Heterogeneity of Commercial Recombinant Human Growth Hormone (r-hGH) Preparations Containing a Thioether Variant

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ABSTRACT: The objective of the present study was to assess (I) the potential presence of a recently discovered thioether variant in commercially available recombinant human growth hormone (r-hGH) preparations, and (II) the impact of the thioether modification on the in-vivo bioactivity and the receptor binding kinetics. Samples were tested employing European (EP) and US Pharmacopeia (USP) Somatropin monograph and mass spectrometry methods. None of the international standards contained this variant. All products conformed to EP specifications but six out of eight lots contained the variant. An artificially enriched thioether sample exhibited a significantly reduced *in vivo* biopotency and altered receptor-binding properties compared with a control. The absence of the variant in the pituitary hGH standard, and the possibility to generate it artificially suggests that it is not naturally occurring and that it may arise from an uncontrolled manufacturing process. Controlled studies may be required to assess its clinical efficacy and safety. EP and USP methods may need to be adapted to reliably detect the presence of the variant. J Pharm Sci 98:4511–4524, 2009

Keywords: growth hormone; bioactivity; immunogenicity; posttranslational modification; protein structure; thioether

INTRODUCTION

Human growth hormone (hGH, somatropin) is a nonglycosylated 22 kDa protein of 191 amino acid residues, containing two disulfide bridges. The first generation of therapeutic hGH was extracted from human pituitary glands; the treatment of some patients with pituitary-derived hGH has

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been related to the onset of Creutzfeldt–Jakob disease.[1] The second generation of hGH preparations is represented by recombinant products produced in *E. coli*, comprising an additional N-terminal methionine (Met-GH) with respect to the human sequence. This Met-GH, whilst efficacious, was however responsible for eliciting anti-hGH antibodies in some patients.[2,27]

Following significant improvements in the *E. coli* production process, almost all of the current, commercially available somatropin preparations were further developed by removing the N-terminal methionine. However, r-hGH derived from *E. coli* production processes exhibited changes in its primary structure, possibly due to transcriptional and/or translational modifications occurring in this cell system.[3–6] Other expression hosts employed to manufacture r-hGH include mammalian cell lines or other eukaryotic systems such as *S. cerevisiae*.[7,8] In general, mammalian protein expression systems can be considered as more advanced since they ensure correct protein folding as well as transcriptional, translational and posttranslational modifications.[9]

Recently, the European Agency for the Evaluation of Medicinal Products (EMEA) and the European Commission (EC) granted marketing authorization for Similar Biological Medicinal Products containing recombinant hGH as the active principle. In the registration of a biosimilar preparation, a comparability exercise is requested to assess the heterogeneity among biopharmaceutical products. It includes physico-chemical and biological characterization of the biosimilar versus the reference medicinal product, together with a description of the production process and of its control parameters.[10] Heterogeneity arising from both process- and product-related impurities can have consequences with regard to the definition of the nonclinical and clinical data requested for the approval of a given biosimilar product.[10] Consequently, it is fundamentally important to closely assess any differences in the impurity profiles between two products by using appropriate analytical protocols.

Recently, a thioether bridge, replacing one of the native disulfide linkages, was identified in an *E. coli*-derived r-hGH preparation.[11] The modification of one of the disulfides to a thioether resulted in an altered spatial arrangement of the C-terminus, reducing the mobility of this region, which in turn may impact on the interaction of the modified molecule with its receptor. Since compendial methods may fail to reveal the presence of this modification, more sensitive methods could be required.

In the present study, both compendial analytical techniques and additional ones, not included in the EP/USP but capable of revealing the presence of the thioether variant, were used to evaluate the quality of commercial somatropin preparations. In order to verify the impact of this novel r-hGH thioether variant on the efficacy and function of the product, artificially enriched samples were generated and these samples were tested for their biological activity (*in vivo* rat weight gain assay) and their receptor binding properties (surface plasmon resonance—SPR, BIAcore). A hallmark of hGH-induced biological activity is a receptor aggregation process involving a receptor homodimerization through a mechanism that requires the two-receptor extracellular domains so as to bind the hormone in a highly regulated sequential order. The sequential order of binding is a consequence of the difference in binding affinities of the hormone to two spatially separated receptor-binding sites.

MATERIALS AND METHODS

In this comparative study the products were tested according to the physico-chemical compendial methods described in the EP and USP. Additional methods that were used in the present study are listed in this section.

Materials

The samples under investigation were commercially available recombinant hGH expressed both in *E. coli* and mammalian cell systems: four batches of Hormotrop®, Bergamo, Dong A-Korea produced in *E. coli* (batch 50897, exp. date August 2007, batch 50793, exp. date July 2007, batch 51026, exp. date October 2007, batch 50923, exp. date September 2007); one batch of Yelit®, Dong A-Korea; produced in *E. coli*, (batch 4684, exp. date June 2006); one batch of Cryotropin®, Cryopharma, BTG-Israel, produced in *E. coli* (batch 50631, exp. date June 2007); and two batches of Saizen®, Merck Serono, Switzerland, produced in a mouse cell line (*C127*), batch SC305D, exp. date April 2006, and batch SC310, exp. date October 2006. One batch of r-hGH drug substance (Merck Serono, Switzerland (*C127*) was used only for thioether variant generation.