International Journal of Pharmaceutics 467 (2014) 48–49

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Dry heat forced degradation of buserelin peptide: Kinetics and degradant profiling

Matthias D'Hondt [a], Maria Fedorova [b], Chien-Yu Peng [c], Bert Gevaert [a], Lien Taevernier [a], Ralf Hoffmann [b], Bart De Spiegeleer [a,*]

[a] Drug Quality and Registration (DruQuaR) Group, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium
[b] Center for Biotechnology and Biomedicine, Institute for Bioanalytical Chemistry, Leipzig University, Deutscher Platz 5, 04103 Leipzig, Germany
[c] Institute of Statistical Science, Academia Sinica, Academia Road 128, 11529 Taipei, Taiwan

ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form 18 March 2014
Accepted 19 March 2014
Available online 21 March 2014

Keywords:
Buserelin
Solid state
Dry heat stress
Degradant identification
Kinetic models
Peptide

ABSTRACT

Buserelin is a GnRH agonist peptide drug, comprising a nine amino acid sequence (pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NH-Et) and most commonly known for its application in hormone dependent cancer therapy, *e.g.* prostate cancer. In order to evaluate its hot-melt extrusion (HME) capabilities, buserelin powder in its solid state was exposed to elevated temperatures for prolonged time periods. A stability indicating UPLC-PDA method was used for quantification of buserelin and the formed degradants. Different solid state kinetic models were statistically evaluated of which the Ginstling–Brounshtein model fitted the data best. Extrapolation to and experimental verification of typical HME-related conditions, *i.e.* 5 min at 100 °C and 125 °C, showed no significant degradation, thus demonstrating the HME capabilities of buserelin.

Mass spectrometric identification of the buserelin-related degradants formed under solid state heat stress was performed. Based upon the identity of these degradants, different degradation hypotheses were raised. First, direct β-elimination of the hydroxyl moiety at the serine residue, followed by fragmentation into an amide (pGlu-His-Trp-NH_2) and pyruvoyl (pyruvoyl-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NH-Et) peptide fragments, was postulated. Alternatively, internal esterification due to nucleophilic attack of the unprotected serine residue, followed by β-elimination or hydrolysis would yield pGlu-His-Trp, pGlu-His-Trp-NH_2 and the pyruvoyl peptide fragment. Degradant pGlu-His-Trp-Ser-Tyr-NH_2 is believed to be formed in a similar way. Secondly, direct backbone hydrolysis would yield pGlu-His-Trp and Tyr-D-Ser(tBu)-Leu-Arg-Pro-NH-Et peptide fragments. Moreover, the presence of Ala-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NH-Et can be explained by hydrolysis of the Trp-Ser peptide bond and conversion of the serine moiety to an alanine moiety. Third and finally, isomerisation of aforementioned peptide fragments and buserelin itself was also observed.

1. Introduction

Gonadotropin-releasing hormone (GnRH) is secreted intermittently from the hypothalamus and binds with its GnRH receptor in the anterior pituitary to regulate production and release of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In turn, these stimulate sex steroid hormone synthesis and gametogenesis in the gonads to ensure reproductive competence. Buserelin is a synthetic GnRH agonist peptide drug, comprising a nine amino acid sequence (pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NH-Et), therapeutically used as an antitumor drug in hormone dependent cancers like prostate and breast cancer (Huhtaniemi et al., 1991; Klijn and De Jong, 1982; Klijn et al., 2001; Nicholson et al., 1984; Seindenfeld et al., 2000). Continuous receptor stimulation results in down regulation of the GnRH receptor and suppression of LH and FSH release and subsequent sex hormones (Harrison et al., 2004; Hazum and Conn, 1988; Millar, 2005). Furthermore, it is also used in the treatment of endometriosis and uterine fibroids, which are both estrogen dependent diseases (Hoellen et al., 2013; Shaw, 1992; Waller and Shaw, 1993). At lower dosage, buserelin can be used in assisted reproduction (Hadziselimovic and Herzog, 1997).

