Research on Generation of Neutralizing Antibodies to Botulinum Neurotoxin Type A Based on Nanobody Technology

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Botulinum neurotoxin type A (BoNT/A) represents a serious biological threat to global public health due to its extreme toxicity and its prolonged persistence. Recently, several camelid single-domain antibodies (or VHHs, or nanobodies) have been reported to exhibit high neutralizing activity against BoNT/A and possess numerous intrinsic advantages such as small size, high stability, ease of production, and especially their ability of multimerization that can improve the potency and confer broad activity against the toxins. This study aims to develop nanobody multimers, based on the reported VHHs, that could neutralize all the subtypes of BoNT/A. To achieve this goal, the light chain (LC) and the receptor-binding domain (HC) of all eight subtypes BoNTA (from A1 to A8) were expressed in Escherichia coli Rosetta™ 2(DE3), and purified using Ni-NTA columns. Additionally, genes encoding 13 VHHs from previously reported studies were codon-optimized, cloned into pET22 vectors and expressed in E. coli BL21(DE3). SDS-PAGE analysis and ELISA results demonstrated that LC and HC domains of all the subtypes of BoNT/A and VHHs have been successfully produced. Besides, ELISA screening identified four VHHs with high affinity for the LC and two for the HC domains of all BoNT/A subtypes. Subsequently, several heterodimeric and heterotrimeric nanobodies were generated from the most promising VHHs. The heterotrimer JPUD12-JPUC1-JPUA5 was found to completely neutralize the LC activity of all eight subtypes at 35 nM, outperforming both monovalent and bivalent forms. This study presents a promising approach for developing a cost-effective antitoxin against botulinum neurotoxins.

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