

Role of Amylin Analogues in Diabetes mellitus: A Review

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ABSTRACT: Diabetes mellitus is hyperglycemic condition that occurs due to defects in insulin secretion or β -cell destruction. Amylin is an 37-amino acid peptide co synthesized and co secreted with insulin which is firstly isolated, purified, and characterized from the amyloid deposits in the pancreases of type 2 diabetics patients. Amylin deficiency occurs in type I and late stage type II diabetes patients due to β -cell destruction. Pramlintide (Symlin®), is an synthetic analogue of the naturally occurring pancreatic hormone amylin, is currently used with insulin in adjunctive therapy for type 1 and type 2 diabetes mellitus. Several synthetic approaches are used for synthesis of pramlintide via microwave irradiation. Amylin agonists could also be useful for weight loss, especially in combination with other agents.

KEYWORDS: Diabetes Mellitus, Amylin Analogues, Co-secreted with, Pramlintide.

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia induced by defects in insulin secretion and/or action. This causes by problems with carbohydrate, fat, and protein metabolism. Current mellitus treatment options include subcutaneous injections of biosynthetic human insulin, insulin analogues, 3 glucagon-like peptide-1 (GLP-1) receptor agonists 4, and amylin receptor agonists. Amylin deficiency occurs in type I and late stage type II diabetes patients due to β -cell destruction. Amylin has a high potential for use in the treatment of diabetes and obesity. The identification of amylin's role resulted in the discovery of pramlintide. The problematic physiochemical property of forming cytotoxic amyloid fibrils was overcome by replacing amylin's amino acids 25, 28, and 29 with pralines in amylin that forms pramlintide. ^[1]

1. DIABETES MELLITUS

Hyperglycemia means the alterations to a metabolism of fat, protein, and carbohydrate are the markers of this metabolic disorder. Diabetes is a worldwide epidemic. With changing lifestyles and increasing obesity, the prevalence of DM has increased worldwide. The global prevalence of DM was 425 million in 2017. ^[2] Of these, 7 million were undiagnosed. With an increase in age, the prevalence of DM also increases.

Type 1 diabetes mellitus (T1DM) accounts for 5% to 10% of DM and is characterized by autoimmune destruction of insulin-producing beta cells in the islets of the pancreas. As a result, there is an absolute deficiency of insulin.

Type 2 diabetes mellitus (T2DM) accounts for around 90% of all cases of diabetes. In T2DM, the response to insulin is diminished, and this is defined as insulin resistance. During this state, insulin is ineffective and is initially countered by an increase in insulin production to maintain glucose homeostasis, but over time, insulin production decreases, resulting in T2DM. ^[3]

Pregnancy is linked to gestational diabetes because of a transient intolerance to glucose uptake. After birth, aberrant glucose metabolism typically recovers to normal. ^[4]

Latent autoimmune diabetes with adult onset (LADA)- Sometimes called type 1.5 DM. These people are frequently misdiagnosed as having type 2 diabetes even if they are not initially insulin dependent.

Diabetes of youth with maturity onset (MODY).-

These can be categorized into several subgroups and exhibit different genetic expressions: The MODY 1, 2, 3, 4, and 5. (with 3 being most prevalent: 70 percent incidence with HNF-1-alpha [12q24] genetic expression) and MODYs 7 and 8 are rare.

Diabetes that is prone to ketosis- Beta cell function that recurs and deteriorates gradually over time. It starts off needing insulin for ketoacidosis, but as the beta cells start working again, the patient can stop taking insulin. Patients who are obese or overweight, under 40 years old, and of African or Afro-Caribbean descent seem to have this variant more frequently.

Secondary diabetes-

- a. Pancreatic disease or resection in secondary diabetes (e.g., cystic fibrosis)
- b. Cushing syndrome or chronic excessive corticosteroid exposure
- c. Glucagonoma
- d. Acromegaly
- e. Alternative uncommon genetic diseases - e.g. mitochondrial diabetes MELAS syndrome. ^[5]

Atypical autoimmune (e.g. type A and B insulin resistance syndrome)-

Diabetes patients are unable to efficiently metabolise glucose and cannot synthesise fatty acids and triglycerides from carbohydrates or amino acids due to insulin secretion or action failure. Because the cells cannot Detect and absorb glucose in the blood, glycolytic, lipogenic, and pentose phosphate enzymes Phosphate pathways are inhibited, while gluconeogenic, glycogenolytic, and lipolytic

pathways are activated. In nondiabetics, activities are increased, reversing the metabolic pathway. There is no cure for diabetes mellitus, and treatment goals include restoring normal metabolic pathways and reversing dyslipidemia. Current treatments include oral glycemic agents, daily insulin injections, diet modification, and physical activity. Amylin is a potent modulator of glycogen synthesis in diabetics patient and glucose uptake in skeletal muscle. It is likely that amylin secretion will disappear along with insulin secretion in type 1 (insulin-dependent) diabetes mellitus (IDDM). Because patients with insulin-treated IDDM frequently experience hypoglycemia, and because amylin acts to modulate insulin action in various tissues, amylin deficiency may contribute to morbidity in insulin-treated IDDM, possibly through the loss of a natural damping mechanism that protects against hypoglycemia.

