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Manipulating Aggregation Behavior of the Uncharged Peptide Carbetocin

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ABSTRACT

Peptides are usually administered through subcutaneous injection. For low potency drugs, this may require high concentration formulations increasing the risk of peptide aggregation, especially for compounds without any intrinsic chargeable groups. Carbetocin was used as a model to study the behavior of uncharged peptides at high concentrations. Manipulation of the aggregation behavior of 70 mg/mL carbetocin was attempted by selecting excipients which interact with hydrophobic groups in carbetocin, and cover hydrophobic surfaces and interfaces. Peptide aggregation was induced by shaking stress and followed over time. Carbetocin solutions showed significant visible particle formation already after 4 h of shaking stress. This particle formation was not due to supersaturation or phase separation but suggested a nucleated aggregation process. None of the excipients prevented carbetocin aggregation, though altered aggregation behavior was observed, such as induction of fibril formation for most, but not all, charged excipients. Sodium dodecyl sulfate was found to accelerate peptide aggregation both below and above the critical micelle concentration in half-filled vials. However, in the absence of an air headspace, sodium dodecyl sulfate above the critical micelle concentration was capable of preventing shaking-induced carbetocin aggregation. Our study highlights the complexity in rational excipient selection to stabilize uncharged peptides at high concentration.

Introduction

Peptides are an important drug class used for various indications such as multiple sclerosis, prostate cancer, organ transplantation, osteoporosis, and diabetes mellitus. Owing to low oral bioavailability, most peptides are administered parenterally.[1] In parental drug delivery, intravenous, subcutaneous (s.c.), and intramuscular administration are frequently used. Often, s.c. is the preferred administration route as it is considered less painful and allows patients to self-administer. However, developing drug products for s.c. delivery can be challenging since the injection volume is generally restricted to <1.5 mL.[2] Depending on potency, it may be necessary to formulate the peptide at a high concentration. Unfortunately, preparation of high concentration peptide solutions also significantly increases the risk of peptide aggregation.[2,3] A standard strategy to mitigate peptide aggregation issues is to select a formulation pH far from the isoelectric point and thereby ensure high-peptide net charge. A high peptide net charge can improve the physical stability via repulsive electrostatic peptide-peptide interactions (PPIs), which counteract attractive hydrophobic PPIs, and thus reduce or prevent peptide aggregation.[4,5] However, if the peptide structure does not include ionisable groups, inducing repulsive PPIs by pH optimization is not possible. Consequently, maintaining a satisfying physical stability at high peptide concentrations may be a challenge, especially since peptides generally do not possess higher-order structure, and the physical stability thus primarily depends on the nature of the PPIs.[6]

Carbetocin is an example of an uncharged peptide (see Fig. 1). It is an oxytocin analogue used to prevent postpartum hemorrhage, a potential fatal condition resulting in heavy bleeding after childbirth. Compared with oxytocin, which is also used for postpartum hemorrhage treatment, the Tyr residue in position 2 is methylated and the first Cys residue is replaced by a synthetic linker in carbetocin (Fig. 1). This results in the removal of the ionisable N-terminus and replacement of the labile disulphide bridge by a more stable thioether. As a result, the recently developed carbetocin drug product currently in clinical trials, CARBETOCIN RTS, can

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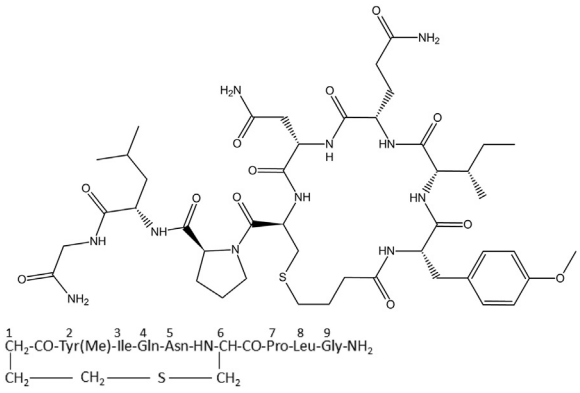

Figure 1. Structure of carbetocin.

