Blood brain barrier shuttle

The present invention relates to ablood brain barrier shuttle that binds receptors on the blood brain barrier (R/BBB) and methods of using the same.

BACKGROUND

Brain penetration of neurological disorder drugs such as e.g. large biotherapeutic drugs or small molecule drugs having a low brain penetration, is strictly limited by the extensive and impermeable blood-brain barrier (BBB) together with the other cell component in the neurovascular unit (NVU). Many strategies to overcome this obstacle have been tested and one is to utilize transcytosis pathways mediated by endogenous receptors expressed on the brain capillary endothelium. Recombinant proteins such as monoclonal antibodies or peptides have been designed against these receptors to enable receptor-mediated delivery of biotherapeutics to the brain. However, strategies to maximize brain uptake while minimizing miss-sorting within the brain endothelial cells (BECs), and the extent of accumulation within certain organelles (especially organelles that leads to degradation of the biotherapeutic) in BECs, remain unexplored.

Monoclonal antibodies and other biotherapeutics have huge therapeutic potential for treatment of pathology in the central nervous system (CNS). However, their route into the brain is prevented by BBB. Previous studies have illustrated that a very small percentage (approximately 0.1%) of an IgG injected in the bloodstream are able to penetrate into the CNS compartment (Felgenhauer, Klin. Wschr. 52: 1158-1164 (1974)). This will certainly limit any pharmacological effect due to the low concentration within CNS of the antibody.

Therefore, there is a need for delivery systems of neurological disorder drugs across the BBB to shuttle the drugs into the brain efficiently.

SUMMARY

The present disclosure provides a blood brain barrier shuttle comprising a brain effector entity, a linker and one monovalent binding entity which binds to a blood brain barrier receptor, wherein the linker couples the effector entity to the monovalent binding entity which binds to the blood brain barrier receptor.

In a particular example of the blood brain barrier shuttle, the monovalent binding entity which binds to the blood brain barrier receptor is selected from the group consisting of proteins, polypeptides and peptides.

In a particular example of the blood brain barrier shuttle, the monovalent binding entity which binds to the blood brain barrier receptor comprises a molecule selected from the group consisting of a blood brain barrier receptor ligand, scFv, Fv, sFab, VHH, preferably a sFab.

In a particular example of the blood brain barrier shuttle, the blood brain receptor is selected from the group consisting of transferrin receptor, insulin receptor, insulin-like growth factor receptor, low density lipoprotein receptor-related protein 8, low density lipoprotein receptor-related protein 1 and heparin-binding epidermal growth factor-like growth factor, preferably transferrin receptor.

In a particular example of the blood brain barrier shuttle, the monovalent binding entity which binds to the blood brain barrier receptor comprises one scFab directed to the transferrin receptor, more particular a scFab recognizing an epitope in the transferrin receptor comprised within the amino acid sequence of Seq. Id. No. 14, 15 or 16.

In a particular example of the blood brain barrier shuttle, the brain effector entity is selected from the group consisting of neurological disorder drugs, neurotrophic factors, growth factors, enzymes, cytotoxic agents, antibodies directed to a brain target, monoclonal antibodies directed to a brain target, peptides directed to a brain target.

In a particular example of the blood brain barrier shuttle, the brain target is selected from the group consisting of β-secretase 1, Aβ, epidermal growth factor, epidermal growth factor receptor 2, Tau, phosphorylated Tau, apolipoprotein E4, alpha synuclein, oligomeric fragments of alpha synuclein, CD20, huntingtin, prion protein, leucine rich repeat kinase 2, parkin, presenilin 2, gamma secretase, death receptor 6, amyloid precursor protein, p75 neurotrophin receptor and caspase 6.

In a particular example of the blood brain barrier shuttle, the brain effector entity is selected from the group consisting of proteins, polypeptides and peptides.

