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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/26, C07K 14/605 — A1

(11) International Publication Number: **WO 95/32730**

(43) International Publication Date: 7 December 1995 (07.12.95)

(21) International Application Number: PCT/DK95/00208

(22) International Filing Date: 26 May 1995 (26.05.95)

(30) Priority Data:
0590/94 26 May 1994 (26.05.94) DK

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(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: A PHARMACEUTICAL PREPARATION COMPRISING GLUCAGON

(57) Abstract

A pharmaceutical preparation comprising glucagon and a stabilizing amount of a pharmaceutically acceptable ampholyte, especially an amino acid or dipeptide or a mixture thereof and optionally an excipient, is stable for extended periods of time.

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A PHARMACEUTICAL PREPARATION COMPRISING GLUCAGON

FIELD OF THE INVENTION

The present invention relates to a stabilized pharmaceutical preparation comprising glucagon.

BACKGROUND OF THE INVENTION

Human glucagon is a polypeptide hormone secreted by α-cells of the pancreatic islets of Langerhans. It is a single-chain polypeptide consisting of 29 amino acid residues the sequence of which is published inter alia in The Merck Index, 10th Edition (1983), Monograph No. 4307.

Glucagon is used for the treatment of hypoglycemia in diabetics due to its glycogenolytic effect on the liver. Glucagon also exerts a spasmolytic effect on smooth muscles which is used clinically in connection with several imaging procedures, especially radiology.

Glucagon for administration by injection is presently marketed in the form of a lyophilized product which is to be reconstituted using a suitable diluent. The lyophilisate contains lactose as the sole excipient.

In the production of those conventional pharmaceutical preparations comprising glucagon, utmost care must be taken in order to avoid decomposition of the glucagon during preparation, dispensing and lyophilization. Furthermore, glucagon undergoes decomposition during storage of the finished product at room temperature. This significantly limits the shelf-life of the conventional preparations.

Thus, there is a need for a more stable formulation of glucagon which retains its activity for extended periods of time at room temperature. Such stabilized preparations com-

prising glucagon are desirable for emergency treatment of acute hypoglycemia rendering it possible for diabetic patients to carry a dose of glucagon in their hand bag enabling themselves or another person present to treat an incidence of hypoglycemia immediately.

BRIEF DESCRIPTION OF THE INVENTION

Surprisingly, it has now been found that a significant improvement of the stability of the glucagon in pharmaceutical compositions comprising this peptide can be achieved by including an ampholyte in the composition.

Thus, in its broadest aspect, the present invention relates to a stabilized pharmaceutical composition comprising glucagon and an ampholyte.

In a preferred embodiment, the invention relates to a pharmaceutical composition for parenteral use.

In another preferred embodiment, the invention relates to a pharmaceutical composition for administration by injection.

In another preferred embodiment, the invention relates to a pharmaceutical composition which is a lyophilisate which has to be dissolved in a prescribed amount of solvent before it is administered by injection.

In another preferred embodiment, the invention relates to a pharmaceutical composition for nasal administration.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to a stabilized pharmaceutical preparation comprising glucagon and a stabilizing amount of a pharmaceutically acceptable ampholyte, especially an amino acid or dipeptide or a mixture thereof and optionally an

excipient. Such preparations retain the glucagon activity at room temperature, e.g. 25°C, for extended periods of time.

US patent No. 5,059,587 (Yamamoto et al.) discloses powder compositions for nasal administration of physiologically active peptides. Various organic acids, i.a. L-glutamic acid and L-aspartic acid, are suggested as absorption promoters. Optionally, a diluent is included in the compositions and among the suggested diluents are amino acids and polyamino acids. However, no composition comprising an amino acid or a polyamino acid is disclosed.

US patent No. 5,023,088 (Wong et al.) discloses a drug delivery system which can be used for the administration of various drugs including pharmacologically active peptides, e.g. glucagon. Sodium bisulfite and histidine hydrochloride are mentioned as examples of stabilizers which may used in case it is desired to include a stabilizer for the drug in the composition.

WO 93/12811 describes a stabilized pharmaceutical composition comprising growth hormone and asparagine.

