

increase the half-life of the triple agonists by substituting an amino acid sequence to increase the resistance to dipeptidyl peptidase-IV (DPP-IV), which decomposes gastrointestinal hormones to get rid of their activities, followed by adding an acyl group to a particular region thereof (Finan B *et al.*, *Nat Med.*, 2015 Jan; 21 (1): 27 - 36). However, their effects of activating three different kinds of receptors were not significant and no triple agonist showed various active ratios thereto.

Accordingly, there is a need for the development of a novel material which can highly activate GLP-1, GIP, and glucagon receptors and has the effects of controlling blood glucose levels and reducing body weight without causing any side-effects such as vomiting and nausea.

Additionally, there is also a need for the development of a novel material which has various active ratios to GLP-1, GIP, and glucagon receptors. For example, there is an increasing need for the development of a material which has an effect of reducing body weight but has a significantly higher effect of controlling blood glucose levels due to high GLP-1 and GIP activities but with relatively low glucagon activity for a hypoglycemic effect; or a material which has high activities for all of GLP-1, GIP, and glucagon, thus having a significantly high effect of reducing body weight.

Additionally, GLP-1, GIP, and glucagon have low *in vivo* stability and thus they have a disadvantage in that they must be administered daily or twice daily when they are applied to humans for therapeutic uses.

[Summary]

[Technical Problem]

An object of the present invention is to provide a conjugate of a peptide having activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor.

Another object of the present invention is to provide a polynucleotide encoding the conjugate, a vector including the polynucleotide, and a transformant including the polynucleotide or the vector.

Still another object of the present invention is to provide a composition containing the conjugate.

Still another object of the present invention is to provide a method for treating a target disease, which includes administering the conjugate or a composition containing the conjugate to a subject in need thereof.

Still another object of the present invention is to provide a use of the conjugate or composition thereof for use in the preparation of a medicament.

[Technical Solution]

To achieve the above objects, in an aspect, the present invention provides a conjugate of a peptide having activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor.

In one aspect, the present invention refers to a conjugate represented by Chemical Formula 1 below:

[Chemical Formula 1]

X - La - F

wherein,

X is a peptide having activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor;

L is a linker;

a is 0 or a positive integer, with the proviso that when a is 2 or greater, each L is independent from each other; and

F is a material capable of increasing the half-life of X.

In another aspect, the present invention refers to a pharmaceutical composition for preventing or treating metabolic syndrome comprising a conjugate as disclosed herein.

In a specific example, X is an analog of native glucagon with a variation selected from the group consisting of substitution, addition, deletion, modification, and a combination thereof, on at least one amino acid of the native glucagon sequence.

In still another specific example, the amino acid sequence with addition is derived from a native GLP-1 amino acid sequence, a native GIP amino acid sequence, or a native exendin-4 amino acid sequence.

In still another specific example, the X is a peptide including an amino acid sequence represented by General Formula 1 below:

Xaa29 is cysteine (Cys, C), glycine (Gly, G), glutamine (Gln, Q), threonine (Thr, T), glutamic acid (Glu, E), or histidine (His, H);

Xaa30 is cysteine (Cys, C), glycine (Gly, G), lysine (Lys, K), or histidine (His, H), or is absent; and

R1 is cysteine (Cys, C), GKKNDWKHNIT (SEQ ID NO: 106), m-SSGAPPPS-n (SEQ ID NO: 107), or m-SSGQPPPS-n (SEQ ID NO: 108), or is absent;

wherein,

m is -Cys-, -Pro-, or -Gly-Pro-,

n is -Cys-, -Gly-, -Ser-, or -His-Gly-, or is absent.

In still another specific example,

in General Formula 1,

Xaa14 is leucine or methionine; and

Xaa15 is cysteine, aspartic acid, or leucine.

