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Research Paper

Stability of lyophilized teriparatide, PTH(1-34), after reconstitution

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ABSTRACT

The peptide teriparatide, also known as parathyroid hormone (1-34), PTH(1-34), was developed for intranasal delivery, requiring extended stability of the reconstituted product for up to four weeks at room temperature. Lyophilized formulations of PTH(1-34), containing glycine and trehalose and using lactate as the buffer, are stable for months upon storage. However, the physical stability of the peptide after reconstitution unexpectedly varied considerably, depending on peptide concentration and storage temperature, with precipitation seen within two to four weeks in some samples. By comparison, equivalent samples that did not undergo lyophilization did not display any precipitation upon storage in the liquid state for as long as twelve weeks. PTH(1-34) appears to adopt a higher order structure that is perturbed by the combined stresses of freezing and drying, leading to greater propensity to aggregate, which is accentuated at higher peptide concentrations and at higher temperatures. The precipitation seems to be correlated with increased amounts of subvisible particles. This study shows the importance of peptide conformation in long-term stability and illustrates the ability of lyophilization to cause increased propensity to aggregate, even in a peptide.

1. Introduction

Few anabolic treatments to build new bone are available for women who have osteoporosis. Forteo® (PTH(1-34), teriparatide [rDNA origin]), is a product approved by the FDA and other jurisdictions for the treatment of women with post-menopausal osteoporosis at high risk of fracture [1]. Forteo® is an aqueous liquid formulation that is stored at 2–8 °C and delivered daily subcutaneously. An intranasal spray formulation of PTH(1-34) was developed that would be more convenient than daily subcutaneous injection. This paper describes a lyophilized formulation of teriparatide, PTH(1-34), that was reconstituted for intranasal delivery. After reconstitution, the product was meant to be kept for at least four weeks at room temperature. However, some reconstituted preparations displayed precipitation upon storage, while others were stable for periods up to twelve weeks.

In general, stabilization and formulation of peptides can be more challenging than for proteins [2,3]. In addition, there is relatively little published information on the stability of lyophilized peptides compared with what has been reported for globular proteins. Moreover, many peptides can have significant physical instability issues, such as aggregation and/or precipitation in aqueous solution. The significantly higher peptide concentration required for intranasal delivery of peptides relative to a parenteral product could increase the risk of aggregation occurring during storage after reconstitution. While Forteo® has a peptide concentration of 0.25 mg/mL, it is anticipated that intranasal delivery will require concentrations of 1–2 mg/mL. Typical commercially available spray pumps had spray volumes of between 50 and 100 μL with 100 μL being the generally most widely used spray volume. Thus an anticipated human 160 μg dose based on preclinical studies would require a PTH(1-34) concentration as high as 1.6 mg/mL.

Previously it was demonstrated in preclinical models that the neutral detergent N-dodecyl-β-maltoside, (DDM; Intravail® A3 [Aegis Therapeutics]) enhanced the intranasal bioavailability of PTH(1-34) (Azelon Therapeutics, data not shown), as it does for other peptides [4]. Thus, all formulations described in this article contain 0.18% DDM. It was determined that lactate, as the buffer species, along with glycine and trehalose are effective stabilizers

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of PTH(1-34) in the presence of DDM. Thus, in the study described here, the formulation compositions are identical. The only parameters that vary are the exposure to freeze-drying and the peptide concentration.

While this formulation of PTH(1-34) was found to be quite stable in the lyophilized state, the challenge was to ensure maintenance of the potency, purity, and physical stability after reconstitution, as the product would be need to be stored for up to four weeks as a liquid at room temperature during patient use. It has been shown previously that PTH(1-34) can be lyophilized, but aggregation of PTH(1-34) occurs in this system as well [5]. This paper describes stability issues associated with reconstituted PTH (1-34) solutions. It appears that the stress of lyophilization can and does lead to physical instability after reconstitution and subsequent storage. This study demonstrates the ability of a PTH(1-34) to adopt higher order structure (HOS) that can be perturbed by freezing and drying stresses, leading to increased propensity to aggregate and form subvisible particles, which ultimately results in precipitation.

