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Research paper

Mucoadhesive liposomes as new formulation for vaginal delivery of curcumin

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ABSTRACT

Local delivery to the affected area represents the optimal means by which advantageous pharmacological properties of curcumin may be fully exploited as currently, due to the biopharmaceutical limitations associated with this polyphenol, its full beneficial effects remain limited. Curcumin-containing liposomes coated with bioadhesive polymers of natural and synthetic origin (chitosan and Carbopol) were evaluated *in vitro*. For these purposes, an *in vitro* model of vaginal mucus was developed allowing the monitoring of curcumin permeability in the conditions mimicking vaginal environment. The model was optimized by varying the amounts of glycoproteins, as compared to the permeabilities determined through isolated bovine mucus. The strength of bioadhesion was evaluated using the isolated bovine mucosa. Both curcumin solution and non-coated curcumin liposomes served as controls. Bioadhesive polymers enabled significantly higher ($p < 0.05$) curcumin permeability through the artificial and isolated bovine mucus compared to the controls. Polymer coating of liposomes resulted in an increase in their bioadhesiveness. Mucoadhesive liposomes can be considered as potential novel drug delivery systems intended for vaginal administration of curcumin.

1. Introduction

Curcumin represents one of 400 natural candidates that are currently being scrutinized as potential chemo-preventive agents and this polyphenolic compound has shown promise in clinical trials for a variety of cancer conditions including multiple myeloma, pancreatic, and colon cancer [1,2]. Being sensitive to environmental conditions (i.e. stability issues) coupled with poor absorption (low solubility, low permeability), and extensive pre-systemic metabolism, attempts to deliver a systemically effective curcumin dose and to achieve health-beneficial effects outside the gut mucosa have failed [3]. Nevertheless, curcumin pharmacological activities appear opportune intriguing a continuous search for a "super curcumin" [2]. Various delivery systems have been proposed as a means to improve therapeutic effects of curcumin. Among them, liposomes able to incorporate poorly soluble molecules and enable their aqueous medium-based administration seem to be among the most promising delivery systems [4]. Although several research groups focused on development of liposomal delivery system for curcumin aiming at either intravenous or oral administration [5–8], to the best of our knowledge only a very limited reports deal with curcumin aiming at vaginal administration [9–11]. This route enabled delivery of effective curcumin amount directly to a diseased area when applied in delivery systems (i.e. liposomes) that ensure satisfactory curcumin stability and solubility [10]. As drug carriers liposomes have shown good potentiality in the anticancer therapy. Owing to their advantages, antitumor efficacy and tolerability have substantially improved while limitations pertaining to conventional anticancer treatments (i.e. poor solubility, irritant properties, lack of stability, rapid metabolism, non-selective drug distribution, poor patient compliance, quality of life, etc.) have elegantly been avoided [12]. Curcumin and many of the anticancer drugs share biopharmaceutical limitations; thus, incorporating curcumin into liposomes seems a reasonable approach to enhance its activity [9].

In our previous *in vitro* study [10], promising pharmacokinetic outcome was achieved when curcumin was applied in liposomes to the vaginal mucosa. Depending on the liposomal size, the concentration of curcumin in different layers of vaginal tissue was significantly higher compared to curcumin applied in solution.

Abbreviations: BCS, Biopharmaceutical Classification System; CPGM, crude pig gastric mucins; MLV, multi-lamellar vesicles; PBS, phosphate buffer saline; PC, phosphatidylcholine; SVF, simulated vaginal fluid.

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Additionally, curcumin tissue retention (from solution and liposomes) was significantly higher compared to the tissue retention of other concurrently assayed highly and low permeable and highly soluble standards at the expense of significantly lower permeability (i.e. curcumin permeability was approximately 1–2 log units below the permeabilities determined for BCS1 standards). Therefore, we concluded that curcumin shows negligible potential for systemic absorption when applied to vaginal mucosa, eliminating the possibility of pharmacokinetic and/or pharmacodynamic interactions in co-medicated patients [10].

The prerequisite for successful topical vaginal therapy is the prolonged residence time of drug-containing formulation on vaginal site [13]. In this study we thus focused on the assessment of the importance of vaginal mucus and its effect on the limited contact time between curcumin-containing liposomes and mucosa. For these purposes liposomes that yielded the highest curcumin tissue retention (and the lowest curcumin tissue permeability) [10] were coated with two bioadhesive polymers (i.e. chitosan and Carbopol) in 0.1% and 0.6% (w/v) concentrations. Chitosan, a linear hydrophilic polymer made of copolymers of *N*-acetyl glucosamine linked by β(1–4) glycosidic bonds and glucosamine, has well known mucoadhesive properties. Carbopol is the polymer of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol and is found in various oral mucoadhesive drug delivery systems due to its ability to interact with the mucus glycoprotein and to remain localized at a specific site [14]. The *in vitro* model mimicking vaginal mucus was optimized varying the concentrations of isolated crude pig gastric mucins type II (CPGM) as a surrogate for vaginal glycoproteins and the corresponding curcumin permeability was determined. Since vaginal mucus is not just a simple aqueous dispersion of glycoproteins, curcumin permeability was additionally determined through isolated bovine mucus, which is structural and functional properties resemble the conditions in the postmenopausal women. Finally, the strength of the system's bioadhesion on isolated bovine mucosa was monitored to further justify the beneficial effects of mucoadhesive coating.

