FUSION PROTEINS IN BRAIN DRUG DELIVERY

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Abstract

This paper reviews the use of fusion proteins for overcoming the blood-brain barrier in the treatment of central nervous system disorders. Various fusion protein strategies, including monoclonal antibodies and peptide fusion partners, are examined, highlighting their potential in enhancing CNS drug delivery.

Key words: *fusion proteins, blood-brain barrier, protein therapeutics.*

Introduction. Fusion proteins, also known as chimeric proteins, join two or more proteins or fragments thereof into a singular polypeptide chain. In nature, these proteins often stem from chimeric genes, which amalgamate different reference genes or gene fragments, possibly due to various mutational events such as insertions, deletions, inversions, translocations, duplications, or chromosomal rearrangements [1]. Additionally, errors during RNA transcription, such as RNA polymerase read-through — when the RNA-polymerase doesn't terminate the gene transcription, can lead to the formation of fusion proteins. Notably, many chimeric proteins and genes are associated with various malignancies [1, 2].

In biotechnology, fusion proteins can be artificially generated through recombinant DNA technology [1]. Initially, fusion proteins found utility in preventing the aggregation of target proteins into inclusion bodies, thereby stabilizing the product and streamlining purification processes [3].

The medical arena heralds one of the most promising domains for recombinant fusion proteins. Already, therapeutic fusion proteins, including chimeric antibodies and albumin fusion proteins, have garnered FDA approval, with numerous promising candidates undergoing development and testing. Marsh and Owen's recent review delineates three primary categories of therapeutic fusion proteins: half-life extenders, therapeutic proteins fused with targeting fragments, and bi-functional chimeric proteins [4]. Central nervous system (CNS) pathologies, such as neurodegenerative disorders and ischemic stroke, present formidable challenges due to the restrictive blood-brain barrier (BBB), which hampers the delivery of therapeutic agents. Consequently, there's a burgeoning demand for fusion proteins engineered to traverse the BBB, facilitating the transportation of therapeutic payloads into the CNS.

Materials and methods. Scopus, PubMed and Google Scholar were used to find papers that contain "fusion protein" and "blood-brain barrier". The chosen papers were analyzed and summarized.

Results and discussion. Large molecules, such as proteins, are unable to cross the BBB without any facilitation. Therefore, to deliver crucial molecules like insulin into the brain parenchyma, there is a special type of transport called receptor-mediated transcytosis. Fusion proteins, combining therapeutic proteins with monoclonal antibodies (MAbs) targeting BBB receptors, exemplify a promising approach to overcome this barrier. For instance, Pabinafusp alfa (JR-141), a recombinant fusion

protein, recently approved in Japan for treating mucopolysaccharidosis II (Hunter syndrome), represents a breakthrough in lysosomal storage disease therapy. Comprising human iduronate-2-sulfatase fused with the Fc domain of a humanized anti-human transferrin receptor IgG, JR-141 is produced by Chinese hamster ovary (CHO) cells. It stands as the first BBB-crossing enzyme-MAb fusion protein for lysosomal storage disease treatment, with numerous similar products under development and testing [5, 6].

MAbs targeting insulin or transferrin receptors serve as vehicles for delivering diverse functional proteins, including neurotrophic factors, avidin, and decoy proteins, into the CNS. MAb targeting insulin (less commonly transferrin) receptors may be fused on the C-terminus (Fc-domain) with neurotrophic factors, such as erythropoietin, glial-derived neurotrophic factor, and brain-derived neurotrophic factor. Short linkers between the protein parts facilitate fusion protein stability. These proteins can be also produced using CHO or COS (monkey kidney tissue cells, immortalized with simian virus 40) cell lines.

Additionally, avidin-MAb fused proteins hold promise in cancer therapy, albeit with ongoing considerations regarding chicken avidin immunogenicity. Decoy molecules, designed to neutralize various substances, present another avenue for therapeutic intervention. For instance, fusion proteins of MAbs with the extracellular domain of the TNF-receptor demonstrate efficacy in neutralizing tumor necrosis factor α (TNF-α), a crucial proinflammatory cytokine implicated in stroke and neurodegenerative processes.

Moreover, bispecific MAbs, engineered by fusing receptor-targeting MAbs with single-chain Fv-fragments specific to amyloid β (Aβ), enhance the pharmacokinetics of A-targeting MAbs, facilitating their distribution across different organs and tissues, including the CNS. This innovation holds promise for optimizing the therapeutic efficacy of A-targeting antibodies [5].

A similar principle was used to deliver Aβ-targeting affibody into cerebrospinal fluid (CSF). Affibody molecules are scaffold proteins that are modified and tested, for instance, using phage display technology, to bind targets with high affinity and specificity. In *Kwon et al.* study, Z_{SYM73}, a heterodimeric affibody was fused through linkers with heavy and light chains of transferrin targeting MAb connected with a linker and albumin-binding domain (ABD), which was used the half-life of the drug. This chimeric protein was produced in CHO, and its pharmacokinetic properties were studied in mice. Although the CSF bioavailability was significantly increased compared to the Z_{SYM73}-ABD fusion protein, the transferrin-fusion protein exhibited a shorter serum half-life due to faster blood clearance, but the further research is needed [7].

In another instance, VHHs (variable domains of heavy-chain-only antibodies) targeting the human transferrin receptor were fused with MAbs targeting Aβ. These fusion proteins effectively reduced Aβ levels in humanized mice, showcasing promising therapeutic potential for Alzheimer's disease treatment in humans [8].

Beyond monoclonal antibodies, the utilization of Tat-peptide, derived from the human immunodeficiency virus (HIV), as a fusion partner facilitated the transportation

of cytoplasmic malate dehydrogenase (MDH1) across the BBB. Produced in *Escherichia coli*, this fusion peptide exhibited enhanced cell viability of HT22 (mouse hippocampal neuronal cell line) cells compared to MDH1 alone following H_2O_2-induced oxidative stress *in vitro*. Moreover, *in vivo* studies demonstrated a reduction in oxidative stress in the gerbil hippocampus after ischemia, highlighting the neuroprotective potential of this fusion protein in mitigating cerebral ischemia-induced damage [9].

Conclusion. This paper provides a brief, but comprehensive review of fusion proteins utilized for delivering protein therapeutics to the CNS.

Highlighting the pivotal role of insulin and transferrin receptors targeting monoclonal antibodies (MAbs) as fusion partners, alongside the potential utility of the HIV-virus tat-peptide, underscores the diverse strategies employed to overcome the BBB and enhance CNS drug delivery.

While these fusion proteins have demonstrated efficacy in preclinical animal models and even clinical trials, their clinical utilization remains in its infancy. However, with ongoing research and advancements in the field, the translation of these promising therapies into clinical practice holds immense potential. Further investigations and collaborations across disciplines are imperative to accelerate the development and eventual emergence of these innovative products on the global market, ultimately offering new avenues for treating CNS disorders and improving patient outcomes.

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