An alternative amylin analogue incorporating N-methylated residues and having a reduced tendency to fibrillation, designated IAPP-GI, has been described by Yan et al. (PNAS, 103(7), 2046-2051, 2006; Angew. Chem. Int. Ed. 2013, 52, 10378-10383; WO2006/042745). IAPP-GI appears to have lower activity than native amylin, however.

WO 2018/046719 describes amylin analogues having, inter alia, a lactam bridge instead of a disulfide bridge, N-methylated residues, and a deletion corresponding to the residues Asn21 and Asn22 of native human amylin. Such analogues have considerably lower tendency towards fibrillation than native amylin, while also having higher potency than the analogues described by Yan et al. (supra). They are typically amenable to formulation at, or near to, physiological pH. However, there is a need to develop improved formulations for these analogues, especially to provide stable formulations capable of long-term storage without undue fibrillation or degradation of the active monomeric form of the peptide.

Summary

Broadly, the disclosure is based on studies described in the examples that led to surprising findings regarding aqueous formulations of the amylin analogue, that are particularly suitable for long term storage. In particular, it was found that a low buffer concentration and a particular pH range are unexpectedly significant for optimal stability.

The disclosure provides a stable aqueous liquid pharmaceutical formulation comprising an amylin analogue, which is:
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNT-Hyp-NH_2

or a pharmaceutically acceptable salt and/or derivative thereof;

wherein the formulation comprises:

(a) the amylin analogue at a concentration of from 0.4 mg/ml to 25 mg/ml; and

(b) a buffer at a concentration of 0.5 mM to 25 mM;

wherein the formulation has a pH of 5.8 to 6.9.

The amylin analogue is present at a concentration of from about 0.4 mg/ml to about 25 mg/ml.

In some examples, the amylin analogue is present at a concentration of at least about 0.4 mg/ml, at least about 0.5 mg/ml, at least about 0.6 mg/ml, at least about 1.2 mg/ml, or at least about 2.5 mg/ml.

The amylin analogue may be present at up to about 25 mg/ml, up to about 20 mg/ml, up to about 15 mg/ml, or up to about 10 mg/ml.

The amylin analogue may be present at a concentration of about 3 mg/ml to about 12 mg/ml, e.g. about 3 mg/ml to about 7 mg/ml or about 8 mg/ml to about 12 mg/ml, e.g. about 5 mg/ml or about 10 mg/ml.

Any suitable buffer may be used, including a phosphate buffer, histidine buffer, TRIS buffer, acetate buffer, arginine buffer, citrate buffer, bicarbonate buffer, diethanolamine buffer, lysine buffer, TAPS buffer, succinate buffer, malate buffer, and α-ketoglutarate buffer. Particularly preferred buffers include phosphate buffer, histidine buffer, citrate buffer and TRIS buffer, especially phosphate buffer and histidine buffer.

Surprisingly, stability was found to be increased at relatively low buffer concentrations. Thus, the buffer is present at a concentration of about 0.5 mM to about 25 mM.

The buffer may be present at a concentration of about 0.5 mM to about 20 mM, e.g. about 0.5 mM to about 15 mM, e.g. about 1 mM to about 15 mM, e.g. about 1 mM to about 12 mM. For example, it may be present at a concentration of about 3 mM to about 7 mM or about 8 mM to about 12 mM, e.g. about 5 mM or about 10 mM, particularly (but not exclusively) when the buffer is phosphate, histidine or citrate.

For TRIS buffer, it may be preferred to be present at about 15 mM to about 25 mM, e.g. about 17 mM to about 23 mM, e.g. about 20 mM, since the buffering capacity of TRIS is lower than that of other buffers such as (e.g.) phosphate and histidine in the relevant pH range.

The pH of the formulation was also found to be significant for stability. The compounds described in WO 2018/046719 were believed to be stable at physiological pH (e.g. around pH 7.4) but it was surprisingly found that chemical stability was increased at lower pH values and physical stability was maintained. Thus, the formulation has a pH of about 5.8 to about 6.9, and may, for example, have a pH of about 6.2 to about 6.8, e.g. about 6.4 to about 6.6, e.g. about 6.5.

The stable aqueous liquid formulation may comprise a tonicity modifier. The tonicity modifier may be ionic or non-ionic. Suitable ionic tonicity modifiers include alkali metal salts (e.g. halides) and earth metal salts (e.g. halides), such as NaCl, NaBr, NaI, KCl, KBr, KI, LiCl, $CaCl_2$ and Na_2SO_4. Non-ionic tonicity modifiers include mannitol (e.g. D-mannitol), propylene glycol, sucrose, glycerol, sorbitol and trehalose.

