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Research paper

Chitosan-coated liposome dry-powder formulations loaded with ghrelin for nose-to-brain delivery

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ABSTRACT

The nose-to-brain delivery of ghrelin loaded in liposomes is a promising approach for the management of cachexia. It could limit the plasmatic degradation of ghrelin and provide direct access to the brain, where ghrelin's specific receptors are located. Anionic liposomes coated with chitosan in either a liquid or a dry-powder formulation were compared. The powder formulation showed stronger adhesion to mucins (89 ± 4% vs 61 ± 4%), higher ghrelin entrapment efficiency (64 ± 2% vs 55 ± 4%), higher enzymatic protection against trypsin (26 ± 2% vs 20 ± 3%) and lower ghrelin storage degradation at 25 °C (2.67 ± 1.1% vs 95.64 ± 0.85% after 4 weeks). The powder formulation was also placed in unit-dose system devices that were able to generate an appropriate aerosol characterized by a Dv50 of 38 ± 6 µm, a limited percentage of particles smaller than 10 µm of 4 ± 1% and a reproducible mass delivery (CV: 1.49%). In addition, the device was able to deposit a large amount of powder (52.04% w/w) in the olfactory zone of a 3D-printed nasal cast. The evaluated combination of the powder formulation and the device could provide a promising treatment for cachexia.

1. Introduction

Ghrelin (GHRL) is a cationic peptide hormone (isoelectric point: 11.5) [1] composed of 28 amino acids. An octanoyl chain is post-translationally grafted onto the main peptide body in Ser-3 position. This fatty acid group is essential for binding GHRL to the "growth hormone secretagogue receptors". The binding of GHRL to these receptors results in the release of neuropeptide Y and Agouti-related peptide (AgRP) [2]. GHRL is involved in numerous physiological regulations and equilibria in the body. Among these, GHRL has positive effects on cachexia by stimulating food intake and decreasing inflammatory cytokine levels [3]. During a whole day, the ghrelin levels are not stable in the body and some secretion peaks are observed before each meal [4].

Cachexia is a complex syndrome usually associated with various severe pathologies such as cancers or heart failure. Following a cachexia consensus conference, cachexia was defined as "weight loss, muscle wasting, loss of appetite and general debility that can occur during a chronic disease" [6]. This syndrome is responsible of a worsened prognosis in these patients. For example, in patients with pancreatic cancer, cachexia would be responsible of 80% of the deaths [5].

Unfortunately, GHRL administration looks challenging due to its physicochemical and biological properties. These properties are similar to those of other biopharmaceutics, such as a short plasmatic half-life (9–13 min for octanoylated human ghrelin) [7]. Therefore, its administration by conventional routes (e.g. oral administration) does not usually provide successful treatment [8]. Alternative routes, such as parenteral administrations, are frequently taken to bypass the limitations encountered with oral administration. However, the invasiveness of such administrations may lead to a poor patient compliance and subsequent treatment failure, especially when chronic administrations are needed.

In this context, nose-to-brain delivery appears to be an attractive option that would encourage compliance. For instance, the nose-to-brain pathway could prevent GHRL from being rapidly cleared from the systemic circulation. In our previous study, a liquid formulation containing chitosan-coated liposomes loaded with GHRL was developed

Abbreviations: AL, anionic liposomes loaded with GHRL; CHOL, Cholesterol; CV, coefficient of variation; DHDP, dihexadecyl phosphate; DoE, design of experiments; FDA, food and drug administration; FBS, foetal bovine serum; GHRL, human octanoylated ghrelin; HBSS, Hank's balanced salt solution; HTCC-AL, AL coated with HTCC; HTCC, N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride; LS100, lipoid® S100; LUV, large unilamellar vesicles; LMV, large multilamellar vesicles; MEM NEAA, minimal essential medium non-essential amino acids; PBS, phosphate buffered saline pH 7.4; PDI, polydispersity index; RH, relative humidity; SEM, scanning electron microscopy; SD, standard deviation; TEM, transmission electron microscopy; TGA, thermogravimetric analysis; TFA, trifluoroacetic acid; TRYP, trypsin; UDS, unit-dose system

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[9]. The formulation provided promising data in terms of entrapment efficiency (56%), enzymatic protection (20.6% and 81.6% in the presence of trypsin and carboxylesterase, respectively) and permeation enhancement (10.8% through a Calu-3 monolayer).

The decision has been made to move to a powder form in orderto achieve a better storage stability of the peptide, and to avoid preservatives and the requirement of a cold chain for shipping and storage [10]. Moreover, permeation in the nasal cavity was found to increase with powders as the low volume of nasal liquid available for dissolution creates a high concentration gradient that positively impacts drug diffusion through the nasal membrane [11–13]. In addition, powders are characterized by a prolonged residence time in the nasal cavity, which offers more opportunity for the peptide to be transferred through the olfactory mucosa [14]. Regarding excipient properties, it has been reported that chitosan derivatives offer a higher transmucosal bioavailability when delivered as a powder [15].

On the other hand, the development of a dry-powder formulation presents some additional issues such as a reproducible and homogenous drug content. Other powder-specific parameters such as density, residual moisture and electrostatic charges can strongly influence and modify the physical behavior of the powder. Nevertheless, even if liquid formulations still represent the majority of nasal medicines, studies that focus on the development of nasal powders are increasing in number [16–18]. Up to now, most nasal powders represented on the market contain corticosteroids for rhinitis management but the number of studies related to nasal powders containing peptides is still very low [19,20].

