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Research Report

Efflux of monoclonal antibodies from rat brain by neonatal Fc receptor, FcRn

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ABSTRACT

Monoclonal antibody (mAb) engineering that optimizes binding to receptors present on brain vascular endothelial cells has enabled them to cross through the blood–brain barrier (BBB) and access the brain parenchyma to treat neurological diseases. However, once in the brain the extent to which receptor-mediated reverse transcytosis clears mAb from the brain is unknown. The aim of this study was to determine the contribution of the neonatal Fc-receptor (FcRn) in rat brain efflux employing two different in vivo drug delivery models. Two mAb variants with substantially different affinities to FcRn, and no known neuronal targets, (IgG1 N434A and H435A) were administered to rats via intranasal-to-central nervous system (CNS) and intra-cranial dosing techniques. Levels of full-length IgG were quantified in serum and brain hemispheres by a sensitive enzyme-linked immunosorbent assay (ELISA). Following intra-nasal delivery, low cerebral hemisphere levels of variants were obtained at 20 min, with a trend towards faster clearance of the high FcRn binder (N434A); however, the relatively higher serum levels confounded analysis of brain FcRn contribution to efflux. Using stereotaxic coordinates, we optimized the timing and dosing regimen for injection of mAb into the cortex. Levels of N434A, but not H435A, decreased in the cerebral hemispheres following bilateral injection into the rat cortex and higher levels of N434A were detected in serum compared to H435A after 24 h. Immunohistochemical staining of human IgG1 in sections of cortex was consistent with these results, illustrating relatively less intense immunostaining in N434A than H435A dosed animals. Using two in vivo methods with direct cranial administration, we conclude that FcRn plays an important role in efflux of IgG from the rat brain.

Abbreviations: FcRn, neonatal Fc receptor; IgG, immunoglobulin G; WT, wild type

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1. Introduction

The blood brain barrier (BBB) is the protective layer separating the systemic circulation from the brain extracellular fluid. It is made up of tight junctions between endothelial cells on cranial capillaries, a thick basement membrane and astrocytic end-feet. The BBB serves to restrict bacteria and other large hydrophilic molecules from entering the brain parenchyma, while allowing small hydrophobic molecules and nutrients to enter.

Pharmacologic treatment of neurologic diseases has relied on brain penetration of small lipophilic molecules. However, where high selectivity and potency is desirable, an alternative therapeutic approach could be the use of monoclonal antibodies (mAbs). Immunoglobulin-Gs (IgGs), along with other plasma proteins, are large hydrophilic molecules that are unable to pass through the BBB in sufficient quantity to be efficacious when systemically administered (Poduslo et al., 1994). Researchers are currently experimenting with receptor-based brain endothelial transcytosis, such as using the transferrin receptor (Bickel et al., 1994; Pardridge et al., 1991; Yu et al., 2011) or insulin receptor (Boado et al., 2007; Pardridge et al., 1995) for IgGs to enter the brain parenchyma. However, once mAbs enter the brain, the extent to which they are cleared by receptor-mediated reverse transcytosis is not well-known.

Evidence of the involvement of an Fc-receptor in the clearance of IgG from the central nervous system (CNS) has been shown by a shorter half-life of IgG, compared to IgM (antibody that lacks Fc region), in both rat and monkey cerebrospinal fluid (Bergman et al., 1998). Moreover, efflux of IgG though the BBB is competitively inhibited by the addition of Fc fragments (Boado et al., 2007; Zhang and Pardridge, 2001). Indeed, the Fc-receptor mediated Aβ-IgG efflux mechanism has been shown to facilitate the clearance of IgG complexes from brains (Deane et al., 2005).