In still another specific example, in General Formula 1,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;

Xaa7 is threonine;

Xaa10 is tyrosine, cysteine, or valine;

Xaa12 is lysine or isoleucine;

Xaa13 is tyrosine, alanine, glutamine, or cysteine;

Xaa14 is leucine, cysteine, or methionine;

Xaa15 is cysteine, leucine, glutamic acid, or aspartic acid;

Xaa17 is glutamine, arginine, isoleucine, cysteine, glutamic acid, or lysine;

Xaa18 is alanine, glutamine, arginine, or histidine;

Xaa19 is alanine, glutamine, valine, or cysteine;

Xaa20 is lysine, arginine, or glutamine;

Xaa21 is glutamic acid, glutamine, leucine, cysteine, or aspartic acid;

Xaa23 is isoleucine or valine;

Xaa24 is cysteine, alanine, glutamine, asparagine, glutamic acid, or aspartic acid; and

Xaa27 is leucine or lysine.

In still another specific example, in General Formula 1,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;

Xaa7 is threonine;

Xaa10 is tyrosine, cysteine, or valine;

Xaa12 is lysine or isoleucine;

Xaa13 is tyrosine, alanine, or cysteine;

Xaa14 is leucine or methionine;

Xaa15 is cysteine or aspartic acid;

Xaa17 is glutamine, arginine, isoleucine, cysteine, or lysine;

Xaa18 is alanine, arginine, or histidine;

Xaa19 is alanine, glutamine, or cysteine;

Xaa20 is lysine or glutamine;

Xaa21 is glutamic acid, cysteine, or aspartic acid;

Xaa23 is valine;

Xaa24 is alanine, glutamine, cysteine, asparagine, or aspartic acid; and

Xaa27 is leucine or lysine.

In still another specific example, in General Formula 1,

Xaa2 is α -methyl-glutamic acid or Aib;

Xaa7 is threonine;

Xaa10 is tyrosine or cysteine;

Xaa12 is lysine or isoleucine;

Xaa13 is tyrosine, alanine, or cysteine;

Xaa14 is leucine or methionine;

Xaa15 is cysteine or aspartic acid;

Xaa16 is glutamic acid;

Xaa17 is arginine, isoleucine, cysteine, or lysine;

Xaa18 is alanine, arginine, or histidine;

Xaa19 is alanine, glutamine, or cysteine;

Xaa20 is lysine or glutamine;

Xaa21 is glutamic acid or aspartic acid;

Xaa23 is valine;

Xaa24 is glutamine, asparagine, or aspartic acid;
Xaa27 is leucine; and
Xaa28 is cysteine, alanine, asparagine, or aspartic acid.

In still another specific example, in General Formula 1,

Xaa1 is histidine or 4-imidazoacetyl;
Xaa2 is α -methyl-glutamic acid or Aib;
Xaa3 is glutamine;
Xaa7 is threonine;
Xaa10 is tyrosine;
Xaa12 is isoleucine;
Xaa13 is alanine or cysteine;
Xaa14 is methionine;
Xaa15 is aspartic acid;
Xaa16 is glutamic acid;
Xaa17 is isoleucine or lysine;
Xaa18 is alanine or histidine;
Xaa19 is glutamine or cysteine;
Xaa20 is lysine;
Xaa21 is aspartic acid;
Xaa23 is valine;
Xaa24 is asparagine;
Xaa27 is leucine;
Xaa28 is alanine or asparagine;
Xaa29 is glutamine or threonine; and
Xaa30 is cysteine, or lysine, or is absent.

In still another specific example,

in General Formula 1,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;
Xaa3 is glutamine;
Xaa7 is threonine;

Xaa10 is tyrosine, cysteine, or valine;
 Xaa12 is lysine;
 Xaa13 is tyrosine;
 Xaa14 is leucine;
 Xaa15 is aspartic acid;
 Xaa16 is glycine, glutamic acid, or serine;
 Xaa17 is glutamine, arginine, cysteine, or lysine;
 Xaa18 is alanine, arginine, or histidine;
 Xaa19 is alanine or glutamine;
 Xaa20 is lysine or glutamine;
 Xaa21 is glutamic acid, cysteine, or aspartic acid;
 Xaa23 is valine;
 Xaa24 is alanine, glutamine, or cysteine;
 Xaa27 is leucine or lysine; and
 Xaa29 is glycine, glutamine, threonine, or histidine.