2. Materials and methods

2.1. Materials

Curcumin, crude pig gastric mucin type II (CPGM), and salts for the incubation saline were from Sigma Aldrich (Diesenhofen, Germany). Human albumins were purchased at Slovenian transfusion agency.

Chitosan® low molecular weight was obtained from Sigma–Aldrich, Chemie GmbH, Steinheim, Germany. Lipoid S 100 (soybean lecithin, >94% phosphatidylcholine) was a gift from Lipoid GmbH, Ludwigshafen, Germany. Carbopol®974P NF was purchased from Noveon Inc., Cleveland, USA.

All chemicals used in this study were of the highest grade available.

2.2. Methods

2.2.1. Preparation and characterization of non-coated and coated liposomes

2.2.1.1. Liposome preparation. Liposomes containing curcumin, coated and non-coated were prepared as previously reported [12]. In brief, (PC; 200 mg) was dissolved in chloroform and mixed with curcumin (20 mg). The solvent was evaporated using rotoevaporator system (Büchi rotavapor R-124 with vacuum controller B-721, Büchi Vac® V-500, Büchi Labortechnik, Flawil, Switzerland) for at least 1.5 h at 50 mmHg and 50 °C. The remaining film was then re-suspended in 10 mL of PBS (pH = 7.4). Prior to coating, liposomal suspension was left overnight at 4 °C.

2.2.1.2. Coating of liposomes. The curcumin-containing liposomes were coated with 0.1% and 0.6% (w/v) chitosan solutions prepared in 0.1% (v/v) glacial acetic acid, respectively [14]. The coating was performed in the presence of free curcumin. An equal volume of chitosan solution was added drop-wise to liposomes under magnetic stirring at room temperature for 1 h. The coated liposomal suspensions were then placed in refrigerator overnight to stabilize. For the preparation of Carbopol-coated liposomes, 0.1% and 0.6% (w/v) Carbopol®974P NF was dissolved in phosphate buffer pH 7.4 [14]. The rest of the coating procedure was as described for chitosan-coated liposomes.

The entrapment efficiency was determined as the percentage of liposomally associated curcumin in comparison with total amount of curcumin taken into the preparation and was determined by HPLC [9].

2.2.1.3. Size distributions. Photon correlation spectroscopy (PCS) technique was employed to determine the vesicle size and size distribution using a Nicomp model 380 particle sizing system (Nicomp Particle Sizing Systems, Santa Barbara, CA). Samples preparation was done according to the guidelines for particle size analysis given by the International Organization for Standardization, and average size of liposomes was determined [15]. Liposomal suspension sample preparation was performed in a laminar airflow bench and each sample was analyzed using data collection times (3 cycles of 5 min) sufficient to ensure statistically sound data base (minimum 1000 counts in the channel). The measurements were performed in triplicates.

2.2.1.4. Zeta potential. Zeta potential measurements were performed on a Malvern Zetasizer Nano Z (Malvern, Worcestershire, UK). The liposomal suspensions were diluted in 1:40 ratio in filtrated water before measurements. All of the results were the average of at least three independent measurements.

2.2.2. Buffers for in vitro experiments

Phosphate buffer pH 7.4 was prepared by dissolving NaCl (8 g/L), KCl (0.2 g/L), Na_2HPO_4 (1.44 g/L), and KH_2PO_4 (0.24 g/L) in MiliQ water and the pH was adjusted to 7.4 with 1 M HCl or 1 M NaOH. On the day of the experiment human serum albumin was added to obtain 4% acceptor solution used in all *in vitro* permeability experiments due to poor curcumin solubility and the tendency for adsorption to plastics. Albumin in the acceptor solution was also used to maintain sink similar to *in vivo* situation.

For simulated vaginal fluid (SVF) NaCl (3.51 g/L), KOH (1.4 g/L), $Ca(OH)_2$ (0.222 g/L), acetic acid (1 g/L), lactic acid (2 g/L), glycerol (0.16 g/L), urea (0.4 g/L), and D-glucose (5 g/L) were dissolved in MiliQ water and pH was adjusted to 4.5 with 1 M HCl [10].

Ringer buffer was prepared from $NaHCO_3$ (2.1 g/L), $NaH_2PO_4 \times H_2O$ (0.055 g/L), NaCl (3.624 g/L), KCl (0.373 g/L), $CaCl_2 \times 2H_2O$ (0.176 g/L), $MgCl_2 \times 6H_2O$ (0.244 g/L), $NaHPO_4 \times 2H_2O$ (0.285 g/L), and D-glucose (1.8 g/L) in MiliQ water and pH was adjusted with NaOH to 7.4 during bubbling with carbogen (O_2/CO_2 95/5) [16].

2.2.3. The in vitro permeability of curcumin

The concentrated liposome samples were sonicated prior to the experiments in an ultrasound bath for 5–10 min. Donor solutions were prepared by diluting concentrated non-coated and coated liposome samples with SVF to obtain identical curcumin donor (20 μM) and similar phospholipid concentrations (0.075–0.082 mg/mL). Curcumin stock solution (i.e. reference formulation) was prepared by dissolving curcumin standard in DMSO and it was