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Development and Characterization of Sodium Hyaluronate Microparticle-Based Sustained Release Formulation of Recombinant Human Growth Hormone Prepared by Spray-Drying

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ABSTRACT

The purpose of this study was to develop and characterize a sodium hyaluronate microparticle-based sustained release formulation of recombinant human growth hormone (SR-rhGH) prepared by spray-drying. Compared to freeze-drying, spray-dried SR-rhGH showed not only prolonged release profiles but also better particle property and injectability. The results of size-exclusion high-performance liquid chromatography showed that no aggregate was detected, and dimer was just about 2% and also did not increase with increase of inlet temperature up to 150°C. Meanwhile, the results of reversed-phase high-performance liquid chromatography revealed that related proteins increased slightly from 4.6% at 100°C to 6.3% at 150°C. Thermal mapping test proved that product temperature did not become high to cause protein degradation during spray-drying because thermal energy was used for the evaporation of surface moisture of droplets. The structural characterization by peptide mapping, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and circular dichroism revealed that the primary, secondary, and tertiary structures of rhGH in SR-rhGH were highly comparable to those of reference somatropin materials. The biological characterization by rat weight gain and cell proliferation assays provided that bioactivity of SR-rhGH was equivalent to that of native hGH. These data establish that spray-dried SR-rhGH is highly stable by preserving intact rhGH and hyaluronate microparticle-based formulation by spray-drying can be an alternative delivery system for proteins.

Introduction

The susceptibility of proteins to physical and chemical degradation in aqueous solution presents a challenge in the development of protein pharmaceuticals. Several stresses such as pH, ionic strength, temperature, light, air-water interface, and agitations can cause deamidation, oxidation, denaturation, or aggregation of protein, which lead to protein instability in aqueous media.[1,2] As a result, many protein drugs are formulated to dry products such as freeze-dried products to prolong their shelf life. Freeze-drying is usually considered to be favorable to the protein than other drying methods.[3,4] However, freeze-drying process has some disadvantages. For example, proteins and buffer ingredients tend to be concentrated during the freeze-drying step and this can result in a change of pH and ionic strength in the protein environment, followed by protein denaturation and aggregation.[5,6] Also, the fact that freeze-drying is a highly energy-intensive and time-consuming process reduces its merit.[7]

Recently, spray-drying technology is widely applied in the industrial field of powder engineering including protein formulations.[8] Spray-drying can produce a dry powder of controllable particle size and shape in a very short time rendering it highly energy efficient compared to freeze-drying.[9] Spray-drying is a transformation process of a given feed solution from fluid state into dried particulate form by spraying the feed into a hot drying gas. It is a continuous particle-processing operation utilizing liquid atomization to create droplets which are further dried into individual particles while moving in the surrounding drying gas. The ease of achieving specific dried particulate properties as well as simple reproducible process is of such importance to industrial process that spray-drying becomes the preferred choice.[10] Also, spray-drying can fundamentally provide the finished product with stability and extended shelf life by reducing the moisture content to minimum levels for the formulation of protein drugs.

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Recombinant human growth hormone (rhGH), which is a protein drug prepared by recombinant DNA technology, is used for the treatment of growth failure in children and metabolic dysfunction in adults with growth hormone deficiency (GHD).[11,12] However, conventional growth hormone therapy requires daily subcutaneous (SC) injections, and various attempts have been made to develop long-acting formulation of rhGH for better patient compliance.[13,14] A new sodium hyaluronate (HA) microparticle-based sustained release formulation of rhGH (SR-rhGH) was developed aiming at once weekly injection to provide enhanced therapeutic efficacy and patient convenience by LG Life Sciences in Korea, which showed a comparable dose response and tolerability profiles to daily-use injectable somatropin for treating children[15] and adult GHD.[16]

In terms of manufacturing process of SR-rhGH, spray-drying technique was applied as an alternative delivery system to produce the form of solid microparticles maintaining intact rhGH and extended shelf life. SR-rhGH contains rhGH as an active ingredient expressed in genetic engineered *Saccharomyces cerevisiae* yeast cells in LG Life Sciences and 2 major excipients, HA and lecithin. HA is a natural polysaccharide polymer to comprise a matrix and release-modifying agent in the microparticle formulation. HA has several desired characteristics as follows. First, HA is a biodegradable polymer which is hydrolyzed by endogenous hyaluronidase enzyme in the body. Second, HA is a biocompatible and non-immunogenic polymer of which degradation compounds are innocuously incorporated in the intermediary metabolism. Third, HA is a nonirritating and noninflammatory material compatible for parenteral administration.[17] Lecithin plays a role of stabilizer during spray-drying. Lecithin is a natural surfactant occurring in animal and plant tissues composed of phospholipids mainly including phosphatidylcholine. A main cause of protein denaturation during spray-drying is known to be the adsorption of protein at the air-liquid interface of spray droplets and the protein denaturation can be overcome by the addition of surfactants and/or divalent metal ions.[7] In SR-rhGH, lecithin is more surface active than the other ingredients and preferentially adsorbed on the surface of the liquid droplets generated in the spray-drying process and prevents rhGH adsorption at the interface.[18,19] Lecithin also acts as a dispersing agent in the formulation showing high affinity with vegetable oils such as medium-chain triglycerides (MCT). Microparticles of SR-rhGH covered with lecithin have an improved affinity to the vegetable oils and are easily suspended.

