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In vitro and *in vivo* preclinical evaluation of a minisphere emulsion-based formulation (SmPill®) of salmon calcitonin

Tanira A.S. Aguirre [a,b], Mónica Rosa [b], Ivan S. Coulter [b], David J. Brayden [a,*]

[a] UCD School of Veterinary Medicine and UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland
[b] Sigmoid Pharma, Dublin City University, Invent Centre, Dublin 9, Ireland

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ABSTRACT

Salmon calcitonin (sCT, MW 3432 Da) is a benchmark molecule for an oral peptide delivery system because it is degraded and has low intestinal epithelial permeability. Four dry emulsion minisphere prototypes (SmPill®) containing sCT were co-formulated with permeation enhancers (PEs): sodium taurodeoxycholate (NaTDC), sodium caprate (C_{10}) or coco-glucoside (CG), or with a pH acidifier, citric acid (CA). Minispheres protected sCT from thermal degradation and the released sCT retained high bioactivity, as determined by cyclic AMP generation in T47D cells. Pre-minisphere emulsions of PEs combined with sCT increased absolute bioavailability (*F*) compared to native sCT following rat intra-jejunal (i.j.) and intra-colonic (i.c.) loop instillations, an effect that was more pronounced in colon. Minispheres corresponding to ~2000 I.U. (~390 μg) sCT/kg were instilled by i.j. or i.c. instillations and hypocalcaemia resulted from all prototypes. The absolute *F* (i.j.) of sCT was 11.0, 4.8, and 1.4% for minispheres containing NaTDC (10 μmol/kg), CG (12 μmol/kg) or CA (32 μmol/kg) respectively. For i.c. instillations, the largest absolute *F* (22% in each case) was achieved for minispheres containing either C_{10} (284 μmol/kg) or CG (12 μmol/kg), whilst the absolute *F* was 8.2% for minispheres loaded with CA (32 μmol/kg). In terms of relative *F*, the best data were obtained for minispheres containing NaTDC (i.j.), a 4-fold increase over sCT solution, and also for either C_{10} or CG (i.c.), where there was a 3-fold increase over sCT solution. Histology of instilled intestinal loops indicated that neither the minispheres nor components thereof caused major perturbation. In conclusion, selected SmPill® minisphere formulations may have the potential to be used as oral peptide delivery systems when delivered to jejunum or colon.

1. Introduction

One of the most common approaches involving lipid emulsions for oral drug delivery is use of self-emulsifying systems (SES). They are composed of oil, surfactant, co-surfactant, and co-solvents in different ratios in an absence of a water phase. SES form transparent isotropic mixtures, which spontaneously form fine oil-in-water (O/W) emulsions in aqueous GI fluids using the agitation provided by gastric motility (Kohli et al., 2010). Improved bioavailability of drugs formulated with self-emulsifying techniques is attributed in part to creation of small oil droplets, which provide a large interfacial area for pancreatic lipase-mediated hydrolysis of triglycerides to promote rapid release of the drug or formation of mixed micelles with bile salts containing the active pharmaceutical ingredient (API) (Kommuru et al., 2001). Lipid-based drug delivery systems have been used successfully for the formulation of small molecules with solubility issues from the Biopharmaceutical Classification System's Class II and IV (Shaikhn et al., 2012). SES may also be applied to Class III molecules as well as for peptides, since they can potentially overcome enzymatic degradation and low epithelial permeability (Kohli et al., 2010). Solid oral dosage forms using self-emulsifying O/W approaches include soft gelatin capsules, pellets, microspheres and minispheres. For example, porous polystyrene minispheres were used as carriers to aid solidification of SES containing Captex 200® (oil), Cremophor EL® (surfactant) and Capmul MCM® (co-surfactant) to formulate laratidine (Patil and Paradkar, 2006). Amongst formulation approaches to obtain biocompatible minispheres are the use of calcium pectinate gels (Günter et al., 2014), chitosan (Shu and Zhu, 2000) and hydrogels (Mohamadnia et al., 2007). These minispheres are typically formed by ionotropic gelation of a solution containing the drug and the gel-forming agent, which is dropped into a crosslinking solution containing coating compounds. However, a problem associated with minispheres is low mechanical strength leading to sub-optimal release profiles (Shu and Zhu, 2000).