In some examples, mannitol (e.g. D-mannitol), propylene glycol and NaCl may be preferred as tonicity modifiers, especially mannitol and propylene glycol.

The aqueous liquid formulation is typically stable for at least 6 months, e.g. at least 12 months, at least 18 months or at least 24 months, at 2-8°C (e.g. at 5°C). Preferably it is stable for at least 18 months, e.g. at least 24 months, at 2-8°C (e.g. at 5°C).

For example, the formulation may display substantially no changes indicative of reduced physical stability (e.g. as demonstrated by turbidity of the solution, or of aggregation, fibrillation or gelling of the amylin analogue) after it has been stored at 2-8°C (e.g. at 5°C) for at least 6 months, at least 12 months, at least 18 months, or at least 24 months. Preferably, the formulation displays substantially no such changes after it has been stored at 2-8°C (e.g. at 5°C) for at least 18 months, e.g. at least 24 months. It will be understood that accelerated conditions may also be employed as a proxy for assessing longer term physical stability as set out in more detail below.

Additionally, or alternatively, at least 80%, more preferably at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the amylin analogue remains in intact monomeric form in the formulation after it has been stored at 2-8°C (e.g. at 5°C) for at least 6 months, at least 12 months, at least 18 months, or at least 24 months. Preferably, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the amylin analogue remains in intact monomeric form after it has been stored at 2-8°C (e.g. at 5°C) for at least 6 months, at least 12 months, at least 18 months, or at least 24 months. More preferably, at least 90%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% of the amylin analogue remains in intact monomeric form in the formulation after it has been stored at 2-8°C (e.g. at 5°C) for at least 18 months, e.g. at least 24 months.

The formulation may further comprise a preservative, especially when it is provided in a multi-use format. Alternatively, and especially when provided in single-use format, the formulation may contain no or substantially no preservative.

The stable aqueous liquid formulation of the disclosure is formulated for parenteral administration, and in particular may be formulated for administration to a subject by injection, e.g. by subcutaneous injection.

In accordance with normal pharmaceutical practice, the formulations of the present disclosure are sterile and/or free (or substantially free, e.g. containing no more than about 100 ppm) of reducing agent.

An individual dose of a formulation of the disclosure may be provided in any suitable volume. For example, an individual dose may have a volume of 0.1 to 1.5 ml, for example 0.3 to 1.5 ml, for example 0.3 ml to 1 ml. Examples of specific individual dose volumes include 0.5 ml and 1 ml (e.g. 1.0 ml). Individual doses may be packaged separately for single use, e.g. in individual vials, cartridges or syringes, as described in more detail below.

By way of illustration, certain specific formulations may comprise or consist of the following:

Amylin analogue at a concentration of about 0.8 mg/ml, phosphate buffer at a concentration of about 5mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 1.2 mg/ml, phosphate buffer at a concentration of about 5mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 2.4 mg/ml, phosphate buffer at a concentration of about 5mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 4.5 mg/ml, citrate buffer at a concentration of about 5mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 9 mg/ml, citrate buffer at a concentration of about 5mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 0.4 mg/ml, citrate buffer at a concentration of about 10 mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 0.6 mg/ml, citrate buffer at a concentration of about 10 mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 1.2 mg/ml, citrate buffer at a concentration of about 10 mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 2.4 mg/ml, citrate buffer at a concentration of about 10 mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 4.5 mg/ml, citrate buffer at a concentration of about 10 mM, and mannitol at a concentration of about 250-260 mM; pH 6.5;

Amylin analogue at a concentration of about 9 mg/ml, citrate buffer at a concentration of about 10 mM, and mannitol at a concentration of about 250-260 mM; pH 6.5.

These formulations may be particularly suitable for a dosing volume of 1.0 ml, although it will be understood that they may be used for other dosing volumes. Alternatively, the concentration of amylin analogue may be adjusted for a different dosing volume.

In these formulations, as elsewhere, the amylin analogue be provided as a chloride salt, e.g. having the formula:
([19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNT-Hyp-NH_2), x(Cl)
where x is 1.0-2.0.

The disclosure further provides a container or delivery device containing a stable aqueous

liquid formulation as described herein. By way of example, suitable containers and delivery devices include a sealed vial, a pre-filled syringe, or a cartridge for an injector device such as an injector pen, an adjustable dose auto-injector, a disposable auto-injector, a wearable injector, or an infusion pump. The delivery device may be a single-use device, such as a pre-filled syringe.