In a particular example of the blood brain barrier shuttle, the monovalent binding entity which binds to the blood brain receptor is selected from the group consisting of proteins, polypeptides and peptides and said monovalent binding entity is coupled to the C-terminal end of the brain effector entity by the linker.

In a particular example of the blood brain barrier shuttle, the brain effector entity comprises a full length antibody directed to a brain target, preferably a full length IgG.

In a particular example of the blood brain barrier shuttle, the blood brain barrier shuttle comprises a full length IgG antibody as brain effector entity, the linker and one scFab as the monovalent binding entity which binds the blood brain receptor, wherein the scFab is coupled by the linker to the C-terminal end of the Fc part of one of the heavy chains of the IgG antibody.

In a particular example of the blood brain barrier shuttle, the effector entity is a full length antibody directed to Aβ.

In a particular example of the blood brain barrier shuttle, the antibody directed to Aβ comprises (a) H-CDR1 comprising the amino acid sequence of Seq. Id. No. 5, (b) H-CDR2 comprising the amino acid sequence of Seq. Id. No. 6, (c) H-CDR3 comprising the amino acid sequence of Seq. Id. No. 7, (d) L-CDR1 comprising the amino acid sequence of Seq. Id. No. 8, (e) L-CDR2 comprising the amino acid sequence of Seq. Id. No. 9 and (f) L-CDR3 comprising the amino acid sequence of Seq. Id. No. 10.

In a particular example of the blood brain barrier shuttle, the antibody directed to Abeta comprises a V_H domain comprising the amino acid sequence of Seq. Id. No. 11 and a V_L domain comprising the amino acid sequence of Seq. Id. No. 12.

In a particular example of blood brain barrier shuttle, the effector entity is a full length antibody directed to Aβ and the monovalent binding entity is a scFab directed to the transferrin receptor, more particular a scFab recognizing an epitope in the transferrin receptor comprised within the amino acid sequence of Seq. Id. No. 14, 15 or 16.

In a particular example of the blood brain barrier shuttle, the first heavy chain of the antibody of the blood brain barrier shuttle directed to a brain target comprises a first dimerization module and the second heavy chain of the antibody of the blood brain barrier shuttle to a brain target comprises a second dimerization module allowing heterodimerization of the two heavy chains.

In a particular example of the blood brain barrier shuttle, the first dimerization module of the first heavy chain of the antibody of the blood brain barrier shuttle directed to the brain target comprises knobs and the dimerization module of the second heavy chain of the antibody of the blood brain barrier shuttle directed to the brain target comprises holes according to the knobs into holes strategy.

In a particular example of the blood brain barrier shuttle, the effector entity is a full length antibody directed to phosphorylated Tau and the monovalent binding entity is one scFab directed to the transferrin receptor.

In a particular example of the blood brain barrier shuttle, the effector entity is a full length antibody directed to alpha synuclein and the monovalent binding entity is one scFab directed to the transferrin receptor.

In a particular example of the blood brain barrier shuttle, the linker is a peptide linker, preferably a peptide which is an amino acid sequence with a length of at least 25 amino acids, more preferably with a length of 30 to 50 amino acids, in particular said linker is $(G_4S)_6G_2$ or $(G_4S)_4$.

The following three example of the disclosure relate to a blood brain barrier shuttle wherein the brain effector entity is a protein, polypeptide or peptide with the proviso that the brain effector entity is not a full length antibody, in particular a full length IgG.

In a particular example of the blood brain barrier shuttle, the monovalent binding entity which binds to the blood brain barrier receptor comprises a CH2-CH3 Ig entity and one sFab which binds to the blood brain barrier receptor, wherein the sFab is coupled to a C-terminal end of the CH2-CH3 Ig entity by a second linker.

In a particular example of the blood brain barrier shuttle, the blood brain barrier shuttle comprises the brain effector entity, the linker, the CH2-CH3 Ig domain, the second linker and one sFab which binds to the blood brain barrier receptor, wherein the brain effector entity is coupled by the first linker to a N-terminal end of the CH2-CH3 Ig domain and the sFab is coupled to a C-terminal end of the CH2-CH3 Ig domain by the second linker.