A novel oral system, SmPill® (Sigmoid Pharma, Ireland), contains emulsions formulated as minispheres, which are subsequently loaded into a gelatin capsule as the final dosage form. SmPill® presents the drug in a dissolved form and the release profile can in theory be designed to target the optimal intestinal region for absorption (Coulter, 2010; Moodley and Coulter, 2008; Sigmoid, 2014). Importantly, the combination of components in the formulation provides sufficient mechanical strength and, pre-solubilisation in the emulsion

* Corresponding author.
E-mail address: david.brayden@ucd.ie (D.J. Brayden).

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enables adaptation for hydrophilic or lipophilic molecules. An additional feature for delivery of poorly permeable molecules is the potential to incorporate intestinal permeation enhancers (PEs), of which C_{10} (Maher et al., 2009), sodium taurodeoxycholate (NaTDC) (Mrestani et al., 2003) and coco glucoside (CG) (Aguirre et al., 2014) were selected here. Acidifying excipients, including citric acid (CA), can also be added in order to protect peptides from serine proteases (Welling et al., 2014). In the final aspect of production, minispheres can be coated with polymers and then presented in capsules designed to target small intestinal or colonic regions of the GI tract, in order to either to respectively maximise systemic absorption or to locally target the drug. A related approach has been used in marketed products in the latter case with coated tablets of budesonide that dissolve when the pH increases to >7.0 in the colon of patients with mild-to-moderate ulcerative colitis (Sandborn et al., 2015). Colonic targeting is the principle behind the SmPill®-enabled cyclosporine A formulation, CyCol®, which is designed to provide local topical delivery of solubilised cyclosporine, but to avoid systemic exposure in the treatment of moderate-severe ulcerative colitis [55]. It has completed two Phase II studies in ulcerative colitis (NCT01033305, 2012; NCT02130414, 2014).

To investigate the potential for SmPill® to enhance systemic bioavailability of a hydrophilic peptide, we used salmon calcitonin (sCT, MW 3432) as a model because, due to its sensitivity to pancreatic serine proteases and poor intestinal permeability, it is useful in evaluating delivery technologies (Maher and Brayden, 2012). Marketed nasal versions of peptides including sCT are estimated to have absolute bioavailability of 1% (Grant and Leone-Bay, 2012), and the most advanced oral formulation of sCT, OSTORA™ (Tarsa Therapeutics, USA), recently completed Phase III to give comparable pharmacokinetics to the nasal comparator (Binkley et al., 2012). OSTORA™ is a Eudragit® L 30 D-55-coated tablet in which sCT and CA are co-localized in vesicles in the core, so it lacked a recognised PE in order to facilitate the regulatory path, although this may partially account for the <1% bioavailability. There appears to be some confusion in the literature over this composition (Lewis and Richard, 2015), because other formulations from Enteris (NJ, USA) based on the same technology include acyl carnitines as PEs and an assumption was made that OSTORA™ was no different. The aims of this study therefore were to produce sCT-SmPill® minispheres which incorporate PEs and/or CA and to assess physicochemical characteristics, *in vitro* bioactivity, and *in vivo* pharmacodynamics (PD) and pharmacokinetic (PK) parameters following intestinal instillation of uncoated minispheres into the jejunum or colon of rats with an ultimate goal of generating oral peptide bioavailability of >1%. We demonstrate that sCT bioactivity was not reduced during minisphere manufacturing and that the inclusion of selected PEs enables significant systemic bioavailability enhancement in both regions.

2. Materials and methods

All chemicals were obtained from Sigma Aldrich, Ireland, except for CG, which was supplied as Plantacare® 818 UP (Cognis, Germany). Media, buffers and supplements were obtained from GIBCO®, Ireland. Synthetic sCT was purchased from Polypeptide Laboratories (Copenhagen, Denmark); it had an average activity of 5100 I.U./mg. Transcutol HP®, Cremophor EL®, and Miglyol 818® were obtained from Gattefossé (France), BASF (Germany) and Sasol (UK) respectively. The Parameter™ cAMP ELISA was obtained from R&D Systems (UK).