A pharmaceutically acceptable ampholyte to be used in accordance with the invention may be selected from the group consisting of amino acids (except histidine) or derivatives thereof such as glycine, N-methylglycine (sarcosine), trimethylglycine hydroxide inner salt (betaine), alanine, β-alanine, valine, leucine, nor-leucine, isoleucine, serine, threonine, aspartic acid, glutamic acid, hydroxyglutamic acid, lysine, hydroxylysine, ornithine, arginine, methionine, asparagine and glutamine; dipeptides such as glycylglycine; pharmaceutically acceptable sulfonic acids or derivatives thereof such as taurine; creatinine, and ethylenediaminetetraacetic acid (EDTA).

An amino acid to be used in accordance with the present invention is preferably a naturally occurring α-amino acid except histidine. Such amino acids may be L- or D-amino acids or glycine or a mixture thereof.

Preferably glycine or glycylglycine or a mixture thereof is used.

A pharmaceutical preparation in lyophilized form according to the invention preferably also comprises an excipient, in order to facilitate on one hand the lyophilization and on the other hand rapid and complete redissolution thereof when the preparation is reconstituted before administration by injection. Usually the solvent used for the redissolution of the lyophilisate is sterile water or sterile water to which a preservative e.g. m-cresol or benzyl alcohol has been added.

An excipient may be selected from disaccharides such as lactose, trehalose, and sucrose, sugar alcohols such as sorbitol or mannitol, polysaccharides such as the polymers commercialized as Dextran® products such as Dextran® 40, Dextran® 70 or Dextran® 75, and Ficoll® and polyvalent alcohols such as polyethylene glycol or polyvinyl alcohol or a combination of two or more of these.

The excipient is preferably present in an amount of from 10 to 600 micromoles per mg of glucagon giving an optimum stabilization. The excipient preferred according to the invention is lactose.

In a further aspect of the invention, the pharmaceutical preparation of the invention is in the form of a stabilized solution of glucagon.

In order to obtain the desired stabilization, the stabilizing amino acid or dipeptide may be present in an amount of from 0.01 to 50 micromoles per mg of glucagon. The amount of

stabilizing amino acid or dipeptide present per dose of pharmaceutical preparation comprising glucagon is according to the invention from 0.1 to 50 micromoles.

A preferred preparation of the invention comprises glycine or glycylglycine in an amount of about 10 micromoles per mg of glucagon. The amount of glycine or glycylglycine is preferably about 10 micromoles per dose.

The pH value of the preparations according to the invention in the form of a reconstituted solution ready for administration is preferably in the interval from 1 to 7. Preferably, the pH value is in the interval from 2 to 4, and most preferred about 2.8.

The invention also relates to a method for the preparation of a pharmaceutical preparation comprising glucagon and a stabilizing amount of a pharmaceutically acceptable ampholyte wherein glucagon is dissolved in a solution of the ampholyte and optional excipient and lyophilized, optionally after sterile filtration.

The dissolution of the glucagon is preferably carried out at a temperature of from 4 to 8°C.

A most preferred preparation according to the invention comprises glucagon, lactose or mannitol as excipient and glycine or glycylglycine or a mixture thereof as a stabilizing agent. Such a preparation has a buffer effect at a pH value of about 2.8 and results in a minimum rate for the decomposition of glucagon. Thus, the invention enables the formulation of a preparation in which glucagon is stable at room temperature.

The amino acid sequence of human glucagon is identical to the amino acid sequence of porcine and bovine glucagon. Hence, glucagon may be isolated by conventional extraction from porcine or bovine pancreatic glands. In the alternative,

glucagon may be prepared by full or partial chemical synthesis or by recombinant techniques, e.g. as disclosed in US patent No. 4,826,763.

The pharmaceutical preparation of the invention may be formulated for administration in any suitable way, e.g. by injection of a solution, by oral administration or by administration to a mucosal membrane, e.g. by nasal administration of a powder or a solution. The pharmaceutical preparation may e.g. be presented in the form of a single dose comprised in a vial or cartridge or any other suitable container.

The amount of the stabilized glucagon composition according to the present invention to be given to a patient in need of such a treatment is identical to the amount which would have to be to be given under identical circumstances of a glucagon composition of the known art.

The invention is explained more detailed in the examples below which illustrate the invention. The examples are not to be considered as limiting the scope of the invention which is defined by the appended claims. The features disclosed in the foregoing description and in the following examples may, either separately or in any combination thereof, be material for realising the invention in diverse forms thereof.

