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(54) Title: STABLE FORMULATION OF MODIFIED GLP-1

(57) Abstract: Pharmaceutical formulations of GLP-1 compounds and methods for preparation thereof.

STABLE FORMULATION OF MODIFIED GLP-1

Field of the invention

The present invention relates to pharmaceutical formulations comprising GLP-1
5 compounds, uses thereof and methods for preparing said formulations.

Background of the invention

Peptides are widely used in medical practice, and since they can be produced by re-
combinant DNA technology it can be expected that their importance will increase also in the
10 years to come.

The hormones regulating insulin secretion belong to the so-called enteroinsular
axis, designating a group of hormones, released from the gastrointestinal mucosa in re-
sponse to the presence and absorption of nutrients in the gut, which promote an early and
potentiated release of insulin. The enhancing effect on insulin secretion, the so-called incretin
15 effect, is probably essential for a normal glucose tolerance. Many of the gastrointestinal hor-
mones, including gastrin and secretin (cholecystokinin is not insulinotropic in man), are insu-
linotropic, but the only physiologically important ones, those that are responsible for the in-
cretin effect, are the glucose-dependent insulinotropic polypeptide, GIP, and glucagon-like
peptide-1 (GLP-1). Because of its insulinotropic effect, GIP, isolated in 1973 immediately at-
20 tracted considerable interest among diabetologists. However, numerous investigations car-
ried out during the following years clearly indicated that a defective secretion of GIP was not
involved in the pathogenesis of insulin dependent diabetes mellitus (IDDM) or non insulin-
dependent diabetes mellitus (NIDDM). Furthermore, as an insulinotropic hormone, GIP was
found to be almost ineffective in NIDDM. The other incretin hormone, GLP-1 is the most po-
25 tent insulinotropic substance known. Unlike GIP, it is surprisingly effective in stimulating insu-
lin secretion in NIDDM patients. In addition, and in contrast to the other insulinotropic hor-
mones (perhaps with the exception of secretin) it also potently inhibits glucagon secretion.
Because of these actions it has pronounced blood glucose lowering effects particularly in pa-
tients with NIDDM.

30 GLP-1, a product of the proglucagon, is one of the youngest members of the se-
cretin-VIP family of peptides, but is already established as an important gut hormone with
regulatory function in glucose metabolism and gastrointestinal secretion and metabolism.
The glucagon gene is processed differently in the pancreas and in the intestine. In the pan-
creas, the processing leads to the formation and parallel secretion of 1) glucagon itself, oc-

cupying positions 33-61 of proglucagon (PG); 2) an N-terminal peptide of 30 amino acids (PG (1-30)) often called glicentin-related pancreatic peptide, GRPP; 3) a hexapeptide corresponding to PG (64-69); 4) and, finally, the so-called major proglucagon fragment (PG (72-158)), in which the two glucagon-like sequences are buried. Glucagon seems to be the only
5 biologically active product. In contrast, in the intestinal mucosa, it is glucagon that is buried in a larger molecule, while the two glucagon-like peptides are formed separately.

While much attention has been focused on the pharmacological properties of acylated GLP-1 compounds, hitherto little is known about their physico-chemical and solution structural properties. Such knowledge is a prerequisite for rational handling during e.g. production, purification and formulation work and is eventually important for understanding of the
10 structural basis for the protraction mechanism.

It is an important technical challenge to ensure prolonged stability during storage (shelf life) of many protein based drug products due to the inherent lability of macromolecules. Hence, proteins are sensitive to both chemical and physical degradation unlike many
15 small molecules. Chemical degradation involves covalent bonds, such as hydrolysis, racemization, oxidation or crosslinking. Physical degradation involves conformational changes relative to the native structure, which includes loss of higher order structure, aggregation, precipitation or adsorption to surfaces. GLP-1 is known to be prone to instability due to aggregation. Both degradation pathways may ultimately lead to loss of biological activity of the protein
20 drug.

GLP-1 and analogues of GLP-1 and fragments thereof are potentially useful *i.a.* in the treatment of type 1 and type 2 diabetes. However, solubility limitations and the low stability against the actions of endogenous diaminopeptidyl peptidase limits the usefulness of these compounds, and thus there still is a need for improvements in this field.

