c retains the binding, internalization and trans-synaptic transport capabilities of undigested TeNT, but does not disrupt any neuronal processes, and is therefore nontoxic.

A need exists for a TeNT-based therapy that avoids the pre-existing anti-TeNT immune response in tetanus toxoid immunised subjects.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in Australia or any other country.

SUMMARY

The inventors have appreciated that exploitation of TeNT has not been fully realised because of the adaptive immune system, which upon administration of a protein therapeutic agent, produces an antibody response, thereby decreasing efficacy of the protein therapeutic agent. The adaptive immune response may be intentional, as a result of vaccination, as demonstrated by many populations that have been immunised against TeNT. Alternatively, the adaptive immune response may be unintentional, resulting from repeated exposure to the protein therapeutic agent.

The inventors have produced a family of modified TeNTs and a treatment regimen that address these problems. Specifically, the invention provides a family of PEGylated TeNTs (PEG-TeNTs) that each evade the immune system, and a tiered treatment regimen in which a different, replacement PEG-TeNT is used for treatment when efficacy of a previously administered PEG-TeNT decreases or when a patient's immunological profile precludes the use of another PEG-TeNT.

In one aspect, the present disclosure refers to a TeNT comprising, relative to SEQ ID NO: 1 R1225E and W1288A, wherein the TeNT is inactive and does not block release of inhibitory neurotransmitters.

In another aspect, the present disclosure refers to a composition, optionally a therapeutic composition, comprising:

(i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and

(ii) a second TeNT, wherein the second TeNT is the TeNT as described herein.

In another asepct, the present disclosure refers to a use of a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG) in the manufacture of a medicament for treating hypotonia in a subject to be administered a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters.

In another aspect, the present disclosure refers to a use of a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters, in the manufacture of a medicament for treating hypotonia in a subject to be administered a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG).

In another aspect, the present disclosure refers to a use of a composition comprising: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters, in the manufacture of a medicament for treating hypotonia in a subject.

In another aspect, the present disclosure refers to a use of : (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters, in the manufacture of a medicament for treating hypotonia in a subject, wherein the first PEG-TeNT is to be administered in combination with the second TeNT.

In yet another aspect, the present disclosure refers to a kit comprising the TeNT as described herein or the composition as described herein.

A first example provides a tetanus neurotoxin (TeNT) or fragment thereof, comprising one or more surface serine to cysteine amino acid substitutions relative to SEQ ID NO: 1.

In one example of the first example, the substituted cysteine is conjugated to polyethylene glycol (PEG).

A second example provides a TeNT or fragment thereof comprising, relative to SEQ ID NO: 1: R1225K; R1225E; W1288A; W1288Y; W1288F; W1288L; R1225K and W1288A; R1225E and W1288A; R1225K and W1288Y; R1225E and W1288Y; R1225K and W1288F; R1225E and W1288F;

CLAIMS

1. A TeNT comprising, relative to SEQ ID NO: 1 R1225E and W1288A, wherein the TeNT is inactive and does not block release of inhibitory neurotransmitters.

2. The TeNT of claim 1, further comprising, relative to SEQ ID NO: 1, E270A; Y374A; E270A and Y374A; E270del; Y374del; or a combination thereof.

3. A composition, optionally a therapeutic composition, comprising:

(i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and

(ii) a second TeNT, wherein the second TeNT is the TeNT of claim 1 or claim 2.

4. The composition of claim 3, wherein the first PEG-TeNT comprises one or more surface serine to cysteine amino acid substitutions relative to SEQ ID NO: 1, and wherein the substituted cysteine is conjugated to polyethylene glycol (PEG).

5. The composition of claim 4, wherein the one or more substituted serine in the first PEG-TeNT comprises one or more of S81C, S120C, S144C, S248C, S335C, S428C, S600C, S963C, S1041C, S1155C, and S1187C relative to SEQ ID NO: 1.

6. The composition of claim 4 or claim 5, wherein the PEG is about 2 kDa, about 5 kDa, about 10 kDa, or about 20 kDa, optionally wherein the PEG is linear or branched.

7. The composition of any one of claims 3 to 6, wherein the first PEG-TeNT comprises a PEGylated TeNT light chain (LC), a PEGylated TeNT heavy chain (HC), or a PEGylated TeNT fragment c (c).

8. The composition of any one of claims 3 to 7, wherein the first PEG-TeNT is PEG-TeNT-HC comprising a PEGylated HC, or is PEG-TeNT-LC-c comprising a PEGylated LC and a PEGylated c.

9. The composition of any one of claims 3 to 8, wherein the first PEG-TeNT is PEG-TeNT-HC, and further comprises PEG-TeNT-LC-c.

10. Use of a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG) in the manufacture of a medicament for treating hypotonia in a subject to be administered a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters.

11. Use of a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters, in the manufacture of a medicament for treating hypotonia in a subject to be administered a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG).

12. Use of a composition comprising: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters, in the manufacture of a medicament for treating hypotonia in a subject.

13. Use of: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A, wherein the second TeNT is inactive and does not block release of inhibitory neurotransmitters, in the manufacture of a medicament for treating hypotonia in a subject,

wherein the first PEG-TeNT is to be administered in combination with the second TeNT.

14. The use of any one of claims 10 to 13, wherein the subject is to be administered:

a PEG-TeNT comprising a PEGylated c (PEG-TeNT-c); and/or

a first PEG-TeNT comprising a PEGylated HC (PEG-TeNT-HC) and a second PEG-TeNT comprising a PEGylated LC-c (PEG-TeNT-LC-c); and/or

a PEG-TeNT comprising a PEGylated HC and a PEGylated LC (PEG-TeNT-LC-HC).

15. The use of claim 14, wherein the subject is to be administered:

a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) until efficacy decreases; then

a PEG-TeNT-HC and a PEG-TeNT-LC-c until efficacy decreases; then

a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC).

16. The use of claim 14 or claim 15, wherein the inactive TeNT, relative to SEQ ID NO: 1, further comprises E270A; Y374A; E270A and Y374A; E270del; Y374del; or a combination thereof.

17. The use of any one of claims 10 to 16, wherein the hypotonia is obstructive sleep apnoea.

18. A kit comprising the TeNT of claim 1 or claim 2, or the composition of any one of claims 3 to 9.