European Journal of Pharmaceutical Sciences 102 (2017) 35–45

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Extensive preclinical evaluation of an infliximab biosimilar candidate

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ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form 30 January 2017
Accepted 31 January 2017
Available online 7 February 2017

Keywords:
Infliximab
Biosimilar
TNF-α
Rheumatoid arthritis
anti-TNF-α
Therapeutic mAb

ABSTRACT

Infliximab is therapeutic monoclonal antibody (mAb) against TNF-α employed in the treatment of immunoinflammatory diseases. The development of biosimilar mAbs is a global strategy to increase drug accessibility and reduce therapy-associated costs. Herein we compared key physicochemical characteristics and biological activities produced by infliximab and infliximab-Probiomed in order to identify functionally relevant differences between the mAbs. Binding of infliximab-Probiomed to TNF-α was specific and had kinetics comparable to that of the reference product. Both mAbs had highly similar neutralizing efficacy in HUVEC cell cultures stimulated with TNF-α. *In vitro* induction of CDC and ADCC were also similar between the evaluated products. *In vivo* comparability was assessed using a transgenic mouse model of arthritis that expresses human TNF-α in a 13-week multiple-administration study. Infliximab and infliximab-Probiomed showed comparable efficacy, safety, and pharmacokinetic profiles. Our results indicate that infliximab-Probiomed has highly similar activities to infliximab in preclinical models, warranting a clinical evaluation of its biosimilarity.

1. Introduction

Monoclonal antibody (mAb)-based therapies have become significant options in the treatment of various pathologies, including cancer and immunoinflammatory diseases. Although mAbs are highly effective, their accessibility is limited, primarily for 2 reasons: i) the high cost per unit, which is unsustainable for patients and health care systems, and ii) their commercial availability, especially in underdeveloped regions of the world (McCamish & Woollett, 2011). These factors are compounded by the fact that mAbs are often used for chronic conditions that require long-term expenditures during treatment (Braun & Kudrin, 2016).

Biosimilar mAbs have comparable efficacy and safety as their original biotherapeutic counterparts and are more affordable for patients (Stevenson, 2015; Gulacsi et al., 2016). Thus, to increase accessibility of such drugs for patients who might benefit from treatment with high-quality mAbs, governments worldwide have established standards for the production, evaluation, and eventual release of biosimilar mAbs. In Europe, the European Medicines Agency (EMA) was the first agency to implement a specific regulatory pathway and guidelines for the development of biosimilars in 2006 and mAbs in 2012. In the US, the Food and Drug Administration (FDA) released a draft guidance on biosimilars in 2012 (Anon, 2014) and its final version in 2015 (Anon, 2015a).

Other countries, such as Japan, Canada, and Australia, have followed the principles of the EMA framework. The World Health Organization (WHO) published guidelines in 2009 to evaluate biosimilars, which formed the basis for legislation in Korea and countries in Latin America (Braun & Kudrin, 2016; da Silva et al., 2014; Goel & Chance, 2016). In Mexico, the Sanitary Risk Authority (COFEPRIS) released the Official Mexican Standard in 2012 and 2013 (Norma Oficial Mexicana NOM-177-SSA1-2013, 2013). All of these guidelines and standards specifically outline the physicochemical characterization and preclinical and clinical requirements for the development of biosimilar mAbs.

As with other licensed and approved biotherapeutics, the patent on infliximab (Remicade®) has expired (Goel & Chance, 2016; Generics and Biosimilars Initiative, 2011), legalizing the development of biosimilars. Infliximab is a chimeric mAb against human tumor necrosis factor-α (TNF-α) that comprises the human IgG1 and κ constant regions and murine variable regions. Infliximab binds to the soluble and transmembrane (m) forms of TNF-α with high affinity (Scallon et al., 2002).

