 300 EP/NF as well as Captex 355 EP/NF were a gift from Abitec, USA. All other materials were of analytical grade and received from commercial sources.

2.2. Preparation and characterization of various self-emulsifying systems

In brief, various lipids, surfactants as well as solvents, summarized in Table 1, were homogenized by using the vortex mixer. After ultrasonication for 30 min, formulations were examined regarding turbidity or phase separation. Afterwards, the most promising SEDD formulations were diluted 1 + 2 with 50 mM lactate buffer pH 4.5 simulating vaginal fluid [5] and incubated for at least 3 h at 37 °C while shaking at 300 rpm. The resulting emulsions were investigated visually regarding phase separation. Within emulsions resulting in one phase, SEED formulations were used for orientating droplet size measurement. The most promising formulations were used to incorporate 1% curcumin (w/v) and characterized regarding droplet size distribution and zeta potential over 24 h at 37 °C using Nicomp PSS 380 DLS/ZLS, Particle Sizing Systems, Inc., Port Richey, Florida with a laser wavelength of 650 nm and an E-field strength of 5 V/cm. Moreover, viscosity of the final SEDD formulations were determined using a thermostatically controlled plate-plate rheometer (ThermoHaake Mars, Haake GmbH, Karlsruhe, Germany).

2.3. Collagen swelling test

In order to investigate the irritation potential of SEDDS for vaginal application, a novel experimental setup was established based on the collagen swelling test. Therefore, a piece of collagen sheet (Suprasorb C; Lohmann & Rauscher) was fixed between a wire thread and immersed in a 5 ml tube containing SEDD formulation A and B diluted 1 + 2 and 1 + 4 with artificial vaginal fluid. Lactate buffer without any SEDD formulation served as control. After 1, 3 and 24 h, the swollen collagen sheets were taken out of the incubation medium and collagen swelling was determined gravimetrically. The swollen collagen was calculated according to the following equation:

$$\text{Collagen swelling } (\%) = [(\text{weight after incubation} - \text{initial weight})/\text{initial weight}] \times 100\% \quad (1)$$

Table 1
Overview of the utilized excipients divided in lipids, surfactants and solvents.

Lipids	Surfactants	Solvents
Captex 300	Caprylic acid	Glycerol 85%
Captex 355	Capmul PG-8	1,3-Dimethyl-2-imidazolidinone
Capmul MCM	Capmul PG-12	Dimethyl sulfoxide
Ethyloleat	Cremophor RH40	PEG 200
Maisine 35-1	Labrasol	PEG 300
Miglyol 840	Labrafil M 1944	Propylene glycol
Triethyl citrate	Cremophor EL	Tetraglycol
	Tween 20	Transcutol
	Tween 80	

2.4. Curcumin mucus binding studies

Curcumin mucus interaction studies were performed to evaluate the binding properties of curcumin to vaginal mucus. Therefore, vaginal tissue of freshly slaughtered cows was collected and vaginal mucus was scraped off utilizing a microscope slide. Afterwards, 300 mg of a curcumin solution (0.025% m/v) in 40% ethanol buffered with 50 mM lactate buffer was added to 300 mg of vaginal mucus and homogenized utilizing the vortex mixer. Samples were incubated at 37 °C over 24 h while shaking at 300 rpm. After 0.5, 1, 2, 3 and 24 h, samples were centrifuged (2000 rpm, 2 min) and 50 µl of curcumin solution were withdrawn. Afterwards, the samples were diluted 1 + 4 with ethanol and fluorescence was measured at an excitation of 445 nm and an emission wavelength of 515 nm using a microplate reader (Infinite™ M200, Tecan, Grödig, Austria). Curcumin solution without mucus served as 100% control value as 300 mg of mucus was replaced by 300 mg of ethanol/lactate buffer. Furthermore, drug mucus interaction study was performed with intestinal mucus following the same procedure.

2.5. Mucus permeation studies using transwell chambers

To investigate the transport of curcumin (1% w/v) incorporated into the two most promising SEDD formulations A and B across the mucus barrier, permeation studies were carried out according to Friedl et al. [6] and slightly modified. Therefore, 24-well plates (Greiner-BioOne, Austria) were used occupying a surface of 33.6 mm^2 covered with 45 mg of mucus. As a lot of vaginal tissues are necessary to obtain enough vaginal mucus, alternatively intestinal mucus was used for this experimental setup. Briefly, SEDD formulation A and B containing curcumin were diluted 1 + 2 with 50 mM lactate buffer and 250 µl of formed emulsions were placed in the donor compartment, whereas 500 µl of lactate buffer were added to the acceptor chamber. The plate was kept on a board (Vibramax 100; Heidolph Instruments, Germany) at 37 °C under continuous shaking at 300 rpm in the incubator for 24 h. At predetermined time points of 0.5, 1, 2, 3 and 24 h 200 µl samples were withdrawn from the acceptor compartment and replaced with the same volume of buffer. The amount of permeated curcumin was determined by fluorometric detection at an excitation of 445 nm and an emission wavelength of 515 nm and calculated in reference to a 100% control value of each SEDD formulation. In order to determine the 100% value, the same procedure was performed using the transwell-system without mucus. Curcumin incorporated in an o/w cream was used as reference formulation.

2.6. Spreading of SEDD formulations over the vaginal mucosa

In order to investigate the spread of SEDD formulations over the vaginal mucosa, a novel experimental setup was generated based on fluorescence imaging utilizing a SLR-camera (Nikon D5200). Therefore, vaginal tissues were cut into pieces and were placed on a petri dish. Before starting the experiment, the vaginal mucosa was photographed and the surface area was determined using