Identification of Recombinant Insulin Analogues by Peptide Mapping Method

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ABSTRACT

Insulin human, containing 51 amino acids, is a small polypeptide hormone that regulates blood glucose homeostasis. Patients with insulin-dependent diabetes mellitus require insulin therapy through the administration of exogenous insulin to avoid ketoacidosis. Utilizing various genetic engineering techniques, pharmaceutical companies have developed a variety of rapid- or long-acting insulin analogues. Analytical methods for various types of insulin and insulin analogues are gradually being included in the United States Pharmacopeia (USP) and the European Pharmacopoeia (Ph. Eur.), but not yet in the Chinese Pharmacopoeia. Usually these insulin analogues only differ by 1 to 3 amino acids, which is too subtle to distinguish by most of analytical methods currently available. In this study a peptide mapping technique was employed to screen insulin analogues for quality assessment. Peptide mapping is capable of identifying single amino acid changes resulted from events such as errors in the reading of complementary DNA sequences or point mutations. Here we analyzed 6 insulin preparations including insulin human, insulin lispro, insulin aspart, insulin detemir, insulin glargine and insulin glulisine using peptide mapping analysis. The peptide fingerprints of the insulin products we tested all corresponded well to those of the standard materials. Our peptide mapping method is more accurate in identifying the subtle differences between the insulin analogues than chromatography is. We concluded that peptide mapping is a valuable initial screening tool for quality assessment of insulin analogues. In the future, we intend to continue to develop this technology for post-marketing surveillance of other biopharmaceuticals and biosimilars, such as somatropin, erythropoietin and G-CSF.

Key words: insulin, insulin analogues, peptide mapping, high performance liquid chromatography

INTRODUCTION

Human Insulin is produced by the beta cells of the pancreas, which are located in the islets of Langerhans(1). Although insulin is active as a monomer, it assembles to dimers and hexamers in the presence of zinc during biosynthesis and storage(2). Because of its evolutionarily conserved gene structure across species, insulin extracted from bovine and porcine pancreas is given to people with diabetes mellitus in early insulin therapy(3). Banting and Best extracted bovine insulin in 1921 and successfully administered it to patients with diabetes mellitus(4). After undergoing evolutionary manufacturing, recombinant insulin was the first approved biotechnology-derived drug product in 1982. Recombinant insulin has subsequently replaced purified insulin from animal sources in clinical therapy. Within 15 years, a further 5 insulin analogues have been developed by pharmaceutical companies providing more choices for diabetes patients (Table 1).

All insulin analogues are modified from the insulin human gene using genetic engineering techniques and produced in E. coli or yeast. Amino acid substitution in insulin lispro, insulin aspart, and insulin glulisine allows a more rapid onset of action and shorter duration of activity. Long-acting basal analogues such as insulin glargine and insulin detemir have a slower onset of action and a longer duration of action(5,6). Depending on disease progression, optional insulin therapy can reduce injection frequency and side effects, and thus improve quality of life(7).

Insulin human is a 2-chain polypeptide hormone composed of 51 amino acids with a molecular weight of 5,808 Daltons (Figure 1A). The A-chain is composed of 21 amino acids and the B-chain is composed of 30 amino acids. The 3-dimensional structure of insulin is further stabilized by disulfide bridges between thiol groups (-SH) on cysteine residues. Insulin has 6 cysteines that form 3 disulfide bridges: 2 form an interchain between the A and B chains (between A7 and B7, and A20 and B19), and 1 forms an intrachain within the A-chain (between A6 and A11)(8,9). Insulin lispro