EXAMPLES

MATERIALS AND METHODS

Preparation and lyophilization of formulations comprising glucagon was carried out using the following procedure:

The glucagon used was recombinant glucagon prepared in Saccharomyces cerevisiae as disclosed in US patent No. 4,826,763.

10.7 g of lactose and the appropriate amount of the amino acid or dipeptide were dissolved in approximately 75 ml of distilled water and the pH value was adjusted to 2.6 - 3.0 using 1 N hydrochloric acid or sodium hydroxide as necessary. The volume was adjusted to 100 ml and the solution was cooled to 4 - 8°C in a refrigerator. Then, 110 mg of glucagon was dissolved in the solution and the pH value was adjusted to 2.6 - 3.0 using 1 N hydrochloric acid while the temperature of the solution was maintained at 4 - 8°C.

The solution was sterile filtered through a 0.2 μm filter (using a 50 ml syringe) and divided into vials, 1 ml in each.

The solution in the vials was lyophilized for a total of 36 hours, using the following procedure:

1) prefreezing at -45°C for 3 - 5 hours, followed by

2) primary drying from -45°C to 20°C and a pressure of 0.1 hPa for 29 hours, and

3) secondary drying at 20°C (and full vacuum) for 4 hours using a Heto CD8 apparatus.

EXAMPLE 1

Preparation of formulations comprising glucagon, lactose and an amino acid or a dipeptide and study of their stability.

In an analogous manner as described above, formulations were prepared from glucagon, lactose and an amino acid or a dipeptide.

As reference was used a formulation comprising glucagon and lactose prepared as described above.

Formulations having the following compositions per vial were prepared:

Reference:

Glucagon	1.1 mg
Lactose USP/Ph Eur	107 mg
1N HCl	ad pH 2.8

Test formulations:

Glucagon	1.1 mg
Lactose USP/Ph Eur	107 mg
Ampholyte	5, 10, or 20 mM
HCl/NaOH	ad pH 2.8

The following ampholytes were tested, each at three concentration levels, 5, 10 and 20 mM, as given under the heading "Conc. AA (mM)" in tables 1 to 3:

Formulation	
A1	Glycine
A2	Glycylglycine
A3	Histidine
A4	Aspartic acid
A5	Glutamic acid
A6	Leucine
A7	Alanine
A8	Asparagine
A9	Valine

Vials containing the test formulations and the reference formulation were incubated at 60°C for a period of up to four weeks. Before the start of the incubation and after one week and four weeks, the contents of one vial from each lot and after two weeks and three weeks, the contents of one vial from most of the lots in the incubator (see tables 1 to 3) were analysed. The degradation of the glucagon, expressed as the percentage of glucagon remaining relative to the amount

of glucagon inserted in the formulations, was analysed by reversed phase high performance liquid chromatography (RPC). For each of the ampholytes tested, a column in one of the tables 1 to 3 (Heading: "[name of ampholyte] RPC %") gives the results which can be compared with the results achieved with the reference.

The results achieved with the ampholytes show that a very pronounced stabilization of glucagon is obtained by adding a stabilizing agent according to the invention.

TABLE 1

		Glycine	Glycylglycine	Histidine	Reference
Week	Conc. AA (mM)	RPC %	RPC %	RPC %	RPC %
0	5	98.00	97.90	97.90	97.60
	10	97.90	97.90	98.00	
	20	97.80	97.90	97.90	
1	5	97.10	96.40	94.70	55.80
	10	92.40	97.00	97.20	
	20	93.40	96.10	94.00	
2	5	91.60	95.20	91.20	29.30
	10	94.50	93.70	94.40	
	20	91.60	90.80	90.90	
3	5	94.80	95.30	88.10	25.30
	10	93.20	94.30	87.60	
	20	91.80	93.60	94.10	
4	5	84.60	91.70	90.90	26.90
	10	93.30	87.50	93.30	
	20	85.40	90.10	94.20	

TABLE 2

		Aspartic acid	Glutamic acid	Leucine	Reference
Week	Conc. AA (mM)	RPC %	RPC %	RPC %	RPC %
0	5	97.70	97.10	97.70	97.80
	10	97.80	97.70	97.60	
	20	97.70	97.70	97.60	
1	5	72.70	60.30	81.00	45.80
	10	95.60	95.80	95.90	
	20	94.80	94.90	95.40	
2	5	65.80	48.10	74.70	33.50
	10	93.30	94.10	94.20	
	20	91.90	93.30	93.60	
4	5	57.80	32.70	67.00	19.20
	10	88.60	90.50	91.00	
	20	86.50	88.20	89.10	

