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Certolizumab pegol does not bind the neonatal Fc receptor (FcRn): Consequences for FcRn-mediated *in vitro* transcytosis and *ex vivo* human placental transfer

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ABSTRACT

Antibodies to tumor necrosis factor (anti-TNF) are used to treat inflammatory diseases, which often affect women of childbearing age. The active transfer of these antibodies across the placenta by binding of the Fc-region to the neonatal Fc receptor (FcRn) may result in adverse fetal or neonatal effects. In contrast to other anti-TNFs, certolizumab pegol lacks an Fc-region. The objective of this study was to determine whether the structure of certolizumab pegol limits active placental transfer.

Binding affinities of certolizumab pegol, infliximab, adalimumab and etanercept to human FcRn and FcRn-mediated transcytosis were determined using *in vitro* assays. Human placentas were perfused *ex vivo* to measure transfer of certolizumab pegol and positive control anti-D IgG from the maternal to fetal circulation.

FcRn binding affinity (K_D) was 132 nM, 225 nM and 1500 nM for infliximab, adalimumab and etanercept, respectively. There was no measurable certolizumab pegol binding affinity, similar to that of the negative control. FcRn-mediated transcytosis across a cell layer (mean ± SD; n = 3) was 249.6 ± 25.0 (infliximab), 159.0 ± 20.2 (adalimumab) and 81.3 ± 13.1 ng/mL (etanercept). Certolizumab pegol transcytosis (3.2 ± 3.4 ng/mL) was less than the negative control antibody (5.9 ± 4.6 ng/mL). No measurable transfer of certolizumab pegol from the maternal to the fetal circulation was observed in 5 out of 6 placentas that demonstrated positive-control IgG transport in the *ex vivo* perfusion model.

Together these results support the hypothesis that the unique structure of certolizumab pegol limits its transfer through the placenta to the fetus and may be responsible for previously reported differences in transfer of other anti-TNFs from mother to fetus.

1. Introduction

Tumor necrosis factor (TNF) alpha is an important cytokine in a number of diseases with a significant inflammatory component, including Crohn's disease (CD), rheumatoid arthritis (RA), axial spondyloarthritis (axSpA) and psoriatic arthritis (PsA) (Wiedmann et al., 2009). Biologic anti-TNF medications, including infliximab (IFX), adalimumab (ADA), etanercept (ETA), certolizumab pegol (CZP) and golimumab are frequently used for the treatment of such diseases.

Inflammatory diseases often affect women of reproductive age, and the risk of adverse pregnancy outcomes correlates with disease activity (Morales et al., 2000; Bush et al., 2004; Norgard et al., 2007; De Man et al., 2009). There is a need for adequate disease control during pregnancy (Ostensen and Forger, 2009; Mahadevan et al., 2011; Ng and Mahadevan, 2013), which has raised concern over the safety of biologic agents for pregnant and breast-feeding women. Current evidence from human studies suggests these agents do not pose a significant clinical risk to conception or early pregnancy (Ali et al., 2010; Fischer-Betz and Schneider, 2010, Mahadevan et al., 2012) or increase the risk of adverse outcomes (Raja et al., 2012;

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Marchioni and Lichtenstein, 2013). However, these reports advise to discontinue treatment in the third trimester to limit placental transfer; while such statements are cautionary through a lack of investigative knowledge, this conclusion is reflected in a number of treatment guidelines (Ostensen and Forger, 2009; Mahadevan et al., 2011; Ng and Mahadevan, 2013; Flint et al., 2016).

CZP has a unique structure compared to the other approved anti-TNF therapies, which may influence fetal exposure during pregnancy. It is a monovalent Fab' fragment of a humanized monoclonal attached to a polyethylene glycol (PEG) chain, which consequently lacks an Fc-region (Fig. 1) (Bourne et al., 2008; Rivkin, 2009). In contrast, the monoclonal antibodies IFX and ADA, and the TNF receptor-IgG Fc fusion protein ETA all possess an IgG1 Fc-region (Fig. 1). Binding of the IgG Fc-region to the neonatal Fc receptor (FcRn) plays an important role in regulating IgG homeostasis by protecting antibodies from degradation (Junghans and Anderson, 1996; Ghetie et al., 1997). In contrast, the plasma half-life of CZP is prolonged by the presence of a PEG moiety (Nesbitt et al., 2007). The IgG Fc-domain is also involved in the active transport of antibodies across the placenta, from maternal to fetal circulation, which is mediated by binding to FcRn (Roberts et al., 1990; Simister and Story, 1997; Saji et al., 1999). Thus, the presence or absence of an Fc-region would be expected to influence binding of biologics to FcRn and affect their active transport across the placenta.

The objective of this study was to determine whether the unique structure of CZP limits its active placental transfer.

2. Materials and methods

2.1. Therapeutic and control reagents

Infliximab (Tanabe Pharmaceutical, Osaka, Japan), adalimumab (Abbvie, Baar, Switzerland) and etanercept (Takeda Pharmaceutical, Osaka, Japan) were all purchased from European distributors. CZP, a control non-PEGylated CZP Fab' (control used in the FcRn binding assay) and P146 antibody (negative control used in the FcRn transcytosis assay) were supplied by UCB Pharma. The P146 negative control antibody was a whole IgG, with specificity to hapten 2,4-dinitrophenol (James and Tawfik, 2003), modified to prevent FcRn binding by mutation of three residues (I253A, H310A, H435A) within the Fc region identified as necessary for binding to FcRn (Roopenian and Akilesh, 2007). A polyclonal human anti-D IgG (supplied under agreement by CSL Behring), known to undergo materno-fetal transfer in the *ex vivo* placental transfer model (Urbaniak et al., 1997), was used as a positive control.