be stored at 30°C for at least 3 years, whereas oxytocin drug products require storage at refrigerated conditions.[7] Removing the need for cold storage of the drug product is an important improvement, especially in regions like sub-Saharan Africa and Asia, where postpartum hemorrhage causes nearly a quarter of all deaths from complications of pregnancy and childbirth, and the possibility of cold storage is limited.[7,8]

Although the therapeutic relevance of a concentrated carbetocin formulation is limited, the peptide is used in this study as a model for the behavior of uncharged peptides at high concentrations. Using a carbetocin concentration of 70 mg/mL, 700 times that of the CARBETOCIN RTS product, the aggregation behavior was investigated under accelerated stress conditions. Following the aggregation investigations, the aim was to better understand how excipients affect the physical stability of carbetocin at concentrated solution conditions and propose potential stabilization strategies.

Experimental

Materials

Carbetocin was kindly donated by Ferring Pharmaceuticals A/S (Copenhagen, Denmark). Sodium hydroxide was purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS), cetyl trimethylammonium bromide (CTAB), sodium benzenesulfonate (SBS), trimethylphenylammonium bromide (TPAB), pentaethylene glycol monododecyl ether (C12E5), methyl-β-cyclodextrin (MBCD), and thioflavin T (ThT) were purchased from Sigma-Aldrich (St. Louis, MO). Water of Milli-Q quality (Advantage A10; Millipore, Molsheim, France) was used throughout the work.

Clear 2R borosilicate glass vials were purchased from Schott (Müllheim, Germany). Coated halobutyl rubber stoppers and flip off caps were purchased from West Pharmaceutical Services (Eschweilen and Stolberg, Germany). The 0.22-μm Durapore® polyvinylidene difluoride membrane filter was purchased from Merck Millipore Ltd. (Cork, Ireland).

Methods

Sample Preparation

All carbetocin samples were prepared with a target concentration of 70 mg/mL, pH 4.6 ± 0.1 unless otherwise indicated. All pH adjustments were done with 0.1 M of NaOH. The samples were prepared by dissolving an accurately weighed amount of carbetocin in the respective solvent. Before filling in sample vials, the prepared solution was filtered through a 0.22 μm filter.

Stress Method

The sample vials were placed horizontally (lying down) in an IKA HS 501 digital table shaker (IKA, Staufen, Germany) and exposed to 200 rpm shaking stress with a 3 cm shaking amplitude. The table shaker was placed in a 25°C stability chamber (Hereaus, Hanau, Germany) to ensure a constant temperature throughout the stress period.

Sampling of Solid Material

Owing to the horizontal orientation of the vials during stress, some of the formed particles stayed on the vial walls after the vial was placed in a vertical (upright) position (see examples in Fig. 2). These particles were collected using a spatula and used for Fourier transform infrared spectroscopy (FT-IR) and High Performance Liquid Chromatography (HPLC) analysis (see below).

Phase Contrast Microscopy

Phase contrast microscopy was performed using an Olympus BX 51 microscope (Olympus, Tokyo, Japan) equipped with an Olympus UC30 camera and a 20× magnifying lens. Approximately, 10-μL solution was placed on a clear glass slit and covered with an objective glass. CellSens Dimension software (version 1.5; Olympus) was used to capture the images.

Visual Inspection

Visual inspection was performed using a VerVide Viewing Booth (VerVide Ltd., Leicester, UK) using the black background and top light setting.

Thioflavin T Fluorescence Spectroscopy

ThT fluorescence spectroscopy was performed using a Varioskan Flash plate reader (Thermo Fisher Scientific, Vantaa, Finland) and black NUNC 96 well plates with optical bottom (Thermo Fisher Scientific, Rochester, NY). A sample volume of 193 μL was added to the well plate and 7 μL of a 1080 μM ThT in water stock solution was added to each sample in the well plate. Temperature was set to 25°C. After an equilibration time of 10 min, samples were excited at 450 nm and a top-top emission scan from 465 to 500 nm was

Figure 2. Representative images of 70 mg/mL carbetocin samples after 0, 4, and 24 h of shaking stress. Excipient conditions are indicated in the figure. The "control" vials contain only carbetocin and water. For excipient details, see Table 1. At all conditions pH is 4.6 ± 0.1.