Generally, peptides are considered to be unstable, prone to a variety of degradation pathways, as discussed by Manning et al. (2010). However, this consideration is mainly based on stability evaluation of peptides in solution, in which the dissolved peptides

* Corresponding author. Tel.: +32 9 264 81 00; fax: +32 9 264 81 93.
E-mail address: Bart.DeSpiegeleer@UGent.be (B. De Spiegeleer).

http://dx.doi.org/10.1016/j.ijpharm.2014.03.043
0378-5173/

are traditionally subjected to a variety of stress conditions, *e.g.* enzymes, elevated temperatures, oxidative reagents, extreme pH (De Spiegeleer et al., 2012a; D'Hondt et al., 2010; Vergote et al., 2008). The limited research of the stability of peptides in their solid state focused on the drying process used, most often freeze-drying, during which again a variety of degradation mechanisms can take place, *e.g.* deamidation, peptide bond cleavage, oxidation, Maillard reaction, β-elimination and aggregation (Chang and Pikal, 2009; Lai and Topp, 1999).

In search for more cost effective and efficient production techniques, the pharmaceutical industry has a renewed interest towards hot-melt extrusion (HME). HME is a fast and cost-efficient solvent-free processing technique with 3 critical aspects towards stability of the active pharmaceutical ingredient (API): high temperature exposure during short time periods, mechanical shear stress influence and polymer/matrix–API interaction (Crowley and Zhang, 2007; Repka et al., 2007). To our knowledge, the evaluation of peptide drug as HME drug candidates is yet to be performed in a structured manner, although some promising preliminary results have been obtained (D'Hondt et al., 2011; Ghalanbor et al., 2010). A first and critical aspect which needs to be evaluated is the heat exposure to the peptide. Therefore, buserelin in its solid powder state was exposed to elevated temperatures for prolonged time periods. The stress settings used in this study encompass those typically encountered during melt extrusion processes. This will allow not only to define a production design space, but also the identification of the formed degradation products and further elucidation of degradation mechanisms. Using appropriate modeling of the observed degradation kinetics, an extrapolation to HME-related conditions can be made to predict the buserelin stability at these conditions.

2. Materials and methods

2.1. Materials

Buserelin acetate, European Pharmacopoeia (Ph. Eur.) quality, (Vergote et al., 2009) was bought at Hybio Pharmaceutical Co. (Shenzhen, China). Acetonitrile (ACN) was obtained from Fisher Scientific (Aalst, Belgium). Water was purified using an Arium 611 purification system (Sartorius, Gottingen, Germany) yielding ≥18.2 MΩ × cm quality water. Formic acid (FA) was obtained from Sigma–Aldrich (Diegem, Belgium). Acquity™ ultra performance liquid chromatography (UPLC) columns and suitable guard columns were purchased from Waters (Zellik, Belgium).

2.2. Liquid chromatography

The UPLC apparatus, implemented for quantification of buserelin and its degradants, consisted of a Waters Acquity™ H UPLC Class Quaternary Solvent Manager, a Waters Acquity™ Sample Manager, combined with a flow through needle, and a Waters Acquity™ UPLC photodiode array (PDA) detector with Empower 2 software for data acquisition (all Waters, Milford, MA, USA). An Acquity™ UPLC BEH 300 C18 (100 mm × 2.1 mm I.D., 1.7 μm particle size) column, thermostated in an oven set at 30 °C ± 3 °C, was used. The flow rate was set at 0.6 ml/min and a gradient was applied (where A = 95% water and 5% ACN with 0.1% FA (m/V), and B = 95% ACN and 5% water with 0.1% FA (m/V)), starting with a 1.5 min isocratic step at 100% A, followed by a linear gradient from 100% A to 79% A in 9.5 min. and ending with a isocratic hold for 7 min. The chromatographic method also included a rinsing step at 80% B, followed by returning to the initial conditions and re-equilibration. The sample compartment was thermostated at 5 °C ± 3 °C and UV detection was performed at 220 nm. The injection volume used was 2 μl. The reporting threshold for the dry heat stressed samples (see Section 2.3) was set at 1% peak area relative to unstressed buserelin.