This study aims to produce and characterize a powder formulation for nose-to-brain delivery that contains chitosan-coated liposomes loaded with GHRL by particle engineering using the spray drying technique. Liposomes are negatively charged and coated with N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) [21]. HTCC derivative has been chosen because of its ability of being positively charged and soluble at any pH [22]. The nose-to-brain administration of liposomes has already shown very satisfactory results thanks to their good entrapment ability, their easy diffusion through nasal mucosa and the timely drug release at the site of action [23]. Such formulation (dry form of HTCC-coated liposomes) should protect GHRL from early degradation, and increase its permeation through the olfactory mucosa due to the formulation's mucoadhesion properties as well as GHRL's long-term storage stability. Lactose is a well-characterized matrix agent in inhalation that allows HTCC-coated liposomes dispersion once in contact with nasal fluids [24]. The spray drying method was already used to produce both nasal powders and inhaled powders containing liposomes [25,26]. Once the drying parameters were optimized, the physicochemical properties of the powder were evaluated. The powder was also introduced in a dry-powder device which was designed to target the olfactory region for optimal nose-to-brain delivery [27]. The aerosol that was generated from this device was studied in depth (e.g. deposition in a nasal cast, aerosol size measurements, etc.). The powder was evaluated in terms of mucoadhesion, permeability through a Calu-3 monolayer and drug and aerosol stability during storage.

2. Materials and methods

2.1. Materials

Synthetic human acylated GHRL (purity ≥ 98%) was purchased from Shanghai Science Peptide Biological Technology co., Ltd (Shanghai, China). Acetonitrile, methanol, trifluoroacetic acid (TFA), dichloromethane (all solvents were high-performance liquid chromatography (HPLC) grade), dihexadecyl phosphate (DHDP) and cholesterol (CHOL) were obtained from Sigma Aldrich (St Louis, MO, USA). Soybean lecithin "Lipoid® S100" (LS100) was purchased from Lipoid® Gmbh (Ludwigshafen, Germany). Trypsin-EDTA 0.25% w/v solution (TRYP), Hank's balanced salt solution (HBSS), certified US origin heat-inactivated foetal bovine serum (FBS), sodium pyruvate 100 mM, L-glutamine 200 mM, penicillin (10,000 U/mL)/streptomycin (10,000 µg/mL), gentamicin 50 mg/mL and minimum essential medium containing no essential amino acids (MEM NEAA) were obtained from Thermo Fisher Scientific (Walthman, MA, USA). Calu-3 lung adenocarcinoma cells (ATCC® HTB-55™) were purchased from ATCC (Manassas, USA). Inserts used for a Calu-3 air–liquid interface culture were composed of a mixed ester cellulose membrane with 0.45 µm porosity and 30 mm diameter adapted for 6-well plates and Amicon® Ultra-15 K centrifugal tubes with a 100 kDa cut-off. These were purchased from Merck Millipore (Darmstadt, Germany). The chitosan derivative was a HTCC derivative with a MW of 92 kDa, a deacetylation degree of 80% and a substitution degree of 33% (Kitozyme, Herstal, Belgium). Lactohale® 210, which is a grade of lactose intended for inhalation, was used as a matrix agent and obtained from DFE Pharma (Goch, Germany).

2.2. Production of HTCC-coated liposomes

Briefly, uncoated liposomes composed of CHOL, LS100 and DHDP (45/45/10% w/w) and loaded with GHRL (AL) were prepared by using a lipid film rehydration method as previously described [9]. Large multilamellar vesicles (LMV) were then sonicated and extruded through membranes with porosities of 1 µm, 0.4 µm and 0.1 µm (EMD Millipore, MA, USA). To prepare chitosan-coated liposomes (HTCC-AL), a suitable amount of HTCC was weighed and dispersed under magnetic stirring in PBS pH 7.4 overnight to obtain a translucent HTCC solution at 10 mg/mL. Then, 9 mL of AL (10 mg/mL) were coated with 1 mL of HTCC (10 mg/mL), which was added dropwise under magnetic stirring at 3000 rpm to obtain a 10-fold dilution of the initial HTCC solution ($[HTCC]_{final}$ = 1 mg/mL) and lead to HTCC coated AL (HTCC-AL). The HTCC-AL dispersion was left for 1 h under magnetic stirring before leaving the formulation overnight at 4 °C. This corresponded to the initial HTCC-AL liquid formulation, which was used in some sections as a reference for comparison with the HTCC-AL powder.

Prior to spray drying, 1.0 g of Lactohale® 210 (DFE Pharma, Goch, Germany) was dissolved in the liposomal dispersion to act as a matrix agent. The preparation was left for 15 min under magnetic stirring for complete solubilization.

2.3. Determination of the spray drying parameters and powder characterizations

A design of experiment (DoE) including four factors, four resolutions and two levels per parameter was established using the statistical software Minitab (*Minitab* Inc., USA). This software selected drying conditions that offered the highest yield as well as a representative fraction of particles larger than 10 µm. For each drying parameter, two levels were defined (minimum "−1" and maximum "+1", Table 1).

The spray drying was performed with a Mini Spray-Dryer B-290 (Büchi, Switzerland) equipped with a 0.7 mm diameter nozzle and a high performance cyclone. Powders were produced by spray drying 10 mL of liquid formulation containing 10% (w/v) Lactohale® 210, 0.1% (w/v) GHRL, 1% (w/v) lipid mixture and 0.1% (w/v) HTCC. The

Table 1
Parameters for the DoE for optimizing the spray drying conditions, with their maximum (+1) and minimum (−1) levels.

Parameters	Level (−1)	Level (+1)
X1 = Inlet temperature (°C)	90	130
X2 = Feed rate (mL/min)	2.6	6.2
X3 = Spray gas flow (L/h)	283	667
X4 = Aspirator rate (m^3/h)	32	38