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(54) Title: SHELF–STABLE FORMULATION OF GLUCAGON–LIKE PEPTIDE–1

(57) Abstract

Glucagon–like peptide–1 (GLP–1) has been shown to be useful in the treatment of diabetes. The invention encompasses a shelf stable formulation that comprises a therapeutically effective amount of GLP–1, a pharmaceutically acceptable preservative, and a tonicity modifier, and that has a pH between about 8.2 to about 8.8.

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SHELF-STABLE FORMULATION OF GLUCAGON-LIKE PEPTIDE-1

Background of the Invention

Glucagon-like peptide-1 (7-37)-OH (GLP-1) is a 31 amino acid hormone that is produced by post-translational processing of the preglucagon gene product in the brain, stomach, intestine, and pancreas. The main physiological function of GLP-1 is to regulate insulin secretion in response to glucose, and thus it has the ability to normalize blood glucose levels. As such, there has been interest in GLP-1, its analogs and derivatives as potential therapeutic agents for the treatment of diabetes. A particular advantage to the use of GLP-1 over other drugs in the treatment of diabetes is that administration of GLP-1 at doses in the 1-5 nmole range exhibit few adverse side effects, such as hypoglycemia. Unexpectedly, GLP-1 also has been shown to work in patients that have secondary failure to sulfonylurea drugs, the most common drug type for the treatment of type II diabetes. GLP-1 also is a potent inhibitor of gastric acid secretion and gastric emptying.

In general, effective therapeutic administration of peptides can be problematic since peptides often are degraded in the gastrointestinal tract by various peptidases. Additionally, certain peptide treatment protocols require either continuous or repeated administration of the peptide agent over an extended period of time. Repeated injections cause both inconvenience and discomfort to the user. Thus, chronic use of the peptide agent, which would be required for patients afflicted with diabetes, would result in inconvenience and discomfort to the user.

The long-term stability of peptides, particularly GLP-1, as components of a pharmaceutical composition for administration to mammals, is questionable. Such a lack of stability adversely affects bioavailability. In fact, when stored at low temperatures of 4° C, by-products of GLP-1(7-37) have been found as early as eleven months after sample preparation (see Mojsov, *Int. J. Peptide Protein Res.*, Vol. 40, pages 333-343 (1992)). Additionally, the biological half-life of GLP-1 molecules, particularly those molecules affected by the activity of dipeptidyl-peptidase IV (DPPIV), is quite short. For example,

the biological half-life of GLP-1(7-37) is only 3 to 5 minutes (see U.S. Patent No. 5,118,666), which is further augmented by its rapid absorption following parenteral administration to a mammal.

Another factor decreasing the bioavailability of GLP-1 is the solubility of GLP-1 when incorporated into an aqueous solution. The solubility of GLP-1 is highly dependent on the environment, such as the choice of buffering system, and the treatment that the peptide has undergone. For example, conversion of a peptide into its salt form plays a role in its solubility. In this regard, synthetic GLP-1 is highly soluble in neutral phosphate buffered saline. Because the solubility of the peptide is high in such aqueous solutions, slow release of the peptide can be difficult to attain unless the peptide is incorporated into a system for slow release.

Stable formulations of therapeutic agents are particularly required for use in delivery devices that expose these agents to elevated temperatures and/or mechanical stress. For example, stable GLP-1 formulations are required for use in continuous infusion systems and pen delivery devices. Current formulations provide only limited stability in these types of delivery devices.

In continuous infusion systems, a fluid containing a therapeutic agent is pumped from a reservoir, usually to a subcutaneous, intravenous, or intraperitoneal depot. The reservoir, which must be refilled periodically, is attached to the patient's body, or is implanted in the patient's body. In either case, the patient's body heat and body motion, and turbulence in the tubing and pump impart a relatively high amount of thermo-mechanical energy to the formulation. In the interest of minimizing the frequency with which the reservoir is refilled, and of minimizing the size of the reservoir, formulations having a relatively high concentration of the therapeutic agent are advantageous.

Injector pens also have been developed to allow diabetic patients to accurately measure and administer controlled doses of insulinotropic agents. Generally, these pens are secured onto a cartridge having a particular quantity of liquid medication sealed therein. The cartridge includes a plunger and a mechanism for advancing the plunger in the cartridge in such a manner to dispense the medication. Injector pens may be reusable or disposable. In reusable pens, a user can change a spent cartridge and reset the leadscrew of the pen back to its initial position. In a disposable pen, the cartridge is permanently

embodiment, the GLP-1 molecule of the formulation is a derivative of GLP-1 and is selected from the group consisting of a peptide having the amino acid sequence:

NH_2-His^7-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-X **(SEQ ID NO:3)**

and a pharmaceutically-acceptable salt thereof, wherein X is selected from the group consisting of Lys and Lys-Gly; a pharmaceutically-acceptable lower alkylester of the peptide; and a pharmaceutically-acceptable amide of the peptide selected from the group consisting of amide, lower alkyl amide, and lower dialkyl amide. In another preferred embodiment, the formulation also comprises a long-acting insulin agent.

The present invention also provides a method of enhancing the expression of insulin in a mammalian pancreatic β-type islet cell in need of such enhancement, comprising administering to the cell, an effective amount of a shelf-stable pharmaceutical formulation, wherein the formulation comprises a therapeutically effective amount of a GLP-1 molecule, a pharmaceutically acceptable preservative, and a tonicity modifier, and wherein the formulation has a pH that is about 8.2 to about 8.8. In a preferred embodiment, the formulation used in the therapeutic method comprises a buffer, such as TRIS. In another preferred embodiment, the formulation used in the therapeutic method further comprises a surfactant, such as Brij-35. In an additional preferred embodiment, the GLP-1 molecule of the formulation thus administered is an analog of GLP-1 and is selected from the group consisting of a peptide having the amino acid sequence:

R_1-X-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Y-Gly-Gln-Ala-Ala^{25}-Lys-Z-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-R_2 **(SEQ ID NO:2)**

and a pharmaceutically-acceptable salt thereof, wherein R_1 is His or desamino-histidine, X is Ala, Gly or Val, Y is Glu or Gln, Z is Glu or Gln and R_2 is Gly-OH. In an especially preferred embodiment, the GLP-1 molecule administered is according to SEQ ID NO: 2, wherein R_1 is L-histidine, X is Val, Y is Glu, Z is Glu, and R_2 is Gly-OH. In an alternative preferred embodiment, the GLP-1 molecule administered is a derivative of GLP-1 and is selected from the group consisting of a peptide having the amino acid sequence:

NH_2-His^7-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-X **(SEQ ID NO:3)**

and a pharmaceutically-acceptable salt thereof, wherein X is selected from the group consisting of Lys and Lys-Gly; a pharmaceutically-acceptable lower alkylester of the peptide;

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