In a particular example of the blood brain barrier shuttle, the CH2-CH3 Ig entity is a CH2-CH3 IgG entity.

In one aspect, the present disclosure relates to a blood brain barrier shuttle comprising a full length IgG antibody directed to a brain target, a linker and one scFab directed to the transferrin receptor, wherein the scFab is coupled by the linker to the C-terminal end of the Fc part of one of the heavy chains of the full length IgG antibody directed to the brain target.

Furthermore, the present disclosure relates to an isolated nucleic acid encoding the blood brain barrier shuttle of the present disclosure, a host cell comprising the isolated nucleic acid encoding the blood brain barrier shuttle and a pharmaceutical formulation comprising the blood brain barrier shuttle.

The blood brain barrier shuttle of the present disclosure can be used as a medicament, in particular it can be used for the treatment of a neurological disorder such as e.g. Alzheimer's disease.

The blood brain barrier shuttle of the present disclosure can be used to transport the brain effector entity across the blood brain barrier.

In a particular example, the heavy chain of the IgG antibody of the blood brain barrier shuttle of the present disclosure coupled at its C-terminal end of the Fc part to the scFab as monovalent binding entity which binds to the blood brain barrier receptor has the following structure:

- IgG heavy chain,
- Linker coupling the C-terminal end of the Fc part of the IgG heavy chain to the N-terminal end of the VL domain of the scFab,
- Variable light chain domain (VL) and C-kappa light chain domain of the scFab,
- Linker coupling the C-terminal end of the C-kappa light chain domain of the scFab to the N-terminal end of the VH domain of the scFab,
- Variable heavy chain domain (VH) of the scFab antibody and IgG CH3 heavy chain domain.

The present disclosure provides a fusion protein to transport a brain effector entity across the blood brain barrier comprising a CH2-CH3 Ig entity, a linker and one sFab directed to a blood brain barrier receptor, wherein the sFab is coupled to a C-terminal end of the CH2-CH3 Ig entity by the linker.

In a particular example of the fusion protein of the present disclosure, the fusion protein of the present disclosure further comprises a linker at the N-terminal end of the CH2-CH3 Ig entity to couple the brain effector entity to the N-terminal end of the CH2-CH3 Ig entity.

In a particular example of the fusion protein of the present disclosure, the brain effector entity is selected from the group consisting of neurological disorder drugs, neurotrophic factors, growth factors, enzymes, cytotoxic agents, antibody fragments or peptides directed to a brain target selected from the group consisting of scFv, Fv, scFab, Fab, VHH, $F(ab')_2$.

In a particular example of the fusion protein of the present disclosure, the sFab directed to the blood brain barrier receptor is a sFab directed to the transferrin receptor, preferably a scFab recognizing an epitope in the transferrin receptor comprised within the amino acid sequence of Seq. Id. No. 14, 15 or 16.

In a particular example of the fusion protein of the present disclosure, the linker is a peptide linker, in particular a peptide which is an amino acid sequence with a length of at least

15 amino acids, more particularly with a length of 20 to 50 amino acids, most particularly said linker has the amino acid sequence $(G_4S)_6G_2$ (Seq. Id. No. 13) or $(G_4S)_4$ (Seq. Id. No. 17).

In a particular example of the fusion protein of the present disclosure, the CH2-CH3 Ig entity is a CH2-CH3 IgG entity.

Furthermore, the present disclosure provides an isolated nucleic acid encoding the fusion protein of the present disclosure and a host cell comprising the nucleic acid encoding the fusion protein of the present disclosure.

The present disclosure provides a conjugate comprising a fusion protein of the present disclosure and a brain effector entity coupled to a N-terminal end of the CH2-CH3 Ig entity of the fusion protein of the present disclosure by a linker.