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http://dx.doi.org/10.1016/j.ejps.2017.01.038
0928-0987/

TNF-α is a proinflammatory cytokine that is secreted primarily by activated monocytes, macrophages, and T cells. The binding of TNF-α to its receptors, TNFR1 and TNFR2, which are expressed on nucleated cells of the immune system and endothelial cells, induces the expression of proinflammatory cytokines (IL-1, IL-6, and IFN-γ), cellular adhesion molecules, and inflammatory markers (Decker et al., 1987; Scallon et al., 1995). Excessive production of TNF-α mediates chronic immunoinflammatory diseases, such as rheumatoid arthritis, Crohn disease, and psoriasis (Tracey et al., 2008). Conversely, the neutralization of TNF-α with biotherapeutics, such as infliximab, improves the pathological state of these diseases in experimental animal models and humans (Scallon et al., 2002; Scallon et al., 1995; Bachmann et al., 2010; Spencer-Green, 2000).

The activity of infliximab has been evaluated extensively in *in vitro* and *in vivo* assays. The binding of infliximab to soluble TNF-α blocks the effects of the latter. In *in vitro* assays (Anon, 1998), infliximab impedes mitogenesis; IL-6 secretion; the priming of human neutrophils; the expression of adhesion molecules, such as E-selectin and ICAM-1; procoagulant activity in human umbilical vein endothelial cells (Siegel et al., 1995); and the adherence of human neutrophils to endothelial cells. Further, the binding of infliximab to the transmembrane form of TNF-α (mTNF-α) induces antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), apoptosis, and cell cycle arrest (Scallon et al., 1995; Horiuchi et al., 2010; Mitoma et al., 2008). *In vivo* studies have reported that the administration of infliximab to transgenic mice that constitutively express human TNF-α reverses the cachectic phenotype and prevents the mortality that is associated with the development of arthritis (Anon, 1998; Siegel et al., 1995).

Several candidates for biosimilars of infliximab are under development. Infliximab-Probiomed is a proposed mAb that is designed to be highly similar to the reference product (Remicade®). It was developed in Mexico per the EU, US, and Mexican guidelines that describe the specific criteria for biosimilars (Anon, 2014; Anon, 2015a; Norma Oficial Mexicana NOM-177-SSA1-2013, 2013). According to this guidance, physicochemical similarity to the reference product is essential in ensuring a comparable clinical efficacy and safety profile. The full characterization of infliximab-Probiomed includes the evaluation of 40 identity, quality, and purity attributes. For example, the amino acid sequences of the 2 mAbs are identical by RP-UPLC-UV (unpublished data) and RP-UPLC-MS. We found that mAbs had similar heterogeneity profiles and secondary and tertiary tridimensional structures. These results indicate that the critical physicochemical attributes of the antibodies are highly similar and warrant the preclinical evaluation of infliximab-Probiomed.

To ensure comparability of the structure-to-function relationships between infliximab and infliximab-Probiomed, we analyzed: i) their affinity and specific binding to TNF-α; ii) the inhibition of TNF-α-induced expression of the cell adhesion molecules ICAM-1, VCAM-1, and E-selectin and the cytokines IL-6 and IL-8, in HUVEC cells; and iii) the *in vitro* induction of CDC and ADCC. In addition, the mAbs have a similar affinity toward FcγR receptors. To assess the *in vivo* comparability of their efficacy, safety, and pharmacokinetic profiles, we used a transgenic mouse model of arthritis that expresses human TNF-α in a 13-week multiple-administration study. Our results demonstrate that infliximab-Probiomed has highly similar activities to infliximab in relevant preclinical models, warranting a clinical evaluation of its biosimilarity.

2. Materials and methods

2.1. Mass spectrometry (MS)

Whole-molecule mass spectrometry analyses were performed on a SYNAPT G2 HDMS coupled to an Acquity UPLC (Waters Corp.; Milford, MA) with an electrospray ionization source (ESI). Samples were diluted to 1.0 mg/mL with 50 mM Tris buffer solution at pH 8.0, and injected in a C18 BEH 300, 2.1 × 150 mm UPLC column using a linear gradient (1% to 99% acetonitrile, with 0.1% formic acid). Acquisition range was set from 500 to 4000 *m/z*. Data processing was performed with BiopharmaLynx software v1.3.1 from Waters Corp.

