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Research Paper

Mucus permeating thiolated self-emulsifying drug delivery systems

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ABSTRACT

Context: Mucus represents a critical obstacle for self-emulsifying drug delivery systems (SEDDS) targeting the epithelial membrane site.
Objective: The aim of the study was the development of a novel SEDDS to overcome the mucus barrier.
Materials and methods: Two novel conjugates *N*-dodecyl-4-mercaptobutanimidamide (thiobutylamidine-dodecylamine, TBA-D) and 2-mercapto-*N*-octylacetamide (thioglycolicacid-octylamine, TGA-O) were synthesized, incorporated into SEDDS and analyzed for stability, cytotoxicity and physico-chemical characteristics using dynamic light scattering. Mucus interaction studies were performed using *in vitro* assays based on multiple particle tracking, rotational silicone tubes and rheology.
Results and discussion: TBA-D was synthesized using dodecylamine and iminothiolane as thiol precursor (yield = 55 ± 5%). TGA-O was obtained via crosslinking of octylamine with SATA ((2,5-dioxopyrrolidin-1-yl) 2-acetylsulfanylacetate) (yield = 70 ± 6%). The chemical structure of target compounds was confirmed via NMR analysis. The thiol-conjugates were incorporated in an amount of 3% (m/m) into SEDDS (Cremophor EL 30%, Capmul MCM 30%, Captex 355 30% and propylene glycol 10%), namely thiolated SEDDS leading to a droplet size around 50 nm and zeta potential close to 0 mV. Thiolated SEDDS with an effective diffusion coefficient ⟨Deff⟩ of up to 0.871 ± 0.122 $cm^2\ s^{-1} \times 10^{-9}$ were obtained. Rotational silicone studies show increased permeation of the thiolated SEDDS A in comparison with unthiolated control. Rheological studies confirmed the mucolytic activity of the thiol-conjugates which differed only by 3% from DTT (dithiothreitol) serving as positive control.
Conclusion: Low molecular weight thiol-conjugates were identified to improve the mucus permeation, leading to highly efficient mucus permeating SEDDS, which were superior to conventional SEDDS and might thus be a new carrier for lipophilic drug delivery.

1. Introduction

Mucus protects the various mucosal surfaces of the body and represents a major hindrance for particulate drug delivery to the underlying mucosal epithelium [1–3]. The mucus hydrogel forms a steric barrier and promotes clearance of pathogens, e.g. viruses, trapped within the mucus network [2,4,5]. Similarly drug carriers that are incapable of diffusing fast enough through the mucus are held within the adhesive network and degraded prior to their delivery to the target absorptive epithelial membrane site [1–4]. Permeating the mucus barrier is a major requirement for successful drug targeting to mucosal tissues. Several strategies to overcome the mucus barrier were recently summarized by Dünnhaupt et al. [6]. Nanoparticulate systems, bearing enzymatically active compounds or systems possessing a 'slippery surface', as well as 'zeta-potential changing systems' are described in the literature [7–10]. Among various approaches, SEDDS emerged to have beneficial features to overcome the mucosal barrier. As shown by Friedl et al. [11], the composition of SEDDS has great impact on their mucus permeating properties. SEDDS consist of an oily phase, containing oil(s), a surfactant(s) and co-surfactant that emulsifies spontaneously upon mixing in an aqueous environment [12,13]. The resulting droplets are typically in the nanometer scale (<100 nm) favorable for the diffusion through mucus as the mucus is reported to express 50% of its pores below a size of 200 nm [14,15]. As the dynamic properties of mucus remain a considerable challenge for drug targeting, novel mucus permeating systems are needed.

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The aim of the study was the development of a novel mucus permeating system based around thiolated SEDDS. The strategy was to equip a nano-emulsion with thiol-moieties that are likely to interact with mucus, leading to a destruction of disulfide bonds of mucus network. This could be realized by the design of a series of novel thiolated emulsifiers based on modified fatty acid derivatives, structure identification of which was assessed via NMR. Ease of incorporation of the novel highly lipophilic thiol-conjugates into SEDDS led to stable thiolated oil-in-water nano-emulsions which were spontaneously formed upon dilution in aqueous environment, namely thiolated SEDDS.

The novel thiolated SEDDS were evaluated for their cytotoxicity and their muco-interactive features. Mucus permeating capacity of the novel designed thiolated SEDDS was evaluated using common *in vitro* models based on multiple particle tracking and on rotating silicone tubes. Mucolytic activity of the novel thiolated conjugates was studied using *in vitro* rheological measurements. In addition, computational predictions of the partition coefficient of the novel thiolated conjugates were provided.

2. Materials and methods

2.1. Materials

Dodecylamine was purchased from Merck Millipore, Austria. Iminothiolane hydrochloride and SATA ((2,5-dioxopyrrolidin-1-yl) 2-acetylsulfanylacetate) were purchased from Santa Cruz, Biotechnology Heidelberg, Germany. Cremophor EL was purchased from BASF, Germany. Captex 355 and Capmul MCM were obtained from Abitec Corporation, United States. Propylene glycol was obtained from Gatt-Koller, Absam, Austria. Fluorescein diacetate (FDA) and *N*-acetylcysteine (NAC) and DTT (dithiothreitol) were purchased from Sigma–Aldrich, Austria. Minimal essential medium (MEM) with and without phenol red was purchased from Sigma–Aldrich, Austria. Lumogen® dye was bought from Kremer, Germany. All other chemicals were of analytical grade and from commercial sources. Silicone tubes were obtained from Lactan, Austria.

