Mechanisms of Antibody Uptake into Central Nervous System

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Antibodies (mAbs) are attractive molecules for their application as a diagnostic and therapeutic agent for diseases of the central nervous system (CNS). mAbs can be generated to have high affinity and specificity to target molecules in the CNS. Unfortunately, only a very small number of mAbs have been specifically developed and approved for neurological indications. This is primarily attributed to their low exposure within the CNS, hindering their ability to reach and effectively engage their potential targets in the brain.

Keywords: antibodies ; central nervous system ; blood–brain barrier ; BCSFB ; non-specific ; Fc receptor

1. Introduction

Monoclonal antibodies (mAbs) have emerged as promising therapeutic and diagnostic candidates for a wide range of diseases due to their ability to target specific molecules with high affinity. They offer advantages including low toxicity, long systemic half-lives, and the capacity for large-scale production with high purity. However, the development of mAbs for central nervous system (CNS) diseases is hampered by the limited access to the CNS caused by protective barriers surrounding the brain such as the blood–brain barrier (BBB). These barriers pose challenges in delivering mAbs to their intended targets within the brain at concentrations necessary for their optimal efficacy. Moreover, mAbs administered directly into the cerebrospinal fluid (CSF) are rapidly cleared from the CNS to the systemic circulation, with reported half-lives from minutes to hours [1][2][3]. Despite these obstacles, recent FDA approvals for treatments of neurological disorders, such as Leqembi® (lecanemab) and Aduhelm® (aducanumab) for Alzheimer's Disease (AD), have demonstrated the potential of mAbs for treating brain disorders. Both mAbs have shown the ability to reduce amyloid plaques in the early stage of AD [4][5]; however, the high intravenous doses of mAb required for achieving sufficient doses in the brain for its efficacy have been associated with damage to the blood–brain barrier (BBB) [6]. Therefore, many researchers are investigating new methods to safely improve the efficiency of mAb delivery to the brain.

Although mAbs possess high specificity, a long systemic half-life, and minimal off-target effects, their potential as therapeutic candidates for neurological diseases is impeded by the restrictive CNS barrier. The physicochemical properties of mAbs (i.e., large size, high hydrogen bonding potential, charge) prevent them from traversing through the BBB to reach potential targets within the CNS. Nevertheless, peripheral administration of mAbs has demonstrated their presence in the CNS with CNS-to-plasma or CNS-to-serum ratios ranging from 0.1% to 0.3% [7][8][9][10][11]. The precise mechanisms by which mAbs in the systemic circulation achieve CNS exposure are speculative, however, several theories have been proposed.

Several mAbs have been approved for use in patients with brain diseases such as Alzheimer's disease (AD), multiple sclerosis (MS), and brain tumors (i.e., glioblastoma, neuroblastoma) [12][13][14]. Several approved therapeutic mAbs have functions to control biological events in the peripheral tissues or outside the brain; thus, they do not need to cross the BBB into the brain for their biological activities. For example, an MS drug, Natalizumab (Tysabri), has activity to inhibit the infiltration of activated immune cells into the brain by blocking immune cell adhesion on the BBB endothelial cells [15]. The two successful mAbs (i.e., Aducanumab, Lecanemab) that target amyloid beta plaques in the brain have been approved for treating AD patients; these mAbs presumably have to cross the BBB to clear the amyloid beta plaques in the brain [4][16][17]. In contrast, several clinical trials of mAbs for the remyelination of axons in MS patients, such as VX15/2503, anti-LINGO-1 (Opicinumab), sHIgM22, and anti-Nogo-A, were terminated due to the lack of efficacy [18][19][20][21]. Similarly, the phase 2 clinical trial of anti-Tau mAb (8E12) in AD patients was stopped due to the lack of mAb efficacy [22]. In some cases, the delivery of mAb to the brain was not efficient because of the difficulty of crossing the BBB from the systemic circulation. In addition, there is still a lack of comprehensive and quantitative studies to compare the efficiency of various methods to deliver mAbs into the brain.

2. Crossing the Blood–Cerebrospinal Fluid Barrier (BCSFB)

To measure brain concentrations, researchers often rely on CSF concentrations to act as a surrogate for widespread brain exposures; however, doing so may produce overestimations of mAb concentrations within the brain parenchyma. Numerous studies have highlighted that molecules administered directly into the CSF experience rapid clearance and achieve minimal penetration into the brain tissue [1][2][3][23]. As a result, measuring antibody CSF concentrations may serve as a representation of molecular transport across the BCSFB but may not provide an accurate prediction of mAb brain deposition and therapeutic efficacy.

