Use of glutamine synthetase for treating hyperammonemia

The present invention relates to the use of glutamine synthetase as a protein therapy (such as enzyme replacement protein therapy) for the treatment of hyperammonemia, and in particular to the systemic administration of glutamine synthetase. The glutamine synthetase is thus administered as a protein, or polypeptide, with the aim of increasing circulating levels of glutamine synthetase. The glutamine synthetase may be provided in conjugated or fusion form, to increase its half-life in the circulation. Also provided is a pharmaceutical composition comprising glutamine synthetase. The invention also relates to the uses, methods and compositions involving a combination of the glutamine synthetase protein and an ammonia lowering agent, such as a nitrogen scavenger.

SUMMARY

In one aspect, the present disclosure refers to a glutamine synthetase protein conjugated to polyethylene glycol (PEG). In another aspect, the present disclosure refers to a pharmaceutical composition comprising a glutamine synthetase fusion protein, comprising i) glutamine synthetase and ii) a peptide comprising a) the sequence HHHHHH (6xHis) and b) a linker comprising 2-10 amino acids, wherein the peptide is provided at the N terminal end of the glutamine synthetase protein and wherein the glutamine synthetase protein is or comprises the protein of SEQ ID NO. 1 or a variant thereof having glutamine synthetase enzyme activity and at least 90% sequence identity to the sequence of SEQ ID NO. 1; and a pharmaceutically acceptable excipient.

In another aspect, the present disclosure refers to a glutamine synthetase protein conjugated at the N-terminal end to a peptide comprising the sequence GGGGS.

In another aspect, the present disclosure refers to a glutamine synthetase protein conjugated at the N terminal end to a peptide comprising the sequence HHHHHH (6xHis) and a linker comprising 2-10 amino acids, wherein the glutamine synthetase protein is the full length protein, preferably comprising 373 or 372 amino acids.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising glutamine synthetase (GS) protein as disclosed herein.

In another aspect, the present disclosure refers to a glutamine synthetase (GS) protein or the pharmaceutical composition as disclosed herein for use in therapy.

In another aspect, the present disclosure refers to a use of a glutamine synthetase (GS) protein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating or preventing hyperammonemia by systemic non-oral administration to a subject.

In another aspect, the present disclosure refers to a use of a pharmaceutical composition comprising glutamine synthetase (GS) protein in the manufacture of a medicament for the treating or preventing hyperammonemia by systemic non-oral administration to a subject.

In another aspect, the present disclosure refers to the use of a GS protein for the manufacture of a composition for use in combination therapy for the treatment or prevention of hyperammonemia, wherein said medicament is to be administered in combination with a compound which removes ammonia or reduces or inhibits production of ammonia.

In yet another aspect, the present disclosure refers to the use of a GS protein in the manufacture of a first medicament and use of a compound which removes ammonia or reduces or inhibits production of ammonia in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for the treatment or prevention of hyperammonemia.

In another aspect, the present disclosure refers to a composition comprising a GS protein and a compound which removes ammonia or reduces or inhibits production of ammonia.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising a glutamine synthetase fusion protein which i) comprises the sequence of SEQ ID NO. 4; or ii) comprises the sequence of SEQ ID NO. 4 with one amino acid deletion and having glutamine synthetase activity; or iii) is encoded by the sequence of SEQ ID NO. 5; iv) is encoded by the sequence of SEQ ID NO. 5 and has one amino acid deletion and has glutamine synthetase activity; and a pharmaceutical excipient.

In another aspect, the present disclosure refers to a kit comprising a GS protein for systemic non-oral administration and compound which removes ammonia or reduces or inhibits production of ammonia or an amino acid or urea cycle intermediate or analogue thereof, preferably wherein the

compound which removes ammonia or reduces or inhibits production of ammonia is selected from the group consisting of a compound which reduces the amount of nitrogen, an ion exchange resin (for example Relapsa), an ammonia absorber (such a liposomal based ammonia absorber, for example Versantis), an engineered microbiome that removes ammonia (for example Kaleido Biosciences KB195), Rifaximin and Lactulose.

In yet another aspect, the present disclosure refers to an expression vector encoding a glutamine synthetase fusion protein as disclosed herein.

In yet another aspect, the present disclosure refers to a use of an expression vector encoding a glutamine synthetase fusion protein as disclosed herein in the manufacture of a medicament for the treatment or prevention of hyperammonemia, wherein the vector is for systemic administration.

In yet another aspect, the present disclosure refers to a kit comprising an expression vector encoding glutamine synthetase or a biologically active fragment comprising at least 80% of the full length sequence of SEQ ID NO 1 or variant having at least 80% sequence identity to SEQ ID NO 1, and a further therapeutic agent, preferably wherein the therapeutic agent is an ammonia lowering agent or an amino acid or urea cycle intermediate thereof. In another aspect, the present disclosure refers to a microbial cell comprising an expression vector as described herein.