There are data to both support and refute the role of the neonatal Fc-receptor (FcRn) in IgG efflux from the brain. Using non-compartment mathematical modeling in mice which lack FcRn functionally, there was no apparent difference in efflux compared to wild-type mice based on labeled IgG and residual blood volume (Abuqayyas and Balthasar, 2013; Garg and Balthasar, 2009). FcRn is visualized by confocal microscopy in brain microvasculature endothelial cells (Schlachetzki et al., 2002), but whether the receptor is involved in efflux in addition to its role in recycling IgG is unknown. In vascular endothelial cells, IgG is taken up from the circulation by non-specific fluid-phase pinocytosis where it binds to FcRn in the acidic endosome. It is recycled to the capillary lumen where it has a long half-life (Roopenian and Akilesh, 2007). It is therefore postulated that expression of FcRn located in brain endothelial cells (Schlachetzki et al., 2002) may be involved in the efflux of IgGs from the brain.

The aim of the current study was to define the role of FcRn in IgG efflux from the rat brain using two variants of a recombinant human IgG1 mAb that either had increased FcRn binding (IgG1 N434A) or decreased FcRn binding (IgG1 H435A) compared to wild-type by incorporating mutations at the 434 and 435 amino acid positions (EU numbering) (Firan et al., 2001; Yeung et al., 2009). The monoclonal antibodies were generated to target respiratory syncytial virus (RSV) and would not be expected to bind to targets in the brain. A human mAb was used to avoid potentially faster clearance of mouse mAb dosed to rats, and enable detection of the human Fc in rat tissues. Studies were 24 h or less to avoid differences in serum levels due to the relationship of FcRn binding affinity and circulating half-life. The two variants have been shown to have rat FcRn binding affinities, of 77 nM for N434A and >1000 nM for H435A at pH 6.0 (Kliwinski et al., 2013).

2. Results

2.1. *Physicochemical characteristics of the FcRn binding variants*

Both variants had identical pI values of 7.2. The circular dichroism (CD) spectra for both the near and far ultra-violet ranges showed very similar secondary and tertiary protein structure for both of the variants. They had the same Size Exclusion Chromatography (SEC) profiles with no covalent aggregates, and were stable at 25 °C for 4 d. There was no interaction with mucins, which would confound their delivery by intranasal route (data not shown).

2.2. *Pharmacokinetics of FcRn binding variants after intranasal-to-CNS administration*

FcRn binding variants (H435A and N434A) were administered intranasally into each nostril of rats (40 nmol/rat) and plasma was collected after 20, 40, and 90 min post-dose. The levels of the FcRn binding variant increased to levels that reached ~200 ng/mL in the circulation at a greater rate than the non-FcRn binding variant (Fig. 1A). Rat brain hemispheres were collected after brain perfusion, at 20, 40, and 90 min post-dose from different rats. FcRn binding variants delivered into the brain (ng/g) were detected by an ELISA-based MSD assay that detects full-length mAb (Fig. 1B). N434A entered the brain at a faster rate than H435A and peaked at a higher level at 20 min. Despite the greater degree of uptake of N434A, levels of this variant dropped to very low levels within the same 90 min timeframe as H435A. Statistical comparison of the AUC values generated for each variant showed a statistically significant difference (N434A AUC 1637 ng min/g vs. H435A AUC 827 ng min/g, $P<0.05$), representing an approximately two-fold faster rate of efflux for N434A compared to H435A.

To monitor that test article was correctly deposited with the tube insertion technique; olfactory epithelia from both nostrils were collected at 20, 40, and 90 min post-dose and analyzed for FcRn binding variants. The PK profiles of each are shown in Fig. 1C and D. In both epithelia, the N434A variant was cleared at a much faster rate than the H435A, and the AUC values for each were significantly different (left AUC H435A 2.2×10^7 ng min/g vs. left AUC N434A 1.4×10^7 ng min/g, $P=0.01$; right AUC H435A 2.6×10^7 ng min/g vs. right AUC N434A 1.6×10^7 ng min/g, $P<0.01$). These data are indicative of an active FcRn mediated efflux mechanism removing the FcRn-binding N434A variant from the olfactory epithelium.