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Formulation and evaluation of novel reverse microemulsions containing salmon calcitonin in hydrofluoroalkane propellants

Ziyun Shan [a,b], Yinhe Tan [a], Lingzhen Qin [a], Ge Li [c], Xin Pan [a], Zhouhua Wang [a], Xiu Yu [a], Qinruo Wang [d], Chuanbin Wu [a,b,*]

[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China
[b] Research and Development Center of Pharmaceutical Engineering, Sun Yat-sen University, Guangzhou, China
[c] Guangzhou Neworld Pharmaceutical Ltd. Co., Guangzhou, China
[d] Faculty of Automation, Guangdong University of Technology, Guangzhou, China

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ABSTRACT

To develop reverse microemulsion as a potential strategy for pulmonary delivery of salmon calcitonin (sCT) in HFA134a propellant of pressurized metered dose inhalers (pMDIs), pluronic P85 (P85) was chosen as the most appropriate surfactant to form microemulsions containing sCT. Formulation parameters, including the surfactant and ethanol content, water content, and sCT loading, were optimized to obtain two desired pMDI formulations A and B with clear and transparent appearance, Tyndall effect, good physical stability and aerosolization properties. Aerosolization properties of the optimized pMDIs were assessed by next generation impactor (NGI) and twin-stage impactor (TSI), and the dose of sCT in each stage was assayed by HPLC. The fine particle fraction (FPF) of formulations A and B were both at the range of approximately 28.0–36.0%. Cytotoxicity studies indicated the cell viability determined by MTT assay only slightly dropped when the A549 cells were exposed to the pMDI formulations. Pharmacological study performed on the male Wistar rats showed the intratracheal administration of the microemulsion pMDIs containing sCT exhibited similar but prolonged hypocalcemic activity compared with the intravenous injection of sCT solution. Therefore, such reverse microemulsions are potential for pulmonary delivery of therapeutic peptides using HFA-pMDIs.

1. Introduction

Inhalation has been a traditional delivering route for drugs that exert therapeutic effects locally in the lung (Chokshi et al., 2009; Courrier et al., 2002). The lung also represents an outstanding route for orally delivering drugs to systemic circulation because of its large alveolar surface area, thin epithelial barrier, extensive vascularization, and relatively low proteolytic activity (Patton and Byron, 2007). Currently, three types of aerosol formulations and devices are available: nebulizers (jet or ultrasound), pressurized metered dose inhalers (pMDIs), and dry powder inhalers (DPIs). Among them, pMDIs have been used as delivery devices for proteins and peptides by many research groups (Bailey and Berkland, 2009; Tan et al., 2011), and are well accepted by both patients and clinicians because of their good compliance such as noninvasiveness, portability, and disposability (Shoyele and Slowey, 2006; Tan et al., 2011). Moreover, pMDIs are the most economical vehicles for oral delivery of drugs to the respiratory tract (Chokshi et al., 2009; Tarara et al., 2004).

In general, pMDIs are formulated as either solutions or suspensions. However, most drugs, including proteins and peptides, exhibit negligible solubility in hydrofluoroalkane propellants used in the preparation of pMDI system, Thus, pMDIs containing these drugs were formulated as either dispersions or solutions with the aid of cosolvents (Chokshi et al., 2009; Rogueda, 2005; Tarara et al., 2004). In addition, reverse microemulsions in propellant-based inhalers have been proposed as possible vehicles for delivery of polar drugs, especially biomolecules, to and through the lungs (Chokshi et al., 2009; Courrier et al., 2002, 2004; Meakin et al., 2006; Patel et al., 2003a,b; Patton and Byron, 2007; Rogueda, 2005; Selvam et al., 2008, 2012). It was reported that formulating high potency drugs within the microemulsion core can protect the drug from interaction with the canister walls of the pMDI (Selvam et al., 2012). Furthermore, this approach may provide an opportunity to deliver drug combinations by saving the extensive reformulation work (Selvam et al., 2012). Despite its potential,

* Corresponding author at: School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China. Tel.: +86 02039943120; fax: +86 02039943117.
E-mail address: chuanbin_wu@126.com (C. Wu).