25 In WO 99/43341 are disclosed certain pharmaceutical formulations comprising GLP-1 having a lipophilic substituent. All of the disclosed formulations are maintained at pH 7.4.

In WO 00/37098 are disclosed shelf-stable formulations comprising GLP-1, a preservative, and a tonicity modifier, at pH 8.2 to 8.8.

30 Human GLP-1 is a 37 amino acid residue peptide originating from proglucagon which is synthesised *i.a.* in the L-cells in the distal ileum, in the pancreas and in the brain. Processing of proglucagon to give GLP-1(7-36)amide, GLP-1(7-37) and GLP-2 occurs mainly in the L-cells. A simple system is used to describe fragments and analogues of this peptide. Thus, for example, Val⁸-GLP-1(7-37) (or Val⁸GLP-1(7-37)) designates a fragment of GLP-1 formally derived from GLP-1 by deleting the amino acid residues Nos. 1 to 6 and substituting the naturally
35 occurring amino acid residue in position 8 (Ala) by Val. Similarly, Lys³⁴(N⁶-tetradecanoyl)-GLP-

1(7-37) designates GLP-1(7-37) wherein the ϵ -amino group of the Lys residue in position 34 has been tetradecanoylated. For convenience the amino acid sequence of GLP-1 (7-37) is given below, wherein the N-terminal His is no. 7 and the C-terminal Gly is no. 37:

5 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-
Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-
Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly.

Where reference in this text is made to C-terminally extended GLP-1 analogues, the amino acid
10 residue in position 38 is Arg unless otherwise indicated, the optional amino acid residue in
position 39 is also Arg unless otherwise indicated and the optional amino acid residue in
position 40 is Asp unless otherwise indicated. Also, if a C-terminally extended analogue extends
to position 41, 42, 43, 44 or 45, the amino acid sequence of this extension is as in the
corresponding sequence in human preproglucagon unless otherwise indicated.

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Summary of the invention

We have discovered that certain modified GLP-1 or analogues thereof when formu-
lated in aqueous solution together with a buffer, are physically stable at high concentrations
of the modified GLP-1 or analogues thereof, when kept in the pH range from about 7 to about
20 10. The present formulations are physically stable within a given shelf life period at the rec-
ommended storage temperature (typically 2-3 years at 2-8°C). Furthermore, the present for-
mulations are physically stable during in-use (typically 1 month at accelerated temperatures
e.g. 25°C or 37°C). The formulations of the invention are also chemically stable thus render-
ing them shelf-stable and suitable for invasive (eg. injection, subcutaneous injection, intra-
25 muscular, intravenous or infusion) as well as non-invasive (eg nasal or pulmonary, trans-
dermal or transmucosal e.g. buccal) means of administration. When the inventive formula-
tion comprising a GLP-1 compound was compared to the same formulation comprising GLP-
1(7-37) substituted for the GLP-1 compound, the physical stability was increased considera-
bly, and typically the shelf-life was increased from a few seconds to several months in the
30 tests used.

One object of the present invention is to provide a pharmaceutical formulation com-
prising a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or
an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic sub-
35 stituent attached optionally via a spacer, wherein said GLP-1 compound is present in a con-

centration from 0.1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10;

Another object of the present invention is to provide a method of preparing a physically stable pharmaceutical formulation of a GLP-1 compound wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, comprising preparing a formulation containing the GLP-1 compound, and a buffer, wherein said GLP-1 compound is present in a concentration from 0.1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10.

In one aspect of the invention the formulation contains a GLP-1 compound in a concentration from 1 mg/ml to 100 mg/ml.

In another aspect of the invention the formulation has a pH from 7.5 to 10.

In one embodiment the GLP-1 compound is Arg³⁴, Lys²⁶(N-ε-(γ-Glu(N-α-hexadecanoyl)))-GLP-1(7-37).

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Description of the invention

In one aspect the invention relates to a pharmaceutical formulation comprising a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 0.1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10;

provided that if an isotonic agent is present and pH is 7.4 then mannitol or NaCl is not the isotonic agent.

In another aspect the invention relates to a pharmaceutical formulation comprising a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10;

provided that if an isotonic agent is present and pH is 7.4 then mannitol or NaCl is not the isotonic agent.

In a further aspect the invention relates to a pharmaceutical formulation comprising a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 0.1 mg/ml or above, and wherein said formulation has a pH from 7.0 to 10.

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