PHARMACEUTICS, PREFORMULATION AND DRUG DELIVERY

Effect of Dimethyl-β-Cyclodextrin Concentrations on the Pulmonary Delivery of Recombinant Human Growth Hormone Dry Powder in Rats

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ABSTRACT: The aim of this article is to prepare and characterize inhalable dry powders of recombinant human growth hormone (rhGH), and assess their efficacy for systemic delivery of the protein in rats. The powders were prepared by spray drying using dimethyl-β-cyclodextrin (DMβCD) at different molar ratios in the initial feeds. Size exclusive chromatography was performed in order to determine protecting effect of DMβCD on the rhGH aggregation during spray drying. By increasing the concentration of DMβCD, rhGH aggregation was decreased from 9.67 (in the absence of DMβCD) to 0.84% (using DMβCD at 1000 molar ratio in the spray solution). The aerosol performance of the spray dried (SD) powders was evaluated using Andersen cascade impactor. Fine particle fraction values of 53.49%, 33.40%, and 23.23% were obtained using DMβCD at 10, 100, and 1000 molar ratio, respectively. *In vivo* studies showed the absolute bioavailability of 25.38%, 76.52%, and 63.97% after intratracheal insufflation of the powders produced after spray drying of the solutions containing DMβCD at 10, 100, and 1000 molar ratio, respectively in rat. In conclusion, appropriate cyclodextrin concentration was achieved considering the protein aggregation and aerosol performance of the SD powders and the systemic absorption following administration through the rat lung. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:5176–5185, 2008

Keywords: dimethyl-β-cyclodextrin; dry powder inhaler; fine particle fraction; recombinant human growth hormone; pulmonary absorption

INTRODUCTION

Recombinant human growth hormone (rhGH), a 191 amino acid protein, is used to treat short stature caused by growth hormone deficiency,

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and promising clinical results have been obtained in the treatment of Turner's syndrome and growth failure due to chronic renal insufficiency. Currently, the administration of hGH involves only parenteral injections, that is, subcutaneously or intramusculary two or three injections per week.[1,2]

A noninvasive route for administration of hGH would provide good compliance and convenience for these patients. The oral bioavailability of hGH has been reported to be extremely low in rats, owing to its large molecular weight ($M_r = 22000$) and the presence of a sophisticated digestive system in the GI tract.[3] The nasal mucosa, although affording a much less proteolytic absorptive environment, similarly exhibited a strong physical barrier toward hGH diffusion leading to a bioavailability value of $<1\%$.[4]

Previous works have indicated that the pulmonary sacs may be the most probable sites for hGH absorption.[5–7] Efficient absorption from the lung results from unique anatomical features of the alveoli (the large absorptive surface area [100 m^2], extensive vasculature, thin layer epithelium [0.1–0.2 μm])[8,9] as well as from aerosol generation systems that successfully bypass particle filtering in upper airways.[10] Macromolecules administered by inhalation of solutions and powders are known to lead to difference in patterns of absorption and deposition of drugs in the lung.[11–13] Dry powder inhalers may be particularly suitable devices for pulmonary administration of proteins like hGH because of their facility of use and the improved drug stability provided by the dry state of the formulation.[14]

Some of the absorption promoters have been investigated for the successful absorption of various proteins from the lung.[15,16] Cyclodextrins as mucosal absorption enhancers have attracted much attention among pharmaceutical scientists, owing to some unique inherent characteristics such as their ability to dissociate and deaggregate protein oligomers and polymers, high tissue compatibility, and wide commercial availability.[17] They are a distinct family of chemical reagents that contain six, seven, or eight monosaccharide units in a cyclized ring with a central cavity that can accommodate other chemicals. A limited number of cyclodextrin derivatives stimulate transmucosal absorption of peptide drugs, and others have no effect.[18] Dimethyl-β-cyclodextrin (DMβCD), is one of the water-soluble cyclodextrin derivatives which its potential as an absorption enhancer of proteins following pulmonary administration has been demonstrated.[19–22] Although cyclodextrins tend to interact with the biomembrane components resulting in extraction of membrane lipids with subsequent shedding of integral proteins, such an effect appears to be relatively mild compared to other types of absorption promoters.[23,24]

In the present study different formulations of rhGH dry powders containing various concentrations of DMβCD were prepared using spray drying method. Spray drying of this sensitive protein in absence of stabilizing agents leads to aggregate formation resulted from exposure to the air–liquid interface during powder preparation process.[25] We have demonstrated previously, the potential of some protein stabilizing agents (lactose, polysorbate, and zinc ion) to protect hGH from aggregation during spray drying.[26] Tavornvipas et al.[27] demonstrated that DMβCD can effectively inhibit the aggregation of rhGH upon exposure to air–water interface created by vortex mixing. Nevertheless, in this work, the effects of DMβCD on (i) protein aggregation during spray drying process, (ii) the aerodynamic behavior of the SD powders and (iii) the systemic absorption of the SD rhGH powders delivered intratracheally in rats were investigated. Additionally, the above parameters were evaluated in the case of rhGH dry powder formulation containing the optimal concentrations of protein stabilizing agents resulted from our previous work.

MATERIALS AND METHODS

Materials

rhGH was purchased from Brasegen (Thebarton, Australia) by molecular mass of 22.13 kDa and was produced from the bacterial fermentation products of a strain of *Escherichia coli*. It was supplied as a 5 mg/mL solution of protein in the ammonium bicarbonate buffer and freezed at $-80^{\circ}C$ temperature.

Dimethyl-β-cyclodextrin was provided from Sigma (Steinheim, Germany). Phosphate buffer, polysorbate 20 and 2-propanol were purchased from Merck (Darmstadt, Germany). Zinc chloride was obtained from Fluka (Buchs, Germany). Lactose monohydrate (pharmatose® 325 M) was supplied by DMV International (Veghel, The Netherlands) and hard gelatin capsule shells (size 2) was supplied by Cipla (Mumbai, India).