AMYLIN

1. BASICS

The pancreatic beta cells of the islets of Langerhans frequently secrete amylin, a significant 37-amino-acid peptide component of islet amyloid, and also with insulin. Other vital organs, including the stomach, spinal ganglia, and the brain, produce amylin in much smaller levels. Amylin's relationship with insulin and other glucoregulatory elements that regulate the metabolism of carbohydrates. Amylin is mainly produced as proinsuline-like material by proteolytic processing. Amylin is produced in response to glucose induced postabsorptive stimulation of pancreatic beta cells. Soluble form of amylin analogue, an amylin analogue's used as supplement to insulin in the treatment of type 1 diabetes. [6] Its helps to reduce postprandial hyperglucemia. Action of amylin is synergistic to insulin.

2. AMYLIN ANALOGUES

Human amylin was first isolated, purified, and identified as a major component of amyloid deposits in pancreatic islets from type 2 diabetes patients

STRUCTURE

Structure of Amylin are as follows:



Fig. no. 1 Structure of Amylin

Amylin is a 37 - amino acid peptide with the general formula:



Wherein, H- at the N- terminus denotes a hydrogen atom, corresponding to the presence of a free amino group on the N- terminal amino acid residue [i.e., the lysine (K) residue at position 1 in the preceding sequence]; and -NH, at the C - terminus denotes that the Fig. no. 1 terminal carboxyl group is in the amide form. [7]

Parentheses indicate the presence of an intramolecular disulfide bridge between the two cysteine (C, Cys) residues at sequence positions 2 and 7().

Directly revealed by the amino acid sequencing of material extracted and purified from human amylin, the human pancreatic amyloid and it's the disulfide bond and the COOH-terminal amidation are two examples of post-translational changes. Amylin's relationship to the calcitonine and is almost 50% the same as CGRP-I. CGRP-II and Amylin's molecular cloning there appears to be only one gene copy per human, as established by the main sequence of the gene. [8] Proamylin, which is found in humans on chromosome 12, has an amidation signal and the usual dibasic sites for processing hormones i.e., a glycine that's also located next to the tyrosine with the COOH end. Strong protection specifically, the gap between it seems that amino acids 20 and 29 create a beta pleated sheet that causes self-aggregation and insoluble plaque. Due to difficulty in work, modified amylin agonist used in clinical trials. [9]

Amylin may be useful in treating of metabolic disorders such as diabetes and/or obesity. Amylin is known to promote gastric emptying, as well as suppress glucagon secretion and food intake, thereby controlling the rate of glucose release into the circulation. [10]

Amylin's role was determined, which led to the development of pramlintide 1, an amylin mimic that differs from amylin in that it contains the amino acids 25, 28, and 29, were replaced out for prolines. By using this replacement method, the problematic physiochemical property of forming human amylin is unsuitable for use as a drug due to cytotoxic amyloid fibrils. Post-meal glucose levels were reduced with pramlintide 1, in both type I and type II DM show that you may control nutrient intake by causing satiety. It is used as an adjunctive for type I DM. Treatment in addition to mealtime insulin alone. Type II late stage DM, it is used in conjunction with mealtime insulin as a therapy. In those who have not been able to regulate their sugar levels despite optimal.

3. PRAMLINTIDE (SYMLIN®)

SYNTHESIS OF PRAMLINTIDE1

The molecular formula for pramlintide acetate is C171H267N51O53S2. [11] Approved by the FDA in 2005, pramlintide acetate, an amylin analogs, also lowers PPG in type 1 and type 2 DM. Several synthetic approach are used for synthesis of pramlintide via microwave irradiation. The peptide coupling or Fmoc (9fluorenylmethoxycarbonyl) removal generally results in more efficient peptide synthesis including human amylin. Amiomethyl polystyrene resin, functionalised with a Rink amide linker (4-[(R, S)-α-[1-(9H-fluoren-9-yl)]-methoxycarbonylamino]-2,4 dimethoxy]phenoxyacetic acid), was used to prepare the linear peptide 20 in combination with a CEM Liberty 12 automated peptide synthesiser. O-(6-Chlorobenzotriazol-1-yl)-N, N, N', - N'-tetramethyluronium hexafluorophosphate (HCTU) and N,N-diisopropylethylamine (iPr2EtN) were employed as the coupling reagents, and 20% piperidine in N,N-dimethylform amide (DMF) was used to remove the Nα-Fmoc protecting groups.