The range and linearity of the UPLC method was verified by injecting 5 increasing buserelin concentrations, ranging from 0.01 to 1.00 mg/ml, and evaluating the peak area. Injection repeatability was characterised by sextuplicate injections of 1.00 mg/ml buserelin. The limit of detection and quantification was determined by the Ph. Eur. method using the 0.01 mg/ml solution, and should be below the applied reporting threshold of 1% relative to the 1 mg/ml buserelin injection (Ph. Eur., 2014). Carry-over was tested by following a 1.00 mg/ml injection by a blank injection. EDQM guidelines stipulate a carry-over specification limit of <0.05% (European Directorate for the Quality of Medicines & Healthcare, 2011). The analytical stability of buserelin samples in the UPLC autosampler, *i.e.* at 5 °C ± 3 °C, is evaluated by comparing the first and last injection of 1 mg/ml buserelin, previously used for injector repeatability (3 h). No significant buserelin degradation, *i.e.* no statistically significant decrease in buserelin peak area nor the formation of degradation products above the applied reporting threshold, is allowed.

2.3. Buserelin dry heat stress samples

Solid state buserelin powder was exposed to various elevated temperatures (De Spiegeleer et al., 2012b; D'Hondt et al., 2011). Approximately 1 mg of buserelin was accurately weighed and transferred into separate glass vials (12 × 32 mm, Borosilicate, Type 1, Class A glass), which were then incubated in a preheated heating block (Stuart, Stone, United Kingdom, temperature accuracy and temperature uniformity within the block both ±1 °C). Temperature settings varied from 150 to 180 °C, with incubation times ranging from 10 to 160 min. Buserelin was exposed to each stress condition in duplicate. After incubation, the stressed samples were immediately placed on ice, in order to prevent further degradation. Unstressed buserelin samples, *i.e.* not incubated in the heating block, were also stored on ice to guarantee identical treatment.

The contents of the unstressed and stressed vials were solubilised using appropriate volumes of solvent consisting of 95% H_2O and 5% ACN mixture with 0.1% FA, and sonicated (Branson™ 2510) to obtain a 1.0 mg/ml buserelin solution. An aliquot was transferred into a HPLC vial and analysed (see Section 2.2). The peak areas of buserelin were used to determine the solid state degradation kinetics.

2.4. Buserelin solid state degradation kinetic

Unlike rate laws in homogeneous kinetics, *i.e.* dissolved state, which usually depends on the reaction order (zero, first, second, third, etc.), reaction kinetics in solid state can depend on other factors such as rate of nuclei formation, interface advance, diffusion and/or geometrical shape of the solid particle. These additional factors result in several models exclusive for solid state reactions (Khawam and Flanagan, 2006). These different models are listed in Table 1: the degradation fraction α, *i.e.* ($\text{Buserelin}_{\text{unstressed}} - \text{Buserelin}_{\text{stressed}}$)/$\text{Buserelin}_{\text{unstressed}}$ obtained at a certain temperature, is modeled in function of the degradation rate constant k and time.

The original degradation data, *i.e.* buserelin peak areas, are used to calculate α. Let $Y_i(t_j|T_l)$ be the integrated form of α, *i.e.* $g(\alpha)$ of the different solid state kinetic models (Table 1), of ith unit at time t_j and stress level T_l of temperature.

$$Y_i(t_j|T_l) = \Theta_i(T_l)t_j + \sigma_B B(t_j) + \varepsilon_{ijl}$$
$$i = 1,2; j = 1,\ldots,4; l = 1,\ldots,5 \quad (1)$$

where $\Theta_i(T_l) \sim N(k(T_l), \sigma_l^2)$ is a random variable representing the unit-to-unit variability of the product and incorporating the