In a further example, the present disclosure provides an article of manufacture or a kit comprising a container holding a stable aqueous liquid pharmaceutical composition of the disclosure.

The present disclosure further provides a stable aqueous liquid formulation as described for use in a method of medical treatment.

The formulations are useful, inter alia, in the reduction of food intake, promotion of weight loss, and inhibition or reduction of weight gain. As a result, they may be used for treatment of a variety of conditions, diseases, or disorders in a subject, including, but not limited to, obesity and various obesity-related conditions, diseases, or disorders, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease. The subject may be affected by obesity accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease. It will be understood that the formulations may thus be administered to subjects affected by conditions characterised by inadequate control of appetite or otherwise over-feeding, such as binge-eating disorder and Prader-Willi syndrome. It will be clear that the formulations can be used for treatment of combinations of the conditions described.

Thus, the disclosure provides a formulation of the disclosure for use in a method of treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing excess body weight. Treatment may be achieved, for example, by control of appetite, feeding, food intake, calorie intake and/or energy expenditure.

The disclosure also provides a formulation of the disclosure for use in a method of treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health complications of obesity or overweight such as infertility. The subject may be affected by obesity

accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

The disclosure also provides a formulation of the disclosure for use in a method of prevention or treatment of Alzheimer's disease, diabetes, type 1 diabetes, type 2 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, hyperglycemia, hypertension, atherogenic dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure, arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, and combinations thereof.

The disclosure also provides a formulation of the disclosure for use in a method of lowering circulating LDL levels and/or increasing HDL/LDL ratio.

Effects of the formulations on these conditions may be mediated in whole or in part via an effect on body weight, or may be independent thereof.

The disclosure further provides use of a formulation of the disclosure in the manufacture of a medicament for treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing excess body weight.

The disclosure also provides use of a formulation of the disclosure in the manufacture of a medicament for treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health complications of obesity or overweight such as infertility. The subject may be affected by obesity accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

The disclosure also provides use of a formulation of the disclosure in the manufacture of a medicament for the prevention or treatment of Alzheimer's disease, diabetes, type 1 diabetes, type 2 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose

tolerance (IGT), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, hyperglycemia, hypertension, atherogenic dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure, arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, and combinations thereof.

The disclosure also provides use of a formulation of the disclosure in the manufacture of a medicament for lowering circulating LDL levels and/or increasing HDL/LDL ratio.

The disclosure further provides a method of treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing excess body weight in a subject, comprising administering a therapeutically effective amount of a formulation of the disclosure to the subject.

The disclosure also provides a method of treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health complications of obesity or overweight such as infertility in a subject, comprising administering a therapeutically effective amount of a formulation of the disclosure to the subject. The subject may be affected by obesity accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

The disclosure also provides a method of prevention or treatment of Alzheimer's disease, diabetes, type 1 diabetes, type 2 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, hyperglycemia, hypertension, atherogenic dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure, arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, and

combinations thereof, in a subject, comprising administering a therapeutically effective amount of a formulation of the disclosure to the subject.

The disclosure further provides a method of lowering circulating LDL levels and/or increasing HDL/LDL ratio in a subject, comprising administering a therapeutically effective amount of a formulation of the disclosure to the subject.

The disclosure further provides the use of a formulation as described above in a method of cosmetic (i.e. non-therapeutic) weight loss.

It will be understood that references to therapeutic uses of formulation and methods comprising administration of formulation may equally be taken to encompass cosmetic uses and administration.

The disclosure further provides a process for producing a stable aqueous pharmaceutical formulation comprising an amylin analogue, which is:
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNT-Hyp-NH_2

or a pharmaceutically acceptable salt and/or derivative thereof;

the process comprising formulating

(a) the amylin analogue, salt or derivative, at a concentration of from about 0.4 mg/ml to about 25 mg/ml; and

(b) a buffer at a concentration of about 0.5 mM to about 25 mM;

to produce the stable aqueous liquid formulation, wherein the formulation has a pH of wherein the formulation has a pH of about 5.8 to about 6.9.

It will be apparent that the process may be applied to any of the formulations of the disclosure described herein.

Thus, for example, the process may further comprise formulating the amylin analogue and buffer with a tonicity modifier as described.

The process may comprise a step of adjusting the pH to the desired value. When the tonicity modifier is a chloride salt, e.g. NaCl, it may be desirable to adjust the pH to the desired value before adding the tonicity modifier to the formulation. This may particularly be the case where the amylin analogue is itself provided in the form of a chloride salt, since the presence of chloride ions above a certain level may promote precipitation or fibrillation of the amylin analogue at pH values around the pI of the peptide (pI = 4.36).

