DESIGN, EXPRESSION, AND CHARACTERIZATION OF AN ASYMMETRIC BISPECIFIC ANTIBODY FUSED TO A SCFV SHUTTLE FOR BRAIN UPTAKE

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We used the “knobs-into-holes” technology to obtain an asymmetric bispecific antibody (BsAb) expressed in CHO cells. A single-chain variable fragment (scFv) was designed from an anti-mTfR (mouse transferrin receptor) antibody sequence and fused to the carboxyl terminus of the knob chain of the IgG1 parental antibody. To express the three genes, we cloned the light chain into the pMono-blasti plasmid and the knob and hole heavy chains in the Freedom pCHO plasmid. Cells were transfected with the pMono-blasti plasmid and selected with blasticidin. In a second step, cells were transfected with the Freedom pCHO plasmid and selected with puromycin and methotrexate. Cells were cloned by serial dilution to select the best producer clones. The BsAb was purified by protein A chromatography and quality was evaluated by SDS-PAGE. The volumetric and specific productivities of the BsAb in fed-batch cultures were 9.6 mg/L and 0.17 pg/cell day, respectively. After clonal dilution selection, a clone with a three-fold higher productivity than the parental pool was obtained. The purified BsAb had a purity of 58%, with two main byproducts, a complete symmetric antibody with two knob heavy (and two anti-mTfR) and two light chains, and a half antibody consisting of the light and knob heavy chain, with 8 and 23 % relative abundance, respectively. These were possibly caused by an overexpression of the knob heavy chain. Tools to better regulate the expression of each gene are ongoing.

The binding of BsAb to mTfR was assessed by indirect ELISA. The apparent affinity of the BsAb to mTfR was 24 nM, and the parental antibody showed no affinity to the receptor. The bioavailability of the BsAb and the parental IgG in the brain was evaluated in a neonatal mouse model. Four-day old neonatal mice were injected intraperitoneally with 67 pmol/g of BsAb or parental antibody. Animals were transcardially perfused at 24, 48, and 96 hours after administration and the brain was extracted for antibody quantification by indirect ELISA. After intraperitoneal injection, the BsAb brain concentration and the elimination rate were three-fold higher than for the parental antibody. It is possible that the homodimer byproduct with two anti-mTfR reduces the transportation of the BsAb to the brain, as Niewoehner et al. (2014) reported that a BsAb with two anti-TfR strongly binds to the TfR, resulting in lysosomal degradation of the BsAb-TfR complex and an effective reduction of the number of receptors. The design of strategies to eliminate the presence of such byproduct and to increase transport to the brain is ongoing.

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