In a particular example of the conjugate of the present disclosure, the brain effector entity is a neurotrophic factor and wherein the linker coupling the neurotrophic factor to the N-terminal end of the CH2-CH3 Ig entity is a peptide linker.

Furthermore, the present disclosure provides a pharmaceutical formulation comprising the conjugate of the present disclosure and a pharmaceutical carrier, the use of the conjugate as a medicament, in particular the use of the conjugate for the treatment of a neurodegenerative disorder, in particular Alzheimer's disease.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1**:** Different format of blood brain barrier shuttles (fusion proteins) used in the examples. 1A: IgG directed to Aβ (mAb31). 1B: single Fab (sFab) directed to TfR coupled to the Fc part of an IgG directed to Aβ (mAb31). 1C: double Fab (dFab) directed to TfR coupled to the Fc part of an IgG directed to Aβ (mAb31). The scFab structure is fused to the C-terminal end of the heavy chain of the IgG antibody.

Fig. 2**:** Binding properties of the fusion proteins towards Aβ structures. The binding affinity was measured using an ELISA setup which shows that the Fab constructs have preserved Aβ binding properties. Binding of mAb31-8D3 constructs to Abeta fibrils. While 8D3 (open squares) does not bind to immobilized Abeta fibrils, mAb31-8D3-dFab (filled squares), mAb31-8D3-sFab (open triangles) and mAb31 (filled triangles) bind with comparable affinities.

Fig. 3**:** Binding properties of the constructs towards the Transferrin receptor (TfR). The binding affinity was measure using an ELISA setup which shows that only the Fab constructs binds the Transferrin receptor (TfR) and the double Fab construct have slightly higher apparent affinity due to the bivalent binding mode. Binding of mAb31-8D3 constructs to mTfR. While

Claims

1. A blood brain barrier shuttle comprising a full length IgG antibody directed to a brain target, a linker and one scFab directed to the transferrin receptor, wherein the scFab is coupled by the linker to the C-terminal end of the Fc part of one of the heavy chains of the full length IgG antibody directed to the brain target.

2. The blood brain barrier shuttle of claim 1, wherein the scFab recognizes an epitope in the transferrin receptor comprised within the amino acid sequence of Seq. Id. No. 14, 15 or 16.

3. The blood brain barrier shuttle of claim 1 or 2, wherein the effector entity is a full length antibody directed to Aβ.

4. The blood brain barrier shuttle of claim 3, wherein the antibody directed to Aβ comprises (a) H-CDR1 comprising the amino acid sequence of Seq. Id. No. 5, (b) H-CDR2 comprising the amino acid sequence of Seq. Id. No. 6, (c) H-CDR3 comprising the amino acid sequence of Seq. Id. No. 7, (d) L-CDR1 comprising the amino acid sequence of Seq. Id. No. 8, (e) L-CDR2 comprising the amino acid sequence of Seq. Id. No. 9 and (f) L-CDR3 comprising the amino acid sequence of Seq. Id. No. 10.

5. The blood brain barrier shuttle of claim 4, wherein the antibody directed to Abeta comprises a V_H domain comprising the amino acid sequence of Seq. Id. No. 11 and a V_L domain comprising the amino acid sequence of Seq. Id. No. 12.

6. The blood brain barrier shuttle of claim 1 or 2, wherein the full length IgG antibody directed to a brain target is a full length antibody directed to phosphorylated Tau.

7. The blood brain barrier shuttle of claim 1 or 2, wherein the full length IgG antibody directed to a brain target is a full length antibody directed to alpha synuclein.

8. The blood brain barrier shuttle of claims 1 to 7, wherein the linker is a peptide linker, preferably a peptide which is an amino acid sequence with a length of at least 20 amino acids, more preferably with a length of 25 to 50 amino acids.

9. The blood brain barrier shuttle of claims 1 to 8, wherein the scFab directed to the transferrin receptor is directed to the human transferrin receptor.