DETAILED DESCRIPTION

Hyperammonemia is a metabolic condition characterised by increased, or excess, ammonia in the blood. It is a dangerous condition, principally since it may lead to increased entry of ammonia to the brain, which in turn causes neurological disorders and neuropsychiatric abnormalities, which can be very severe, leading to, *inter alia,* brain damage, seizures, retardation, coma and even death. Indeed, encephalopathy is a common and hazardous complication of hyperammonemia. Although precise mechanisms for encephalopathy/brain damage are not yet understood, astrocytic osmotic stress caused by the increased ammonia is believed to play a role, leading to cerebral oedema and increased intracranial pressure. Hyperammonemia may be congenital or acquired, and may be primary or secondary.

Primary (congenital) hyperammonemia arises from various inborn errors of metabolism characterised by reduced activity of any of the enzymes or transporter proteins of the urea cycle. Indeed, such inborn errors of metabolism form a group of diseases called urea cycle disorders (UCD). Ammonia (NH_3), which may co-exist in the body with its charged form ammonium (NH_4^+) depending on pH, is a product of the catabolism of proteins and other nitrogenous compounds. It is converted by the

Claims:

1. A pharmaceutical composition comprising a glutamine synthetase fusion protein, comprising i) glutamine synthetase and ii) a peptide comprising a) the sequence HHHHHH (6xHis) and b) a linker comprising 2-10 amino acids, wherein the peptide is provided at the N terminal end of the glutamine synthetase protein and wherein the glutamine synthetase protein is or comprises the protein of SEQ ID NO. 1 or a variant thereof having glutamine synthetase enzyme activity and at least 90% sequence identity to the sequence of SEQ ID NO. 1; and a pharmaceutically acceptable excipient.

2. A pharmaceutical composition wherein the glutamine synthetase protein comprises 373 or 372 amino acids.

3. A pharmaceutical composition according to claim 2 wherein the glutamine synthetase protein lacks the N terminal methionine.

4. A pharmaceutical composition according to any one of claims 1 to 3 wherein the linker comprises the sequence GGGGS.

5. A pharmaceutical composition according to any one of claims 1 to 4 wherein the fusion protein is linked to a moiety.

6. A pharmaceutical composition according to claim 5, wherein the moiety is selected from a protein, a peptide, a non-protein polymer or an affinity tag.

7. A pharmaceutical composition according to claim 6, wherein the moiety is PEG.

8. A pharmaceutical composition according to claim 7, wherein PEG is conjugated to the peptide.

9. A pharmaceutical composition according to claim 7 or 8 wherein PEG is a 20kDa N-terminal aldehyde PEG.

10. A pharmaceutical composition according to any one of claims 1 to 9 wherein the peptide is, or comprises, the sequence MGSSHHHHHHGGGGS or GSSHHHHHHGGGGS.

11. A pharmaceutical composition comprising a glutamine synthetase fusion protein which
i) comprises the sequence of SEQ ID NO. 4; or
ii) comprises the sequence of SEQ ID NO. 4 with one amino acid deletion and having glutamine synthetase activity; or
iii) is encoded by the sequence of SEQ ID NO. 5;
iv) is encoded by the sequence of SEQ ID NO. 5 and has one amino acid deletion and has glutamine synthetase activity;
and a pharmaceutical excipient.

12. A pharmaceutical composition according to claim 1, wherein the glutamine synthetase fusion protein is conjugated to polyethylene glycol (PEG).

13. A pharmaceutical composition according to claim 2, wherein the PEG is linked to the glutamine synthetase fusion protein at the N terminus.

14. A pharmaceutical composition according to claim 1, wherein the glutamine synthetase fusion protein is conjugated to 20 kDa N-terminal aldehyde PEG.

15. A pharmaceutical composition according to any one of the preceding claims, wherein the glutamine synthetase fusion protein is in a form suitable for systemic non-oral administration.

16. A pharmaceutical composition according to any one of claims 1 to 15, wherein the glutamine synthetase fusion protein is in a form suitable for parenteral or subcutaneous administration.

17. A pharmaceutical composition according to any one of claims 1 to 16, wherein the glutamine synthetase fusion protein is in a multimeric form or monomeric form.

18. A pharmaceutical composition according to any one of claims 1 to 17 for use in therapy.

19. Use of a glutamine synthetase (GS) protein in the manufacture of a medicament for treating or preventing hyperammonemia by systemic non-oral administration to a subject.

20. The use according to claim 19 wherein the glutamine synthetase protein is a glutamine synthetase fusion protein as defined in any one of claims 1 to 17.

21. The use according to claims 19 or 20, wherein said medicament is to be administered in combination with a compound which removes ammonia or reduces or inhibits production of ammonia.

22. The use according to any one of claims 19 to 21, wherein the GS protein is not to be administered to muscle.

23. The use according to any one of claims 19 to 22, wherein the hyperammonemia arises due to a urea cycle disorder (UCD) and/or glutamine synthetase deficiency, or is associated with organ failure or arises due to non-alcoholic fatty liver disease, hepatic encephalopathy, acute liver failure, liver cirrhosis, and/or kidney dysfunction and/or failure.