2.2. In vitro *human FcRn binding assay and quantification*

A label-free surface plasmon resonance-based assay (Biacore™, GE Healthcare UK) was used to determine the kinetics (on and off rates) and affinity (binding strength) of the interaction between the anti-TNFs and human FcRn. Binding of a non-PEGylated CZP Fab' was also assessed to determine whether PEGylation affected binding of the CZP Fab' to FcRn.

Human FcRn extracellular domain (IgG Fc-binding domain) was expressed in CHO cells transiently transfected with human FcRn alpha chain and beta 2-microglobulin (B_2M). The FcRn-B_2M heterodimer was prepared by affinity chromatography on a column of human IgG. The human FcRn extracellular domain was immobilized on the test chip by amine coupling to a level of 267 RU (response units). Samples were passed over the FcRn-coated chip (30 μL/min) in running buffer (0.02 M phosphate/0.15 M sodium chloride, pH 6.0/0.05% v/v polysorbate P20) for 5 min at a range of concentrations (0 nM, 21 nM, 42 nM, 84 nM, 168 nM, 335 nM and 670 nM) to determine the binding on-rate; pH 6 buffer was used to allow optimum binding. The FcRn binding model represents the intracellular environment and endosomal internalisation of IgG bound to the extracelluar FcR domain, which is known to be optimal at pH 6 and inactive at physiological pH (Chaudhury et al., 2003). The off-rate was followed for a further 5 min by running buffer alone over the chip. A zero control sensorgram was run every second cycle in order to account for baseline drift and bulk buffer effects; a total of 30 such controls were tested and all produced overlying sensorgrams close to baseline. The chip surface was regenerated between cycles using 3 × 80 s pulses of regeneration buffer (200 nM sodium chloride/100 mM TRIS, pH 8.0). Data were normalized by subtracting blank flow cell data and mean zero control cycle data. The average dissociation constant (K_D) for each compound was calculated from a global fit of association and dissociation kinetics measured over six concentrations (12 replicate experiments).

2.3. In vitro *human FcRn transcytosis assay and quantification*

Madin Darby Canine Kidney (MDCK-II) cells transfected with human FcRn and B_2M (Claypool et al., 2002) were cultured in minimal essential medium (MEM) supplemented with 10% v/v fetal bovine serum (FBS), 2 mM L-glutamine, 1% w/v non-essential amino acids, and 1% w/v sodium pyruvate (Invitrogen) at 37 °C/5% CO_2. The cells were cultured for 3 days in a 24-well transwell plate (BD Falcon), until an intact monolayer was formed, confirmed by measuring trans-epithelial resistances, which were at least 150 Ωcm^2 (Claypool et al., 2002).

The cells were washed with pH 7.2 Hanks' balanced salt solution (HBSS; Invitrogen), and biotinylated anti-TNFs (biotinylated using a kit from Roche) were added to the apical surface in HBSS pH 5.9 with 1% v/v bovine serum albumin (BSA) (pH adjusted with 10 mM 4-morpholineethanesulfonic acid [MES]) at 10 μg/mL. HBSS pH 7.2 with 1% v/v BSA (buffered with 10 mM HEPES) was added to the basolateral side. The use of 1% v/v BSA in the apical and basolateral surfaces was consistent with the methods described in the literature (Claypool et al., 2004). Anti-TNF concentration was quantified in the basolateral supernatant after 4 h' incubation at 37 °C. The 4-h sampling time was optimal for the assay to establish the maximum signal from the transcytosis assay without interfering with the integrity of the monolayer and was validated for specific FcRn-dependent transport with the P146 control (FcRn binding abolished) antibody in all assays. The amount of each anti-TNF transcytosed was measured using a meso scale discovery (MSD) electrochemiluminescent assay. The biotinylated anti-TNFs were captured on an MSD plate with an anti-human IgG antibody (Jackson Labs), then detected with a streptavidin sulpho-tag reagent (MSD). The electrochemiluminescent signal was determined using an MSD Sector Imager 6000 plate reader. Levels of each test anti-TNF were determined by comparison to a standard curve for each corresponding anti-TNF tested. Average (arithmetic mean) amount of anti-TNF transcytosed and the standard deviation (SD), were calculated for each anti-TNF from 3 replicate experiments.

2.4. Ex vivo *placental transfer model*

The collection of human placentas was subject to North of Scotland Research (NORES) Ethical Committee (Ethics Reference 09/S0801/006) and National Health Service (NHS) Grampian Research and Development Office approval and was audited (June 2010).

Freshly delivered placentas from elective caesarean sections were used after obtaining patients' written informed consent. Placental perfusion was carried out according to published methods (Duncan et al., 1995), previously validated for assessing IgG materno-fetal antibody transport (Armstrong-Fisher et al., 1997). Briefly, a placental lobule was cannulated from fetal and maternal