The role of HOS in PTH(1-34) biological activity has been widely investigated [6–8]. While it is clear that that two α-helical segments exist in aqueous solution, the ability of PTH(1-34) to adopt an organized tertiary structure has been debated [9,10]. This study indicates that this higher order, organized structure is perturbed upon lyophilization, leading to greater propensity to form aggregates, which result in the precipitation in the least stable samples.

2. Materials and methods

2.1. Materials

PTH(1-34), was supplied by PolyPeptide Laboratories (Torrance, CA) and dodecyl maltoside (DDM) was supplied by Aegis Therapeutics (San Diego, CA). All other chemicals used were reagent grade or better.

2.2. Liquid formulation screening

Formulations containing reconstituted peptide solution were visually examined at each indicated time point. Vials containing any amount of precipitate or gelation were noted and removed from accelerated stability chambers. Each vial was turned to a 45° angle to determine gelation.

2.3. Lyophilization of the clinical lots

Three lots of PTH(1-34) intended for clinical use were formulated and freeze-dried in Type 1 glass vials (5 mL nominal volume (8 mL to lip) 22.8 mm OD), labeled as lots 1, 2, and 3. The fill volume was 3 mL to be reconstituted with 4 mL distilled water to give a pH 4.8 composition comprised of DDM (0.18%), EDTA (0.1%), lactic acid (20 mM), trehalose (80 mg/mL), and glycine (15 mM) and peptide concentrations of 1.4, 1.2, and 0.8 mg/mL after reconstitution (which corresponds to concentrations of 1.86, 1.60 and 1.06 mg/mL in the 3 mL that were lyophilized). Lyophilization of the clinical lots used the following parameters.

Freezing:
0.6 °C/min ramp rate to −50 °C then hold for 4 h

Primary drying:
Drop pressure to 120 mTorr
Ramp to −20 °C at 0.2 °C/min
Hold at −20 °C for 51 h at 120 mTorr (note lot 1 at 1.4 mg/mL primary drying for 63 h)

Secondary drying:
Ramp at 0.5 °C/min to +25 °C
Drop pressure to 70 mTorr when reach +25 °C
Hold at +25 °C least for the 13 h used in first run; stopper under nitrogen

2.4. Size exclusion chromatography (SEC)

The SEC analyses were performed according to a method from the literature [11]. A mobile phase composed of 80% 0.2 M sodium chloride containing 0.1% TFA and 20% acetonitrile and a TSK-Gel G2000SWxl 300 mm × 7.8 mm column were used for all separations. Detection was performed at 215 nm, at a flow rate of 0.5 mL/min. An injection volume of 2 μL was used and chromatograms from buffer blanks were subtracted from each peptide run.

2.5. RP-HPLC method

A Phenomonex Jupiter 3 μm C18 150 × 4.6 mm column with a C18 guard column was used for all separations. 5.8 μL of sample was injected for all separations. System peaks before 5 min retention time were excluded from percent monomer calculations. Mobile phase A consisted of 10% acetonitrile, 90% 0.2 M sodium sulfate pH 2.3, and mobile phase B consisted of 50% acetonitrile, 50% 0.2 M sodium sulfate pH 2.3. The gradient profile was as follows:

Time (min)	% Mobile phase A
0	100
5	65
35	60
45	0
46	100
55	100

2.6. Moisture content

Karl Fisher 870-KF Titrator (Metrohm) was used for measuring the percentage of moisture in the samples. At the start of sample analysis the titration vessel was flushed and filled twice with 75 mL of fresh anhydrous methanol. The titration vessel was then conditioned by addition of Composition 5 (Metrohm reagent) until the drift equilibrated below 20 μL/min. The Metrohm KFT Ipol analysis program was used for all analysis.

A standard of 2 μL of water was used to monitor the instrument and correct for day to day variations in moisture content readings. Prior to each sample measurement, the titration cell was rinsed once with methanol and re-equilibrated with Composition 5 (Metrohm reagent) reagent. A mass of powder sample between 80 and 100 mg was weighed and the resultant value entered into the instrument display. Sample was then added to the titration cell, and the titration was begun. When sample masses were limited, single run analysis was performed.

2.7. Differential scanning calorimetry (DSC)

Lyophilized samples were prepared for DSC by addition to a sealable 6 mm aluminum hermetic pan. Mass of the power sample