Finally, cleavage from the resin and global removal of side-chain protecting groups was carried out using trifluoroacetic acid/triisopropylsilane/water/ 3, 6-dioxa-1, 8-octane-dithiol at room temperature, which afforded linear pramlintide 20 with a crude

purity of 49%. Disulfide bond formation was performed directly on crude 20 using a 0.1 M tris(hydroxymethyl)aminomethane (Tris.HCl) buffer (pH 8.4) in water at various concentrations which afforded the desired product 1 after 24 h. But the poor solubility of starting material, yield of peptide will be low. For that subsequently attempted using a mixture of 0.1 M Tris.HCl buffer (pH 8.4) in a mixture of dimethyl sulphoxide (DMSO) which helps to improve solubility and water (1 : 3), at a concentration of 5 mg mL⁻¹ cyclization of crude linear pramlintide 20 was complete in 20 min when treated with DPDS in DMSO at a high concentration. RP-HPLC used to check purification of pramlintide 1 and which is in 99% purity. ^[12]

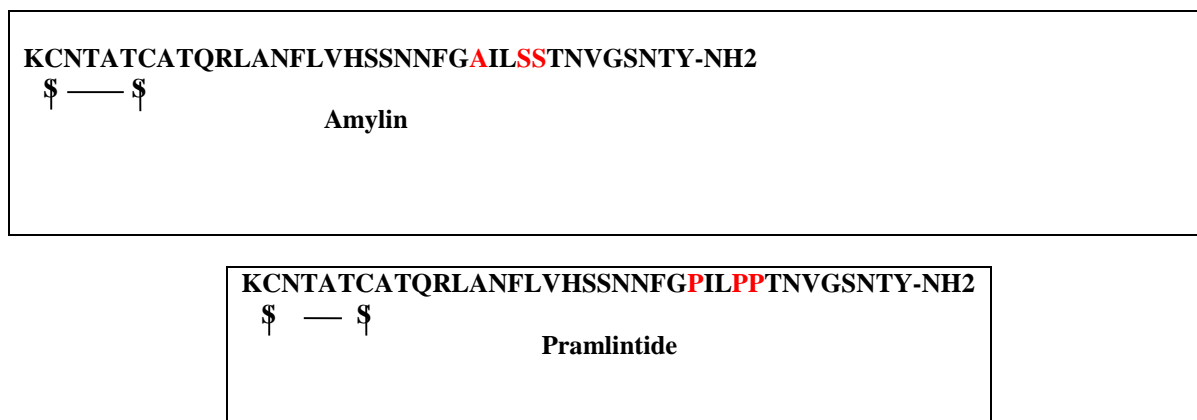


Fig. No.3. Primary sequence of human amylin and amylin analogue pramlintide 1. ^[13]

Pramlintide's benefits include decreasing postprandial levels of glucose and A1C, possible decrease in potential weight loss and the total daily insulin dose. Hyperglycemia following a meal has allegedly been linked to an increase in atherosclerotic events unrelated to its impact on A1C. ^[14]

The major drawbacks of pramlintide treatment include nausea, hypoglycemia (when combined with insulin), and its Subcutaneous administration method. The preliminary clinical studies used insulin with pramlintide fixed dosage Symlin has been linked with an increased risk of severe hypoglycemia when administered with premeal insulin. ^[15]

CARE SHOULD BE TAKEN

- 1) To reduce the risk, the premeal insulin dose should be decreased by 50% when Symlin is started, and then adjusted as necessary going forward low blood sugar levels.
- 2) Patients should always have glucagon and/or hypoglycemic medications on hand.
- 3) Patients are also advised to always carry a medical alert bracelet.
- 4) Any meal that contains more than 250 kcal or 30 g of carbohydrates is followed by an injection of Symlin into the thigh or belly before the first bite is taken.
- 5) Symlin must be injected at least 2 inches away from the site of the premeal insulin injection. With each injection, the sites ought to be switched.
- 6) It is crucial to closely monitor blood glucose levels, before driving a motor vehicle or heavy machinery, blood sugar levels should be monitored before and/or after each meal, and before going to sleep.
- 7) The most frequent negative side effect of using Symlin is nausea, usually this gets better within a month.
- 8) Tolerability can be enhanced by beginning therapy with a low dose, switching to once daily dosing during the largest meal, and slowly titrating the dose.
- 9) Prior to adding a second or third daily dose of Symlin, doses should be raised to the targeted dose. Continue until the three target daily doses are attained.
- 10) Once Symlin is taken at the right dose and tolerated, insulin dosages should be changed to attain the ideal blood glucose levels, while the dosage of Symlin remains unchanged.
- 11) Symlin pens it hasn't been used should be kept cold. Before injecting, let the drug reach room temperature. Once Symlin pens are stable for thirty days when left open (and at room temperature). The medication need to be transparent. It should not be employed if it is overcast, tinted, or has any precipitation. Keep Symlin from freezing.
- 12) Patients with a history of frequent, severe hypoglycemia should not use Symlin, nor should those with asymptomatic hypoglycemia.
- 13) Patients with gastroparesis, women who are pregnant or who are trying to get pregnant and nursing mothers shouldn't use Symlin. ^[16]