2.1. sCT-SmPill® minispheres preparation

sCT minispheres were prepared following a standard operation procedure with minor modifications (Coulter et al., 2010). Approximately 1700 mg of oil phase composed of Transcutol HP®, Cremophor EL®, and Miglyol 818® at a ratio of 57: 24: 19 (w/w) respectively, was mixed with approximately 24 mL of aqueous phase, comprising gelatin, PE (NaTDC, C_{10}, or CG), or CA to form a homogeneous pre-minisphere emulsion. 1 mL of a 20 mg/mL sCT solution in water was added to the emulsion and mixed at 65 °C for 5 min. The sCT-containing final emulsion was then extruded into cold oil to form minispheres. Minispheres were kept in oil at 2–8 °C for 30 min, separated with a sieve and allowed to dry at 2–8 °C. The theoretical percentage of each component in dried minispheres is shown (Table 1).

2.2. Rotational viscometer

Rheological characteristics of sCT-SmPill® pre-minisphere emulsions were determined using a rotational viscometer (DV-II + PRO Digital Viscometer, Brookfield Instruments, UK) with an UL adapter and a LV1 spindle. Analysis was carried out at 60 °C, varying the speed from 0.1 to 0.6 RPM in order to maintain the torque lower than 100%.

2.3. Multiple light scattering

The physical stability of sCT-SmPill® pre-minisphere emulsions was analysed by multiple light scattering using Turbiscan® Lab equipment (Formulaction Ltd, France). Samples were dispensed into the equipment glass cell and were analysed at 50 °C with scans every 5 min for 20 min. The detection is made for the whole height of the sample from the bottom to the top of the cell every 40 μm. Data were acquired with synchronous detectors which receives transmitted light (T) through the sample (at 180°) and backscattered light (BS) by the sample (at 45°). The light source was an electro luminescent diode in the near infrared ($\lambda = 880$ nm). This technique is useful for identification of particle migration (sedimentation and creaming) and particle size variation (coalescence and flocculation).

2.4. Scanning electron microscopy of sCT-SmPill® minispheres

Shape and surface of minispheres were examined with a scanning electron microscopy (SEM) (Jeol Scanning Microscope, JSM-5800, Tokyo, Japan). The minispheres were carbon and gold sputtered (Jeol Jee 4B SVG-IN, Tokyo, Japan) before analysis.

2.5. sCT in vitro cyclic AMP bioactivity assay in T47D cells

A concern over formulating sCT in SmPill® was maintenance of sCT bioactivity after incorporation into emulsions at 65 °C, followed by release. Two prototype sCT minispheres were prepared with gelatin, sorbitol and the three oil phase components, but without any PE or CA. The influence of temperature was assessed by examining the effects of incubating sCT in the emulsion at 65 °C for either 5 min or 15 h. Negative control minispheres without sCT were prepared under parallel conditions. Dried minispheres were dissolved in PBS at 37 °C using an orbital shaker prior adding to cells. T47D cell culture was carried out as described elsewhere (Ryan et al., 2009), with the density of seeded cells altered to 2.5×10^5 cells/well. The theoretical loading of the two prototype formulations was approximately 0.4 mg sCT/100 mg minispheres (Table 1), and individual calculations were carried for

Table 1
Ratios by weight of components in four dried minisphere prototypes.

	CA	NaTDC	C_{10}	CG
sCT	0.40	0.40	0.13	0.40
Transcutol HP®	20.3	20.7	15.0	19.7
Cremophor EL®	8.7	8.9	6.4	8.4
Miglyol 818®	6.6	6.7	4.9	6.4
D-Sorbitol	5.4	5.2	5.5	5.5
Gelatin	52.4	52.9	50.2	50.2
CA	6.2	-	–	–
NaTDC	–	5.2	–	–
C_{10}	–	–	17.9	–
CG	–	–	–	9.4