Evidence to support the BCSFB crossing of mAbs includes the relative “leakiness” of the BCSFB compared to the BBB. While the BCSFB and BBB have distinct permeability profiles based on specific transporter expression on their respective membranes, the BCSFB has been found to be more permeable compared to the BBB [24]. This increased permeability manifests as leakage of plasma proteins across the barrier and lower electrical resistance of the cellular barrier [24][25].

3. Non-Specific Endocytosis

In a recent study conducted by Van De Vyver et al., pharmacokinetics in the brains of healthy rats were modeled to analyze the effects of non-targeting mAbs administered via intravenous (IV) or intracerebroventricular (ICV) route [26]. Pathway analysis from their study suggested that antibody exposure in the interstitial fluid (ISF) of the brain is predominantly mediated by mAbs traversing the BBB rather than entering the ISF directly from the CSF, regardless of route of administration [26]. While some researchers have speculated that transcytosis of IgG antibodies may be facilitated by receptors on brain endothelial cells, such as the neonatal Fc receptor (FcRn), several studies have refuted this hypothesis [8][9][27]. Alternatively, other researchers have proposed that antibody uptake across the BBB occurs non-specifically via endocytic vessels in the brain [27][28].

Researchers supporting the non-specific uptake of antibodies across the BBB have highlighted that the magnitude of circulating mAb uptake into the CNS (0.1–0.3%) is comparable to other endogenous circulating proteins, such as serum albumin [28][29]. In line with this notion, several studies have reported that increasing antibody dosage leads to an increase in CNS exposure in a non-saturating fashion [27][30]. Conversely, an independent investigation examined the transport of IgG antibodies across human brain microvascular endothelial cells in an in vitro BBB model and discovered that antibody transport was saturable and reliant on macropinocytosis [29]. These findings collectively indicate that the uptake of IgG occurs through non-specific, charge-based adsorption of IgG to the negatively charged endothelial cell surface, followed by subsequent macropinocytosis. The relationship between charge and brain uptake has been demonstrated in other studies for mAbs [27][31] as well as other macromolecules such as albumin [32].

4. Antibody Clearance from the CNS

The administration of most therapeutic mAbs for neurological disorders is performed intravenously. This is because strategies to bypass the BBB through delivery directly into the CSF of the CNS have demonstrated that mAbs rapidly efflux from the CSF back into the serum with limited penetration into the brain parenchyma. This is also true for the administration of mAbs directly into the brain parenchyma, where rapid clearance half-lives have been reported and minimal diffusion throughout the whole brain tissue [33][34]. The rapid clearance of direct CNS delivery, therefore, causes these more invasive administration methods to have similar mAb exposure profiles as the IV administration. Therefore, it is imperative to improve our understanding of the potential mAb clearance mechanisms limiting their brain exposure in order to develop long-acting therapeutics for the brain.

5. Neonatal Fc Receptor

The neonatal Fc receptor (FcRn) is a class of Fc receptors recognized for its crucial role in antibody transport and recycling. FcRn facilitates passive immunity transfer from mother to young by enabling the transcytosis of IgG antibodies across the placental and intestinal mucosa barrier. The receptor is expressed on the cell surface of various cell types, including endothelial cells, epithelial cells, and antigen-presenting cells [35]. A study by Schlachetzki et al. [36] demonstrated that FcRn is expressed on the microvasculature in the brain, raising inquiries about its involvement in the transport of IgG antibodies across the BBB.

While FcRn may facilitate the bidirectional transport of antibodies across a barrier, multiple studies have found no evidence of FcRn contributing to the influx of antibodies from blood to the brain, leading to higher CNS exposure [8][9][27][37]. However, Pardridge and colleagues have suggested that FcRn may mediate brain-to-blood efflux of IgG and have

demonstrated Fc-dependent elimination of IgG from the brain after intracranial administration in rats [33][38]. Similar studies by Cooper et al. observed reduced clearance of an IgG with attenuated FcRn binding following intracranial administration in rats [39]. Additionally, brain clearance of endogenous amyloid beta following intravenous administration of anti-amyloid beta (anti-Aβ) mAb was found to be reduced in *FcRn*$^{-/-}$ mice [40].

While investigations by Balthasar and co-workers have challenged the idea of FcRn-mediated brain efflux, [8][9] it is important to note that study design differences may have contributed to these conflicting findings. Balthasar's studies tracked whole brain concentrations following intravenous administration of radiolabeled mAbs in FcRn knockout mice and observed no difference in brain-to-blood AUC ratios between *FcRn*$^{-/-}$ mutants and control animals [8][9]; however, whole-brain concentrations may inaccurately reflect antibody concentrations in the parenchyma, where FcRn-mediated efflux across the BBB is speculated to occur and may reflect CSF concentrations from mAb crossing the BCSFB, as discussed in previous sections.

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