TABLE 3

		Alanine	Asparagine	Valine	Reference
Week	Conc. AA (mM)	RPC %	RPC %	RPC %	RPC %
0	5	97.30	97.30	97.40	97.00
	10	97.50	97.60	97.60	
	20	97.30	97.60	97.30	
1	5	93.60	93.90	85.90	68.10
	10	96.30	96.20	96.10	
	20	95.90	96.00	95.80	
3	5	90.90	91.90	80.00	44.00
	10	94.30	94.40	94.00	
	20	93.20	93.80	92.80	
4	5	89.90	91.40	75.40	35.90
	10	93.40	93.60	93.50	
	20	92.30	93.00	92.10	

CLAIMS

1. A pharmaceutical preparation comprising glucagon and a stabilizing amount of a pharmaceutically acceptable ampholyte except histidine.

2. A pharmaceutical preparation as claimed in claim 1, wherein the ampholyte is selected from the group consisting of glycine, N-methylglycine (sarcosine), glycylglycine, trimethylglycine hydroxide inner salt (betaine), alanine, β-alanine, valine, leucine, nor-leucine, isoleucine, serine, threonine, aspartic acid, glutamic acid, hydroxyglutamic acid, lysine, hydroxylysine, ornithine, arginine, methionine, asparagine, glutamine, taurine, creatinine, and ethylenediaminetetraacetic acid (EDTA).

3. A pharmaceutical preparation as claimed in claim 2, wherein the ampholyte is a pharmaceutically acceptable, naturally occurring amino acid, except histidine, or a dipeptide or a mixture thereof.

4. A pharmaceutical preparation as claimed in claim 3, wherein the ampholyte is glycine or glycylglycine or a mixture thereof.

5. A pharmaceutical preparation as claimed in any of claims 1-4 further comprising a pharmaceutically acceptable excipient.

6. A pharmaceutical preparation as claimed in claim 5 wherein the excipient is selected from disaccharids such as lactose, trehalose, and sucrose and sugar alcohols such as sorbitol or mannitol, polysaccharides such as the polymers commercialized as Dextran® products and Ficoll®, and polyvalent alcohols such as polyethylene glycol or polyvinyl alcohol or a combination of two or more of these.

7. A pharmaceutical preparation as claimed in claim 6 wherein the excipient is lactose.

8. A pharmaceutical preparation as claimed in any of claims 5-7 wherein the excipient is present in an amount from 10 to 600 micromoles per mg of glucagon.

9. A pharmaceutical preparation as claimed in claim any of claims 1-8 wherein the glycine or glycylglycine is present in an amount of from 0.01 to 50 micromoles per mg of glucagon.

10. A pharmaceutical preparation as claimed in claim 9 wherein the glycine or glycylglycine is present in an amount about 10 micromoles per mg of glucagon.

11. A pharmaceutical preparation as claimed in any of claims 1-10 which is a solution with a pH value in the interval from 2 to 7.

12. A pharmaceutical preparation as claimed in claim 11 wherein the pH value is in the interval from 2.3 to 3.8.

13. A pharmaceutical preparation as claimed in claim 12 wherein the pH value is a 2.8.

14. A pharmaceutical preparation as claimed in any of claims 1-10 which is a lyophilisate which on dissolution in the amount of solvent prescribed for administration by injection has a pH value as in the interval from 2 to 7, preferably from 2.3 to 3.8, more preferred a pH value of 2.8.

15. A method for the preparation of a pharmaceutical preparation comprising glucagon and a stabilizing amount of a pharmaceutically acceptable ampholyte wherein glucagon is dissolved in a solution of the ampholyte and optional excipient, and lyophilized, optionally after sterile filtration.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00208

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/26, C07K 14/605
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5023088 A (PATRICK S. L. WONG ET AL), 11 June 1991 (11.06.91), column 3, line 53 - column 4, line 11; column 6, line 13 - line 15	1-10
Y	WO 9312811 A1 (NOVO NORDISK A/S), 8 July 1993 (08.07.93), page 5 - page 6	1-10
A	US 5059587 A (NAKAYUKI YAMAMOTO ET AL), 22 October 1991 (22.10.91)	1-10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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INTERNATIONAL SEARCH REPORT

31/07/95

International application No.
PCT/DK 95/00208

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