2.2. Methods

2.2.1. Synthesis of thiolated conjugates

2.2.1.1. Synthesis of TBA-D. In a round bottom flask 1.816 mmol dodecylamine (1 eqv), 1.816 mmol iminothiolane hydrochloride (1 eqv) and 1.998 mmol triethylamine (1.1 eqv) were dissolved in a mixture of 10 ml acetonitrile and 4 ml methanol. The solution was stirred at room temperature for approximately 4 h and the solvent was removed under reduced pressure. The crude product was purified via chromatography over silica as stationary phase and using diethyl ether 100% as mobile phase to yield the product as a colorless liquid (yield = 55 ± 5%) (Fig. 1A). Finally the product was stored under inert gas at −20 °C until further use.

2.2.1.2. Synthesis of TGA-O. In the first reaction step, 2 mmol octylamine (1 eqv), 2 mmol SATA (1 eqv) and 0.2 mmol triethylamine (0.1 eqv) were dissolved in 10 ml acetonitrile in a round bottom flask. The solution was stirred at room temperature and monitored with thin layer chromatography. After 2.5 h, the solvent was removed under reduced pressure. The crude product was purified via chromatography over silica as stationary phase using a mixture of petroleum ether and ethyl acetate (7:3) as mobile phase to yield the protected thioester (product A) as a white solid (Fig. 1B). In the second step the thioester was hydrolyzed by suspending TGA-octylamine-thioester, hydroxylamine hydrochloride, EDTA (2,2′,2″,2‴-(ethane-1,2-diyldinitrilo)tetraacetic acid) and sodium hydrogen carbonate in a mixture of water and methanol (1:1). The pH was adjusted to pH 7 and the suspension was stirred at room temperature under thin layer chromatographic monitoring. After approximately 3 h, when the reaction had reached completion the product was extracted with dichloromethane. The organic phases were combined and dried with anhydrous sodium sulfate and the solvent was removed under reduced pressure. The product was purified via chromatography over silica as stationary phase using a mixture of dichloromethane and ethyl acetate (6:4) as mobile phase to yield the TGA-O as a colorless liquid (yield = 70 ± 6%). Finally the product was stored under inert gas at −20 °C until further use.

2.2.2. Identification of thiolated compounds: NMR analysis

The thiol conjugates were analyzed by ^{1}H and ^{13}C NMR spectra (Varian Gemini 200 spectrometer (^{1}H: 199.98 MHz, ^{13}C: 50.29 MHz). The center of the solvent multiplet ($CDCl_3$) was used as internal standard, which was related to TMS δ = 7.26 ppm for ^{1}H and δ = 77.00 ppm for ^{13}C.

2.2.3. Formulation of SEDDS

The new thiol-conjugates TBA-D and TGA-O were incorporated in a SEDDS-formulation previously developed by our research group [16]. The original SEDDS formulation consists of Cremophor EL 30%, Capmul MCM 30%, Captex 355 30% and propylene glycol 10%. The SEDDS preconcentrate was spiked with 3% (m/m) of thiol-conjugate referring exemplarily to NAC as control. The other thiol-conjugates, TBA-D and TGA-O, were incorporated in an equivalent molar amount. This results of course in different masses of the thiol-conjugates due to their different molecular masses. NAC was chosen, as it is reported to be the most commonly used mucolytic agent and provides only one thiol moiety on the molecule and is thus best comparable to the novel thiol conjugates. The prepared liposolution consisting of emulsifier, lipid phase and co-solvent was thoroughly mixed to guarantee a homogeneous formulation and then diluted with 0.1 M bis-tris-buffer of pH 6.8 in a ratio of 1:100.

2.2.4. SEDDS characterization

The stability of SEDDS was confirmed via a centrifugation test of 12,100*g* for 20 min using minispin centrifuge, Eppendorf, Germany. In addition, the particle size was monitored prior to experiment via an extended shelf-life study up to 12 h using bis-tris buffer pH 6.0, 0.1 M in a 1:100 dilution. To estimate stability of SEDDs in cell culture medium, preliminary tests were performed exemplarily for SEDDS A using white MEM as dispersing agent.

Size, zeta potential and polydispersity index of SEDDS formulations were determined by photon correlation spectroscopy using Nicomp PSS 380 DLS/ZLS, Particle Sizing Systems, Inc., Port Richey, Florida, with a laser wavelength of 650 nm and an E-fields strength of 5 V/cm. The experiment was performed at room temperature.

2.2.5. Cell viability assay-resazurin assay

The potential cytotoxic effect of the thiolated SEDDS was evaluated using the resazurin assay on a human colon carcinoma cell line monolayer according to a protocol previously reported by our research group [17]. Caco-2 cells were cultured over a period of 14 days in 24-well plates. After seeding the cells ($d = 25 \times 10^3$ cells/well) they were fed with 500 µl of minimal essential medium (MEM) supplemented with 20% fetal bovine serum (FBS) at 37 °C in 5% CO_2 environment. Every second day the medium was replaced by fresh medium. The assay was performed with resazurin which is reduced to fluorescent resorufin. Before incubating the cells with the test medium, they were washed with 500 µl of phosphate buffered saline (PBS) and then incubated with 500 µl of 0.5% sample solution (prepared with white MEM, which consists of MEM omitting the indicator). After incubating the samples for 4 h, the cells were washed again with PBS and were