As aforementioned, spray-drying has advantages as a controllable delivery system and also provides the protein drugs with stability by reducing the moisture content. Nevertheless, one major challenge for applying spray-drying technique to proteins is the concern that proteins would not be able to withstand the heat during the spray-drying process.[7,20] Fundamentally, protein drugs are susceptible to denaturation when they are exposed to high temperature. The maintenance of the structural integrity of proteins during the manufacturing process is an essential requirement for the development of the protein drugs because biological activity of proteins depends entirely on their 3-dimensional conformation.[1,2]

In this study, characteristics of spray-dried SR-rhGH with freeze-dried formulation were compared in terms of morphology and *in vitro* release, and the background of selection of spray-drying technology to prepare a once weekly injection formulation was described. The stability of SR-rhGH prepared by spray-drying was investigated by comparing with the somatropin reference materials such as somatropin standard of United States Pharmacopoeia (USP) and somatropin chemical reference substance (CRS) of European Pharmacopoeia (Ph. Eur.). For this characterization study, 3 batches of SR-rhGH were used to confirm its structural stability with respect to reproducibility and consistency. In order to evaluate the structural integrity, peptide mapping by liquid chromatography-mass spectrometry (LC-MS), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and circular dichroism (CD) methods were conducted. With respect to physicochemical characterization, size-exclusion high-performance liquid chromatography (SE-HPLC), reversed-phase high-performance liquid chromatography (RP-HPLC), and the modified RP-HPLC method were conducted to evaluate the potential product-related variants as impurities. Biological activities of SR-rhGH were measured by using rat weight gain (RWG) assay and cell proliferation assay systems compared to international standard.

Materials and Methods

Materials

Materials used included: rhGH bulk solution (LG Life Sciences, Korea); mannitol (Sigma-Aldrich, St. Louis, MO); glycine (Sigma-Aldrich); sodium phosphate (Sigma-Aldrich); HA (LG Life Sciences); water-for-injection (WFI; LG Life Sciences); lecithin (Lipoid, Germany); MCT (Sasol GmbH, Germany); sodium chloride (Sigma-Aldrich); absolute ethanol (J.T. Baker, USA); *n*-propanol (Sigma-Aldrich); Tris(hydroxymethyl)aminomethane (Merck); USP somatropin standard (2.88 mg/vial, Lot No. F0E191, United States Pharmacopeial Convention); Ph. Eur. somatropin CRS (3.86 mg/vial, Batch 3.0, EDQM); trypsin (Sigma-Aldrich); dithiothreitol (DTT; Sigma-Aldrich); SDS (Sigma-Aldrich); silver nitrate (Sigma-Aldrich); ammonium bicarbonate (Sigma-Aldrich); international standard for somatropin (National Institute for Biological Standards and Control 98/574, UK); Ba/F3-hGHR cells (LG Life Sciences); Dulbecco's phosphate-buffered saline (Gibco-BRL). We also used: RPMI-1640 assay medium (Gibco-BRL); 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (Promega); phenazine methosulfate (Sigma-Aldrich).

Methods

Preparation of SR-rhGH by Spray-Drying

rhGH bulk solution containing 2.25% mannitol, 0.5% glycine (Sigma-Aldrich), and 10 mM sodium phosphate, pH 7.5 was buffer exchanged to 3 mM sodium phosphate, pH 7.5 by using ultrafiltration system having 10 KD of membrane cut-off size (Merck Millipore, Billerica, MA) and then filtered through 0.2-μm sterile filter (Merck Millipore). Sterile HA powder was dissolved in WFI using a mechanical stirrer under aseptic conditions. Lecithin was predispersed in WFI using a magnetic stirrer and then homogenized with M-110Y microfluidizer (Microfluidics). HA solution and lecithin dispersed solution were mixed with a mechanical stirrer and then rhGH solution was added to the mixed solution. The final mixture solution was spray-dried under aseptic conditions using a Production Minor™ Spray Dryer (GEA Niro, Denmark).

To assess the thermal effect on SR-rhGH during spray-drying, the respective SR-rhGH samples were obtained at different inlet temperatures of 100, 110, 120, 130, and 150°C with the same operating conditions of 360 kg/h process air rate, 15 kg/h atomizing rate, and 4.3 kg/h feed rate.

Preparation of SR-rhGH by Freeze-Drying

The formulation and preparation procedures of final mixture solution for freeze-drying were the same as those for spray-drying. The final mixture solution was placed in glass vials with 1 mL and loaded onto the center shelf of the VirTis Genesis freeze dryer (SD Scientific, USA). Freezing was performed at 1°C/min to −40°C and held for 4 h. The shelf temperature was ramped at 0.3°C/min to −30°C at a chamber pressure of 100 mTorr and these conditions