Macedonian pharmaceutical bulletin, 68 (Suppl 1) 251 - 252 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.120
Short communication

Selective costimulation modulator abatacept-design, therapeutic applications and quality assessment

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Introduction

The principal aim of this paper is to address the design, therapeutic application and quality assessment of a recombinant CTLA4-Fc fusion protein, abatacept. Abatacept is the first developed medicine within the class of selective T-cell co-stimulation modulators. Unlike other biologics designed to reduce inflammation, abatacept does not block proinflammatory cytokines but it modulates a T- cell activation. Since its authorization by the FDA in 2005, abatacept has been used successfully for the treatment of various autoimmune diseases, including rheumatoid arthritis, polyarticular juvenile idiopathic arthritis and psoriatic arthritis. (EMA, 2022; FDA 2022). Recently, FDA approved abatacept in combination with a calcineurin inhibitor and methotrexate for the prophylaxis of acute graft versus host disease. Abatacept is available as injection for intravenous and subcutaneous use. (FDA, 2022).

Design and structure-activity relationship

Abatacept (CTLA4-Ig, rDNA) is a fusion protein produced by rDNA technology in mammalian CHO cells. It is soluble homodimer consisting of two identical subunits covalently linked by a disulfide bond. Each subunit consists on the ECD of human cytotoxic lymphocyte associated antigen 4 (CTLA-4) and the modified sequence of the human IgG_1 Fc fragment (hinge, C_H2 and C_H3 domain). The molecular weight of abatacept is 92.3 kDa of which 15 % is composed of carbohydrates. Modifications to the original sequences were made to avoid disulfide bridge formation and to decrease Fc-mediated effector functions and complement activation (EMA, 2022). ECD of CTLA-4 is responsible for interaction with CD80/CD86 (B7) receptors of T-lymphocyte. On the other hand, IgG_1 portion endows prolonged circulating half-life of abatacept and the ability for the purification by protein A chromatography. (Isaacs et al., 2009; Ramos-de-la-Peña et al., 2019). Abatacept selectively modulates a key co-stimulatory signal required for full activation of T lymphocytes expressing CD28. Full activation of T lymphocytes requires two signals provided by antigen presenting cells (APCs): recognition of a specific antigen (MHC complex found on APCs) by a T cell receptor (antigen-specific, signal 1), and a second, costimulatory signal. A major costimulatory pathway involves the binding of CD80 and CD86 molecules on the surface of APCs to the CD28 receptor on T lymphocytes (signal 2). The antigen-specific signal is not sufficient to generate a full response, and in the absence of a second signal may lead to clonal inactivation or anergy and T-cells apoptosis. By binding to CD80/CD86, CTLA4-Ig selectively inhibits the costimulatory pathway resulting in immunosuppression. (EMA, 2022; Linsley et al., 1991).

Structural characterization

Abatacept is produced in the CHO cells, and therefore it is a complex protein containing numerous structural variants which arise during the production process in cell culture, posttranslational modifications and

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modifications resulting from degradation of the molecule. (FDA, 2022). Therefore, a combination of more than one analytical technique, including MS, HPLC and electrophoresis technique is required for its comprehensive characterization (Duivelshof et al., 2021).

Multiple charged isoforms in the native abatacept ranging from pI 4.5 - 5.5 are detected using isoelectric focusing (IEF) (Nebija et al. 2011). Literature data revealed that the drug substance is a mixture of different iso- and glycoforms of the protein and in addition there is N-terminal and C-terminal heterogeneity (Duivelshof et al., 2021). Abatacept contains N-linked and O-linked oligosaccharides in each chain. Three N-linked glycosylation sites are confirmed by LC-MS/MS peptide mapping to occur at asparagine (N) residues N-76, N-108 (both at CTLA4 region) and N-207 (Fc region) and four O-linked glycosylation sites have been identified at serine (S) residues, S- 129, S-136, S-139, and S-148. It was shown that N-linked oligosaccharides are neutral asialo biantennary complex type, while O-linked oligosaccharides have different degree of sialidation. (Bongers et al., 2011; Zhu et al., 2014). Glycosylation differences have been shown to impact the pharmacokinetics and the clearance of product and terminal sialic acids are important for pharmacokinetics of the protein (Greve et al., 1996).

CE and HPAEC-PAD were used to study the oligosaccharide mixture of abatacept, whereas electrophoresis of fluorophore-conjugated carbohydrates was used for the examination of oligosaccharide composition of different lots of CTLA4-Ig (Greve et al., 1996). SDS-PAGE and 2-D electrophoresis complemented with MALDI TOF analysis were used for the assessment of identity, purity and structural integrity of abatacept. (Nebija et al., 2011). Analytical development program of abatacept employed orthogonal analytical methodologies for the characterization of the primary structure, glycosylation and physicochemical properties. (ICH, 2022; FDA, 2022). LC tandem MS peptide mapping was used for the demonstration of identity and primary structure, CE, IEF, for the demonstration of molecular identity, glycoforms, IEX HPLC, monosaccharide analysis, to confirm of the consistency of glycosylation, and bioassay for the release and stability testing (FDA, 2022; ICH, 2022)

Conclusion

Abatacept is an immunosuppressive and anti-inflammatory medicine used for the treatment of autoimmune diseases. The effects are based on the inhibition of T cell activation. Due to its complexity, detailed analysis of primary and higher order structure and posttranslational modifications can be achieved by a combination of various analytical techniques.

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