24. Use of a GS protein for the manufacture of a composition for use in combination therapy for the treatment or prevention of hyperammonemia, wherein said medicament is to be administered in combination with a compound which removes ammonia or reduces or inhibits production of ammonia.

25. Use of a GS protein in the manufacture of a first medicament and use of a compound which removes ammonia or reduces or inhibits production of ammonia in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for the treatment or prevention of hyperammonemia.

26. The use according to claims 24 or 25, wherein the hyperammonemia arises due to a urea cycle disorder (UCD) and/or glutamine synthetase deficiency, or is associated with organ failure or arises due to non-alcoholic fatty liver disease, acute liver failure, liver cirrhosis, and/or kidney dysfunction and/or failure.

27. The use according to any one of claims 24 to 26, wherein the GS protein is a glutamine synthetase fusion protein as defined in any one of claims 1 to 17.

28. The use according to any one of claims 24 to 27, wherein the compound which removes ammonia or reduces or inhibits production of ammonia is selected from the group consisting of a compound which reduces the amount of nitrogen, an ion exchange resin (for example Relapsa), an ammonia absorber (such a liposomal based ammonia absorber, for example Versantis), an engineered microbiome that removes ammonia (for example Synlogic), Rifaximin and Lactulose.

29. The use according to any one of claims 24 to 28, wherein the compound which reduces the amount of nitrogen is selected from the group consisting of: a pharmaceutically acceptable salt of phenylacetic acid or a pharmaceutically acceptable pro-drug thereof, a pharmaceutically acceptable salt of phenylbutyric acid pharmaceutically acceptable pro-drug thereof, glycerol phenylbutyrate pharmaceutically acceptable pro-drug thereof, a pharmaceutically acceptable salt of benzoic acid or a pharmaceutically acceptable pro-drug thereof, and ammonia binding resin, most suitably wherein the pharmaceutically acceptable salt of phenylacetic acid is sodium phenylacetate.

30. A composition comprising a GS protein and a compound which removes ammonia or reduces or inhibits production of ammonia.

31. A composition according to claim 30, wherein the composition is in a form suitable for systemic non-oral administration, and preferably suitable for non-muscular administration.

32. A composition according to claim 30 or 31, wherein the GS protein is a glutamine synthetase fusion protein as defined in any one of claims 1 to 17.

33. A composition according to any one of claims 30 to 32, wherein the compound which removes ammonia or reduces or inhibits production of ammonia is selected from the group consisting of a compound which reduces the amount of nitrogen, an ion exchange resin (for example Relapsa), an ammonia absorber (such a liposomal based ammonia absorber, for example Versantis), an engineered microbiome that removes ammonia (for example Synlogic), Rifaximin and Lactulose.

34. A composition according to claim 33, wherein the compound which reduces the amount of nitrogen is selected from the group consisting of: a pharmaceutically acceptable salt of phenylacetic acid or a pharmaceutically acceptable pro-drug thereof, a pharmaceutically acceptable salt of phenylbutyric acid pharmaceutically acceptable pro-drug thereof, glycerol phenylbutyrate pharmaceutically acceptable pro-drug thereof, a pharmaceutically acceptable salt of benzoic acid or a pharmaceutically acceptable pro-drug thereof, and ammonia binding resin, most suitably wherein the pharmaceutically acceptable salt of phenylacetic acid is sodium phenylacetate.

35. A kit comprising a GS protein for systemic non-oral administration and compound which removes ammonia or reduces or inhibits production of ammonia or an amino acid or urea cycle intermediate or analogue thereof, preferably wherein the compound which removes ammonia or reduces or inhibits production of ammonia is selected from the group consisting of a compound which reduces the amount of nitrogen, an ion exchange resin (for example Relapsa), an ammonia absorber (such a liposomal based ammonia absorber, for example Versantis), an engineered microbiome that removes ammonia (for example Kaleido Biosciences KB195), Rifaximin and Lactulose.

36. A kit according to claim 35, wherein the compound which reduces the amount of nitrogen is as defined in claim 35 and/or the GS protein is a glutamine synthetase fusion protein as defined in any one of claims 1 to 17.

37. An expression vector encoding a glutamine synthetase fusion protein according to any one of claims 1 to 17.

38. Use of an expression vector encoding a glutamine synthetase fusion protein according to any one of claims 1 to 17 in the manufacture of a medicament for the treatment or prevention of hyperammonemia, wherein the vector is for systemic administration.

39. The use according to claim 38, wherein the vector further encodes a compound which removes ammonia or reduces or inhibits production of ammonia or is used in combination with a compound which removes ammonia or reduces or inhibits production of ammonia.

40. A kit comprising an expression vector encoding glutamine synthetase or a biologically active fragment comprising at least 80% of the full length sequence of SEQ ID NO 1 or variant having at least 80% sequence identity to SEQ ID NO 1, and a further therapeutic agent, preferably wherein the therapeutic agent is compound which removes ammonia or reduces or inhibits production of ammonia or an amino acid or urea cycle intermediate thereof.

41. A microbial cell comprising an expression vector according to claim 37.