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microemulsion in propellant-based inhaler also has drawbacks, such as low fine particle fraction (FPF) due to its low vapor pressures (Brambilla et al., 1999; Smyth, 2003), high viscosity, and sometimes cytotoxic effects due to high content of surfactant and cosolvent.

By far, only few studies reported the formulation of reverse microemulsions in propellants (Chokshi et al., 2009; Courrier et al., 2004; Meakin et al., 2006; Patel et al., 2003a,b; Selvam et al., 2008, 2012; Sommerville et al., 2000), and even fewer studies prepared microemulsions in hydrofluoroalkanes (HFAs) (Chokshi et al., 2009; Meakin et al., 2006; Patel et al., 2003a,b; Selvam et al., 2008, 2012; Steytler et al., 2003), which are the only propellants accepted by FDA for use in pMDIs. The fluorinated surfactants, which were mostly used in researches on microemulsions in HFA-based pMDIs (Patel et al., 2003a,b; Steytler et al., 2003), are less appealing to pulmonary drug delivery applications (Lawrence and Rees, 2000). In recent years, Chokshi et al. (2009) and Selvam et al. (2008, 2012) investigated the ability of ethoxylated non-ionic amphiphile to form reverse microemulsions in HFA134a with ethanol as cosolvent. Selvam et al. (2012) also studied the aerosol characteristics of HFA-based microemulsion formulations in the presence of a water-soluble model solute, methyl orange (MO) and the effects of excipients on the aerosol characteristics. Meakin et al. (2006) incorporated salbutamol sulfate into the HFA-based microemulsion formulations and determined the particle size of microemulsions. However, the fraction of the fine particles in these microemulsions was relatively low (<20%, Meakin et al., 2006; Selvam et al., 2012). And unfortunately, pharmaceutical evaluation of these preparations has not been carried out, and no therapeutic protein has been incorporated in these systems.

In the last several decades, peptides and proteins have become more and more important therapeutic drugs to improve the quality of life of patients (Davis, 1999; Yang et al., 2012). However, because of their low absorption rate and instability in the gastrointestinal tract, administration of these active agents is mainly limited to parenteral routes. Compared with other non-invasive administration routes, lung is the most promising alternative to injection for delivering biopharmaceuticals to achieve systemic absorption as mentioned above. For example, salmon calcitonin (sCT), a polypeptide of 32 amino acids which has been approved for the treatment of hypercalcaemia and bone disorders such as osteoporosis and Paget's disease, is currently marketed as both injectable solutions (Calcimar®, Miacalcin®) and nasal sprays (Fortical®, Miacalcin®). In comparison with the patient unfriendly intramuscular or subcutaneous injection, the nasal formulations showed a wide window of bioavailability, ranging from 0.3% to 30% relative to injection (Stevenson, 2009). Because of the unique properties of the lung as mentioned above, the patient friendly pulmonary administration may offer more advantages than injection and nasal spray in improving the performance of sCT.

Therefore, reverse microemulsions in HFA134a containing sCT were formed in this study and investigated as a potential strategy for pulmonary delivery of therapeutic peptides using HFA-pMDIs. In order to improve the lung deposition and physical stability of sCT in hydrofluoroalkane propellants, formulation parameters such as the content of surfactant, ethanol, water, and sCT were optimized. Aerosolization properties of the prepared pMDIs were assessed using next generation impactor (NGI) and twin-stage impactor (TSI), and the dose of each fraction of stages was quantified by HPLC. Cytotoxicity of the formulations was evaluated on A549 cells, an alveolar type II epithelial cell line. The pharmacological effect of the formulations on serum calcium level of male Wistar rats was also evaluated. To authors' knowledge, this is the first time a therapeutic protein was incorporated in reverse microemulsions in HFA134a propellant and was evaluated deeply. The outcome of this research may address the question whether a reverse microemulsion in hydrofluoroalkane propellant offers a greater potential to deliver peptide drugs to and through the lung.