4. CAGRILINTIDE:

Cagrilintide is a non-selective agonist designed for subcutaneous injection once per week and formulation at low PH. In the vast scheme of things, cagrilintide provides interesting clinical results regarding body weight and is meant to help with the treatment of obesity almost certainly in conjunction with semaglutide. A specific emphasis was placed on the use of semaglutide and cagrilintide together as the Therapy to use both amylin and GLP-1 HAS BEEN PROPOSED. UTILISE PARTIALLY COMPLEMENTARY SYSTEMS TO FUNCTION. ^[17]

SYNTHESIS OF CAGRILINTIDE

Utilizing the manufacturer's instructions for Fmoc peptide synthesis using common protected amino acids, peptides were created on the commercial peptide synthesizers Prelude and Liberty acids. OxymaPure/DIC was the coupling agent, and the resin was Amide resin rink. 25% piperidine in N-methyl pyrrolidone (NMP) coupling was done after the Fmoc group was removed using by adding DMF and 0.3 M Fmoc-amino acid to 0.3 M OxymaPure DIC (3 M) in excess of 68 equivalents and activated by 68

equivalents 34 equivalents of collidine (3 M solution in DMF) and DMF). 30–60 minutes were required for coupling at room temperature prelude. Cysteine was trityl-protected, and resin was treated with an iodine solution to create the disulfide bridge (further details are in the Supporting Information). Trifluoroacetyl (TFA) %, 2.5 percent water, and 2.5 percent triisopropylsilane were used for the cleavage, which lasted for up to three hours. After that, diethyl ether precipitation and preparative HPLC on C18 reverse phase columns using % TFA in water and % TFA in acetonitrile as eluents were done, and then the product was lyophilized. The peptides' purity was greater than 95% (Supporting Information). Prior to in vitro analysis utilising a continuously running insulin-based standard, the peptide content of the lyophilized product was evaluated by an ultra-performance liquid chromatography (UPLC) system outfitted with a chemiluminescent nitrogen-specific HPLC (Antek 8060 CLND) nitrogen detector. The supporting information contains further details concerning the synthesis, purification, purity, representative liquid chromatography, mass spectrometry, and UPLC data.^[18]

KCNTATCATQ RLAEFLRHSS NNFGPILPPT NVGSNTP-amide

Str. Cagrilintide

5. PEGYLATED OR GLYCOSYLATED AMYLIN

To improve the effectiveness of amylin mimetics by modify the peptide chain, coupling it to molecular scaffolds such as polyethylene glycol/by glycosylation. As compared to native amylin PEGylated amylin has a prolonged glucose-lowering effect. Further, glycosylation of the amylin analog pramlintide also provides increased half-life compared to the native peptide.^[19]

6. DAVALINTIDE

Davalintide is an potent amylinomimetics, efficacious and long duration of action than amylin. It has greatly enhanced duration of action and is more potent than amylin and has role in reducing food intake and body weight.^[20] Amylin's relationship with insulin and other glucoregulatory elements that regulate the metabolism of carbohydrates. Amylin is mainly produced as proinsuline-like material by proteolytic processing. Amylin is produces in response to glucose induced postabsorptive stimulation of pancreatic beta cells. Soluble form of amylin analogue, an amylin analogues is used as supplement to insulin in the treatment of type 1 diabetes.^[22] It helps to reduce postprandial hyperglucemia. Action of amylin is synergistic to insulin. The role of CGRP neurons to control meal ending satiation.^[23]

CONCLUSION-

In this review, we concluded that amylin play an important role in complication of diabetes mellitus like Obesity. Amylin and its analogues is known to promote gastric emptying, as well as suppress glucagon secretion and food intake, thereby controlling the rate of glucose release into the circulation, it act by reducing appetite. Soluble form of amylin analogue, like Pramlintide used as supplement to insulin in the treatment of type 1 diabetes with improved physicochemical properties.

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