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Design of salmon calcitonin particles for nasal delivery using spray-drying and novel supercritical fluid-assisted spray-drying processes

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ABSTRACT

The overall aim of this study was to prepare a nasal powder formulation of salmon calcitonin (sCT) using an absorption enhancer to improve its bioavailability. In this work, powder formulations for nasal delivery of sCT were studied using various absorption enhancers and stabilizers. Powders were prepared by two different methods: conventional spray-drying (SD) and novel supercritical fluid-assisted spray-drying (SASD) to investigate the role of CO_2 in the particle formation process. The prepared sCT powder formulations were characterized by several analyses; powder X-ray diffractometry (PXRD), scanning electron microscopy (SEM), and the Fourier transform infrared (FT-IR) spectroscopy method. The particle size distribution was also evaluated. *In vivo* absorption tests were carried out in Sprague-Dawley rat using the prepared powder formulations, and the results were compared to those of raw sCT. Quantitative analysis by high-performance liquid chromatography (HPLC) indicated that sCT was chemically stable after both the SD and SASD processes. Results of PXRD, SEM, and FT-IR did not indicate a strong interaction or defragmentation of sCT. The *in vivo* absorption test showed that SD- and SASD-processed sCT powders increased the bioavailability of the drug when compared to the nasal administration of raw sCT. In addition, SASD-processed sCT exhibited higher nasal absorption when compared with SD-processed sCT in all formulations due to a reduction of particle size. The results from this study illustrate that the preparation of nasal powders using the SASD process could be a promising approach to improve nasal absorption of sCT.

1. Introduction

Biopharmaceuticals such as peptides and proteins are highly potent and selective drugs with a wide range of therapeutic applications and have been rapidly developing in the last two decades (Frokjaer and Otzen, 2005; Walsh, 2005). Due to the peculiar properties of peptide and protein drugs that include large molecular size and susceptibility to enzymatic degradation, most of these biopharmaceuticals are mainly administered via injections for systemic effect. Injections are not always the desired dosage form due to patient compliance, safety and disease management; therefore, safe and effective non-invasive administration routes are required as an alternative to injection.

Among the non-invasive routes, nasal administration offers promising potential as a viable alternative for the delivery of some peptide drugs. The nasal epithelium has a relatively high permeability, and only two cell layers separate the nasal lumen from the dense blood vessel network in the lamina propria. Some of the major advantages offered by nasal delivery include rapid absorption, high bioavailability, fast onset of therapeutic action, avoidance of hepatic first pass metabolism, reduced risk of overdose, improved patient compliance and ease of administration (Dondeti et al., 1996). However, most peptides and proteins are not

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well absorbed from the nasal cavity when administered as a simple solution. Limiting factors of nasal drug absorption are (i) the enzymatic barrier of the nasal mucosa, (ii) the physical barrier of the epithelium, (iii) the rapid mucociliary clearance limiting the time available for absorption, and (iv) the mucus layer itself (Schmidt et al., 1998). Thus, intranasal formulation development requires special attention to bioavailability, which can be improved by absorption enhancers such as bile salts, fusidate derivatives, fatty acids, surfactants or cyclodextrin (Schipper et al., 1993b, 1995b; Shao and Mitra, 1992).

Another key factor for nasal delivery is the dosage form, *i.e.*, liquid or powder. The solid form of the active pharmaceutical ingredient (API) is preferable since it provides good stability, comes in a small packaging size and is easy to handle in comparison to liquid forms (Marttin et al., 1997). In fact, some previous studies showed that the powder forms have better chemical stability and enhanced nasal absorption (Ishikawa et al., 2002; Matsuyama et al., 2006; Schipper et al., 1993a).

