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Characterization of a complex glycoprotein whose variable metabolic clearance in humans is dependent on terminal N-acetylglucosamine content

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Abstract

Glycoproteins can be cleared from circulation if they carry oligosaccharide structures that are recognized by specific receptors. High-mannose type and asialo complex oligosaccharides are cleared by the mannose and asialoglycoprotein receptors, respectively. This paper presents the protein and terminal saccharide characterization for nine batches of a glycoprotein developed for pharmaceutical use. Each of these batches was evaluated in human pharmacokinetic (PK) studies, and had similar terminal elimination half-lives, but the initial clearance of this glycoprotein varied between batches. The protein is lenercept, an immunoadhesin comprising the Fc domain of human IgG1 and two tumor necrosis factor (TNF) binding domains derived from the extracellular portion of the TNFR1(p55). Lenercept is manufactured in Chinese hamster ovary (CHO) cells and is extensively *N*-glycosylated but is devoid of high-mannose glycans. The pharmacokinetic variability between these lots *only* correlated with terminal *N*-acetylglucosamine and not with terminal galactose, sialic acid or any polypeptide related parameter. The data emphasize the need for appropriate analytical methods for the characterization of glycoproteins, especially those designed for long half-lives, and show that assessment of the content of all three terminal saccharides is sufficient to ensure consistency of their PK performance properties.

Keywords: Lenercept; TNF receptor; Terminal *N*-acetylglucosamine; Glycoprotein clearance

1. Introduction

Approval to market a pharmaceutical, whether developed using biotechnology or ''traditional'' methods, requires the demonstration of reproducibility of its safety and efficacy as well as demonstration of manufacturing consistency. This typically requires, in addition to adequate clinical trials that show patient benefit, validation of both the manufacturing process and the control system employed to assess the batch-to-batch reproducibility of its operation. Batches of final product are assayed, using validated methods, to ensure that they meet specifications for identity, purity, potency, strength and quality. Many specifications are set on a case-by-case basis and are guided by these considerations and manufacturing process history, to ensure that marketed batches will have the same properties as the batches used for clinical demonstration of safety

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and efficacy. For glycoprotein pharmaceuticals, however, the situation is more complex due to the generally observed product microheterogeneity imparted by the heterogeneity of the attached glycans [1]. The glycans can influence either the intrinsic biological activity or the pharmacokinetic (PK) properties or both [2]. Consequently, additional analytical methods are required for assessment of the key carbohydrate features, which could cause variability from batch-to-batch in either potency or PK properties.

In contrast to the biosynthesis of the polypeptide portion of a glycoprotein, which can be controlled at the DNA level, the biosynthesis of the glycans is controlled by a complex set of enzyme-mediated reactions that can be influenced by genetic, metabolic, and environmental factors [3]. Assessment of the reproducibility of the glycosylation status of a glycoprotein pharmaceutical, especially during the scale-up phase of manufacturing process development, is therefore of paramount importance to ensure maintenance not only of potency, but also of PK properties. This is recognized by the importance of ''equivalence'' assessment if a change is made during the development of the manufacturing process, usually performed by comparative PK studies in animals or humans, even if only slight differences could be detected during the analytical comparisons of the final products obtained from the two processes [4]. When differences are observed in PK parameters, they are frequently attributable to differences in glycosylation [5] although there are examples where small changes in polypeptide structure can also have significant effects.

For the pharmaceutical, which is the subject of this paper, PK changes were observed when the glycoprotein product expression level in the production clone was increased in response to anticipated commercial needs after initial demonstration of clinical efficacy. However, PK variability was also observed from batch-to-batch when the optimized cell culture process was performed reproducibly with tight control of raw materials and manufacturing operations [4]. This variability was established by a series of human PK volunteer trials measuring the PK properties of nine separate batches of drug. These studies allowed the identification of a biochemical parameter, terminal *N*-acetylglucosamine (tGlcNAc), that correlated with the clearance. The data presented here demonstrate that no polypeptide structural feature was responsible for the PK variability and that analysis of terminal saccharide composition was sufficient to attribute the variability to the tGlcNAc content.

The drug is a tumor necrosis factor alpha (TNF) antagonist, with a generic name of lenercept. It is an immunoadhesin comprising the extracellular domain of the human TNF receptor 1 (p55) fused to the CH2 and CH3 domains of human IgG1 [6]. The amino acid sequence is shown in Fig. 1. Expressed in CHO cells, lenercept spontaneously forms a disulfide-linked dimer, creating an Fc domain, which carries two TNF receptor domains [6] and forms a stable complex with TNF in which the activity of the TNF is neutralized [7]. The receptor domain contains three N-linked glycans and one O-linked site while the site in the Fc domain is glycosylated similarly to non-

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1   LVPHLGDREKRDSVCPQGKYIHPQNNSICCTKCHKGTYLY
          L1       L2          L3       L4
41  NDCPGPGQDTDCRECESGSFTASENHLRHCLSCSKCRKEM
                        L5                 L6
81  GQVEISSCTVDRDTVCGCRKNQYRHYWSENLFQCFNCSLC
           L7                        L8       ss
121 LNGTVHLSCQEKQNTVCTCHAGFFLRENECVSCSNCKKSL
                              L9             L10
161 ECTKLCLPQIENVKGTEDSGTTDKTHTCPPCPAPELLGGP
   L11      L12       L13            L14
201 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
           L15           L16
241 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
     L17     L18         L19                L20
281 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
    L21 L22  L23   L24 L25          L26
321 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
       L27           L28                 L29
361 DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
                L30           L31
401 KSLSLSPGK
         L32
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Fig. 1. Amino acid sequence of lenercept. Numbers down the left edge are residue numbers. Expected peptides from digestion with Lys-C, i.e., those ending in **K**, are numbered L1–L32 under the sequence. For clarity, cleavage sites are indicated by **K**, odd-numbered peptides are underlined and glycosylation sites are boxed.

engineered IgG [8]. Initial efficacy of the molecule was demonstrated in a baboon model of septic shock [9]. Lenercept has been clinically evaluated in rheumatoid arthritis and septic shock indications [10,11].

In the work presented herein, we show that the bioactivity of the drug is reproducible from batch-to-batch, that polypeptide properties are either constant from batch-to-batch or have a variability that is unrelated to the PK variability. The status of the glycans was assessed by direct measurement of the three saccharides, which can be terminal in complex-type N-linked glycans, i.e., sialic acid, galactose, or GlcNAc; the first by chemical analysis and the other two by enzymatic methods. In a separate paper [12], we directly show that lenercept molecules carrying the tGlcNAc moieties in the receptor domain were selectively cleared in the initial rapid elimination phase of the PK profile and that this occurs similarly in humans and cynomolgus monkeys. Taken together, these papers provide an important link between certain aspects of glycan structure and a large (and, to our knowledge, unprecedented) dataset from human PK trials. Further, they show the utility of the methods now available to characterize the glycoprotein (both before and after injection) and their potential as tools for monitoring glycosylation, control and consequences.