2. Materials and methods

2.1. Materials

Salmon calcitonin (sCT, purity 99.5%) was purchased from Chengdu Kaijie Biopharm Co., Ltd. (Chengdu, China). HFA134a (pharmaceutical grade, purity 99.99%) was obtained from INEOS Flour Ltd. (Runcorn, Cheshire WA74JE, UK) and 2H, 3H-perfluoropentane (HPFP, 99.9%) was purchased from DuPont Company (Wilmington, USA). HPLC grade methanol and acetonitrile were received from Honeywell (China) Co., Ltd. (Shanghai, China). Calcium carbonate, lanthanum chloride, tetramethylammonium hydroxide (TMAH) were purchased from Aladdin Reagent Database Inc. (Shanghai, China). Pluronic L64 and pluronic P85 were obtained from Nanjing Well Chemical Co., Ltd. (Nanjing, China). Solutol® HS 15, Cremophor® EL, Cremophor® RH 40, poloxamer P407, and poloxamer P188 were kindly donated by BASF (Ludwigshafen, Germany). The A549 cell line was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), penicillin and streptomycin solution, and 0.25% trypsin solution were obtained from Gino Biomedical Tech. Co., Ltd. (Zhejiang, China). Fetal bovine serum (FBS) was purchased from Thermo Fisher Scientific Inc. (MA, USA). MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide) was purchased from MP Biomedicals (CA, USA). Water was distilled *via* a water purification system (PureLAB option, ELGA Lab Water Inc., UK). All the other chemicals used were analytical reagents.

2.2. Methods

2.2.1. Preparation of reverse microemulsions

Reverse microemulsions containing sCT in hydrofluoroalkane propellants were prepared and the formulation parameters were optimized. In a plastic coated glass bottle, sCT, water, surfactant, and ethanol were added sequentially. After 1 min of agitation by a vortex mixer to fully dissolve the surfactant in water, a 50 μL metering valve (Valois Dispensing Systems Co., Ltd., Jiangsu, China) was immediately fixed onto the bottle using a semi-automatic bottle crimper (Zhongshan Zhihua Aerosol Equip. Co., Ltd., Guangdong, China) at 22–26 °C. Then the propellant HFA134a (10 g) was added through a valve stem using an aerosol filling machine (Zhongshan Zhihua Aerosol Equip. Co., Ltd., Guangdong, China) to form pMDI and the final weight of each finished formulation of pMDI was recorded.

The emulsification performance of different surfactants, including the non-ionic ethoxylated copolymers Solutol® HS 15, Cremophor® RH 40, Cremophor® EL, pluronic P85, pluronic L64, poloxamer P188, and poloxamer P407, in HFA propellants was studied through visual observation after filling of HFA134a. A typical microemulsion is clear and shows Tyndall effect.

Formulation parameters, including the total content of surfactant and ethanol (surfactant/ethanol at a fixed ratio of 1/2 or 1/3), water content, and sCT loading, were optimized to obtain desired microemulsions with good physical stability and aerosolization properties. The composition changes were described as follows: (a) the total content of surfactant and ethanol varied from 11.80%, 12.50%, 13.00%, 14.00%, to 14.50% (w/w), with water content of 0.64% and sCT loading of 1.30 mg; (b) the water content varied from 0.55%, 0.60%, 0.65%, 0.68%, to 0.72% (w/w), with the total content of surfactant and ethanol at 13.15% and sCT loading of 1.30 mg; (c) sCT loading varied from 1.00 mg, 1.10 mg, 1.30 mg, 1.50 mg, to 1.60 mg, with the total content of surfactant and ethanol at 13.15% and water content of 0.64%.