2.2. Capillary isoelectric focusing (cIEF)

cIEF analyses were performed on a ProteomeLab PA800 instrument using standard procedures recommended by the manufacturer (Beckman Coulter Inc.). Briefly, samples at 5 mg/mL were mixed with 2.4 M urea-cIEF gel, 4.8% 3–10 carrier ampholytes, 40 mM L-arginine, 1.6 mM iminodiacetic acid, and pI markers (pI 10, pI 7, and pI 4.1). Separation was performed on a neutrally coated capillary (50 μm I.D., 30.2 cm long) from Beckman Coulter Inc. Focusing was performed with 30 kV for 30 min at 25 psi and 20 °C. Detection was performed at 280 nm.

2.3. Circular dichroism (CD)

Analyses were performed on a Jasco-815 spectropolarimeter from Jasco Inc. Samples were diluted with 10 mM phosphate buffer solution at pH 7.0 to 0.1 mg/mL for far- UV CD spectra (190–300 nm) and to 3.3 mg/mL for near-UV CD spectra (240–350 nm). Spectrum was acquired as an average of 3 scans at 1 nm data pitch, 1 nm bandwidth, and 50 nm/min of scan speed. Blank was 10 mM phosphate buffer solution at pH 7.0.

2.4. TNF binding

Binding experiments were performed on a Biacore T200 system (GE Healthcare) that was equilibrated at 25 °C. For direct covalent immobilization of infliximab or infliximab Probiomed (ligands), we used a CM5 sensor chip (GE Healthcare BR-1005-30) and an amine coupling kit (GE Healthcare BR-1000-50). Antibodies were diluted in immobilization buffer (10 mM sodium acetate, pH 4.5) and injected over the chip surface to reach approximately 2200 resonance units (2237 RU for infliximab and 2215 RU for infliximab-Probiomed. Human recombinant TNF-α (BD-Bioscience 554618) and human recombinant TNF-β (R&D 211-TBB-050/*CF*) (analytes) were prepared in degassed and ready-to-use HBS-EP buffer (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) (GE Healthcare BR-100188), which was also used as the running buffer. The interactions were assessed in a range of 0–200 nM of analyte. Samples at each concentration of TNF-α and TNF-β were injected for 10 min at a flow rate of 25 μL/min. All injections were performed in duplicate and CV for replicates was always ≤10.8%. After injection of each analyte, surface regeneration was performed with 10 mM glycine, pH 3 (GE Healthcare BR-1003-57) at a flow rate of 30 μL/min.

The data were analyzed with BIAevaluation 2.0 (GE Healthcare BR-1005-97). To calculate the affinity constants and other kinetic parameters, the data were fitted to a Langmuir 1:1 binding model. Three (TNF-β) or 4 (TNF-α) independent experiments were performed, and the data were expressed as averages and standard deviations. The kinetic parameters of the ligands were compared by student's *t*-test; a significant difference was considered at a P value < 0.05.

2.5. Expression of cell adhesion molecules and cytokines in endothelial cell cultures

HUVECs (ATCC CRL_1730) were cultured in F12K medium (ATCC 30-2004) that was supplemented with 10% heat-inactivated fetal bovine serum ([FBS]; GIBCO16000-044), 0.1 mg/mL heparin (Sigma H3149), 0.03 mg/mL endothelial growth factor (Gibco PHC9391), and 0.1 mg/mL Normocin (Invitrogen Ant-nr-1) at 37 °C in a humidified atmosphere with 5% CO2. HUVECs were cultured overnight on 0.1% gelatin-coated wells and then exposed for 8 h to 10–1000 μg/mL of mAbs in the presence of human recombinant TNF-α (BD Biosciences 554618 or R&D 210-TA-100/CF). The neutralizing activity of the therapeutic mAbs was analyzed by quantifying the expression of ICAM-1 (anti-