Also provided is a formulation produced by the process described.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the disclosure and apply equally to all aspects and embodiments which are described.

Throughout this specification, the term "about" in relation to a numerical value is optional and means, for example, +/-10%.

Detailed Description of the Invention

Throughout the description and claims the conventional one-letter and three-letter codes for natural amino acids are used. All amino acid are of the L-configuration unless stated otherwise.

Amylin analogue

The amylin analogue (Compound 1; "CPD 1") present in the formulations of the present invention is described in WO 2018/046719 and has the formula:

[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNT-Hyp-NH_2

Gly(Me):	N-methylglycine [also known as sarcosine (Sar)]
Ile(Me):	N-methylisoleucine
Aad:	2-aminoadipic acid, e.g. (2S)-2-aminoadipic acid [also (2S)-2-aminohexanedioic acid] , also known as homo-glutamic acid
[19CD]-:	19-carboxy-nonadecanoyl-

Parentheses "()" shown after the symbols for particular amino acid residues indicate residues whose side chains participate in an intramolecular lactam bridge. Thus, the

Claims:

1. A stable aqueous liquid pharmaceutical formulation comprising an amylin analogue, which is:
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNT-Hyp-NH_2

or a pharmaceutically acceptable salt and/or derivative thereof;

wherein the formulation comprises:

(a) the amylin analogue at a concentration of from 0.4 mg/ml to 25 mg/ml; and

(b) a buffer at a concentration of 0.5 mM to 25 mM;

wherein the formulation has a pH of 5.8 to 6.9.

2. The stable aqueous liquid formulation of claim 1, wherein the amylin analogue is present at a concentration of at least about 0.4 mg/ml, at least about 0.5 mg/ml, at least about 0.6 mg/ml, at least about 1.2 mg/ml, or at least about 2.5 mg/ml.

3. The stable aqueous liquid formulation of any one of the preceding claims, wherein the amylin analogue is present at up to about 25 mg/ml, up to about 20 mg/ml, up to about 15 mg/ml, or up to about 10 mg/ml.

4. The stable aqueous liquid formulation of any one of the preceding claims, wherein the amylin analogue is present at a concentration of 3 mg/ml to 12 mg/ml, e.g. 3 mg/ml to 7 mg/ml or 8 mg/ml to 12 mg/ml, e.g. about 5 mg/ml or about 10 mg/ml.

5. The stable aqueous liquid formulation of any one of the preceding claims, wherein the buffer is present at a concentration of 0.5 mM to 20 mM, e.g. 0.5 mM to 15 mM, e.g. 1 mM to 15 mM, e.g. 1 mM to 12 mM.

6. The stable aqueous liquid formulation of any one of the preceding claims, wherein the buffer is present at a concentration of 3 mM to 7 mM or 8 mM to 12 mM, e.g. about 5 mM or about 10 mM.

7. The stable aqueous liquid formulation of any one of the preceding claims, wherein the buffer is phosphate, histidine or citrate.

8. The stable aqueous liquid formulation of any one of claims 1 to 6, wherein the buffer is TRIS buffer, and wherein the buffer is present at 15 mM to 25 mM, e.g. 17 mM to 23 mM, e.g. about 20 mM.

9. The stable aqueous liquid formulation of any one of the preceding claims, wherein the pH is 6.2 to 6.8, e.g. 6.4 to 6.6, e.g. about 6.5.

10. The stable aqueous liquid formulation of any one of the preceding claims, further comprising a tonicity modifier.

11. The stable aqueous liquid formulation of any one of the preceding claims, wherein the formulation is stable at 2-8°C for at least 6 months, at least 12 months, at least 18 months or at least 24 months.

12. The stable aqueous liquid formulation of any one of the preceding claims, wherein the formulation displays substantially no turbidity, or aggregation, fibrillation or gelling of the amylin analogue, after storage at 2-8°C for at least 6 months, at least 12 months, at least 18 months or at least 24 months.

13. The stable aqueous liquid formulation of any one of the preceding claims, wherein at least 80%, more preferably at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the amylin analogue remains in intact monomeric form in the formulation after it has been stored at 2-8°C for at least 6 months, at least 12 months, at least 18 months or at least 24 months.

14. The stable aqueous liquid formulation of any one of the preceding claims, wherein the amylin analogue is provided as a chloride salt.

15. The stable aqueous liquid formulation of any one of the preceding claims, wherein the formulation is formulated for administration to a subject by injection, e.g. wherein the injection is subcutaneous injection.