Protein and peptide drug powders can be prepared by several methods. Spray-drying (SD) is a useful and widely applied method to prepare dry protein and peptide particles, where microparticles and nanoparticles can be formed directly from a drug solution with greater control over particle size, morphology and powder density (Johnson, 1997; Ståhl et al., 2002; Vehring, 2008). In addition, the application of supercritical fluids (SCFs) to particle design has emerged as a promising technique for producing powders for inhalation (Moshashaée et al., 2003a,b,b; Okamoto and Danjo, 2008; Reverchon et al., 2007b). SCFs can be defined as a substance existing as a single fluid phase above its critical temperature and pressure. The particle preparation process of SCFs can take advantage of some specific properties of gases at supercritical conditions, like the modulation of the solubilization power, large diffusivities, solventless or organic solvent-reduced operation and the consequent possibility of controlling powder sizes and distribution. In particular, supercritical carbon dioxide (SC-CO_2) is widely used to prepare powder formulations of biopharmaceuticals because of its mild process conditions and non-flammable, non-toxic and inexpensive properties. The techniques proposed are the rapid expansion of the supercritical solution (RESS), the aerosol solvent extraction system (ASES) and supercritical anti-solvent precipitation (SAS). However, in many previous studies, the solubility of many peptides and proteins in SC-CO_2 is relatively low, and thus SC-CO_2 was used as an anti-solvent for the precipitation of proteins and peptides (Chattopadhyay and Gupta, 2002; Jung and Perrut, 2001; Moshashaée et al., 2003a,b; Winters et al., 1997). For successful SAS precipitation, however, the complete miscibility of the liquid in the SC-CO_2 and the insolubility of the solute in it are essential. For this reason, SAS often uses organic solvents such as dimethylsulfoxide, which might cause perturbation of the secondary structure and is not applicable to the water solution of proteins and peptides due to the very low solubility of water in CO_2 (Thiering et al., 2000). Recently, supercritical carbon dioxide-assisted atomization techniques were proposed: carbon dioxide-assisted nebulization with a bubble dryer (CAN-BD) and supercritical assisted atomization (SAA) (Reverchon et al., 2007a; Sievers et al., 2003). Based on these theories, a novel supercritical fluid-assisted spray-drying (SASD) process was developed by Hwang's group as a valid alternative technique to the conventional SD process and SAS process for preparation of nanoparticles (Hwang et al., 2009). In SASD process, SC-CO_2 acts both as a co-solvent being partially miscible with solvent to be treated, as well as a pneumatic agent to atomize the solution into fine particles. Therefore, it allows the micronization of the compound from an aqueous solution as well as a water-organic solvent mixture.

Salmon calcitonin (sCT) is a polypeptide hormone comprised of 32 amino acids with a molecular weight of 3431.88 Da, with a single disulfide bond (Cys1—Cys7) at the NH_2-terminus. It is currently formulated as a sterile solution for intramuscular or subcutaneous injection and as a nasal spray in the management of several bone-related diseases including Paget's disease, hypercalcemia and osteoporosis (*e.g.*, Miacalcic®). It is reported that the bioavailability of market nasal salmon calcitonin is about 3%. Using an absorption enhancer has shown promising results in improving systematic bioavailability of sCT by *in vivo* study on animals and humans via nasal administration (Gordon et al., 1985; Matsuyama et al., 2006; Schipper et al., 1995a). There are a few studies of the dry powder formulation of sCT using the SD process. These studies showed that the physiochemical stability of sCT powder was not affected during the SD process (Chan et al., 2004; Yang et al., 2007a). However, particle design and formulation of sCT with proper additives using the SD and SCFs processes for nasal delivery with the aim of increasing permeability, bioavailability and maximizing chemical and physical stability have not been systematically investigated.

Thus, the objectives of this study were: (i) preparation of sCT nasal powder formulation using the SD and SASD processes with proper additives to improve penetration and bioavailability and (ii) evaluation of physicochemical characteristics and *in vivo* bioavailability of sCT powders from the SD and SASD processes.

2. Materials and method

2.1. Materials

sCT was kindly supplied by Polypeptide Laboratories A/S (Hillerod, Denmark). Inulin (Frutafit® TEX) was gifted from Sensus (Roosendaal, Netherlands). Trehalose, sodium taurocholate hydrate and heptakis ((2,6-di-*o*-methyl)-β-cyclodextrin) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Chitosan glutamate [Protasan UP G 213; Mw: 200–600 kDa, DD (degree of deacetylation): 75–90%] was purchased from Nova Matrix/FMC Biopolymer (Sandvika, Norway). Carbon dioxide (CO_2, purity 99.9%) was purchased from Hanmi Gas Co., Ltd. (Korea). All organic solvents were of HPLC grade. All other chemicals in this study were analytical reagent grade.

3. Powder preparation

3.1. Powder preparation using the SD process

Aqueous solutions (total concentration: 10 mg/ml) of sCT, absorption enhancer, and stabilizer in different weight ratios were prepared as described in Table 1. Inulin and trehalose were used as stabilizers; chitosan, sodium taurocholate, and beta-cyclodextrin were used as absorption enhancers. The SD process was carried out using a laboratory-scale spray dryer (Mini Spray

Table 1
Formulations of sCT powders.

	Quantity (mg) per formulation					
	sCT	Stablizer		Absorption enhancer		
		Inulin	Trehalose	Chitosan	Sodium taurocholate	Beta-cyclodextrin
F1	50	400	–	50	–	–
F2	50	400	–	–	50	–
F3	50	400	–	–	–	50
F4	50	–	400	50	–	–
F5	50	–	400	–	50	–
F6	50	–	400	–	–	50