In still another specific example, X is a peptide including an amino acid sequence represented by General Formula 2 below:

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Xaa10-Ser-Lys-Xaa13-Xaa14-Xaa15-
 Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-
 Xaa30-Xaa31- Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 2, SEQ ID NO:
 104)

In General Formula 2,

Xaa1 is 4-imidazoacetyl, histidine, or tyrosine;
 Xaa2 is glycine, α -methyl-glutamic acid, or Aib;
 Xaa10 is tyrosine or cysteine;
 Xaa13 is alanine, glutamine, tyrosine, or cysteine;
 Xaa14 is leucine, methionine, or tyrosine;
 Xaa15 is aspartic acid, glutamic acid, or leucine;
 Xaa16 is glycine, glutamic acid, or serine;

Xaa17 is glutamine, arginine, isoleucine, glutamic acid, cysteine, or lysine;
Xaa18 is alanine, glutamine, arginine, or histidine;
Xaa19 is alanine, glutamine, cysteine, or valine;
Xaa20 is lysine, glutamine, or arginine;
Xaa21 is cysteine, glutamic acid, glutamine, leucine, or aspartic acid;
Xaa23 is isoleucine or valine;
Xaa24 is cysteine, alanine, glutamine, asparagine, or glutamic acid;
Xaa28 is lysine, cysteine, asparagine, or aspartic acid;
Xaa29 is glycine, glutamine, cysteine, or histidine;
Xaa30 is cysteine, glycine, lysine, or histidine;
Xaa31 is proline or cysteine; and
Xaa40 is cysteine or is absent.

In still another specific example, in General Formula 2,

Xaa13 is alanine, tyrosine, or cysteine;
Xaa15 is aspartic acid or glutamic acid;
Xaa17 is glutamine, arginine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa21 is cysteine, glutamic acid, glutamine, or aspartic acid;
Xaa23 is isoleucine or valine;
Xaa24 is cysteine, glutamine, or asparagine;
Xaa28 is cysteine, asparagine, or aspartic acid;
Xaa29 is glutamine, cysteine, or histidine; and
Xaa30 is cysteine, lysine, or histidine.

In still another specific example, X is a peptide including an amino acid sequence represented by General Formula 3 below:

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Xaa13-Leu-Asp-Glu-Xaa17-Xaa18-Xaa19-Lys-Xaa21-Phe-Val-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-Xaa30-Xaa31-Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 3, SEQ ID NO: 105).

In General Formula 3,

Xaa1 is histidine or tyrosine;

Xaa2 is α -methyl-glutamic acid or Aib;

Xaa13 is alanine, tyrosine, or cysteine;

Xaa17 is arginine, cysteine, or lysine;

Xaa18 is alanine or arginine;

Xaa19 is alanine or cysteine;

Xaa21 is glutamic acid or aspartic acid;

Xaa24 is glutamine or asparagine;

Xaa28 is cysteine or aspartic acid;

Xaa29 is cysteine, histidine, or glutamine;

Xaa30 is cysteine or histidine;

Xaa31 is proline or cysteine; and

Xaa40 is cysteine or is absent.

In still another specific example, R1 is cysteine, GKKNDWKHNIT (SEQ ID NO: 106), CSSGQPPPS (SEQ ID NO: 109), GPSSGAPPPS (SEQ ID NO: 110), GPSSGAPPPSC (SEQ ID NO: 111), PSSGAPPPS (SEQ ID NO: 112), PSSGAPPPSG (SEQ ID NO: 113), PSSGAPPPSHG (SEQ ID NO: 114), PSSGAPPPSS (SEQ ID NO: 115), PSSGQPPPS (SEQ ID NO: 116), or PSSGQPPPS (SEQ ID NO: 117), or is absent.

In still another specific example, in General Formulas 1 to 3, the 16th amino acid and the 20th amino acid from the N-terminus together form a ring.

In still another specific example, X is a peptide including an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 102.

In still another specific example, F is selected from the group consisting of a polymer, fatty acid, cholesterol, albumin and a fragment thereof, an albumin-binding material, a polymer of repeating units of particular amino acid sequences, an antibody, an antibody fragment, an FcRn-binding material, an *in vivo* connective tissue, a nucleotide, fibronectin, transferrin, a saccharide, heparin, and elastin.

In still another specific example, F is a polymer selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer, a lipid polymer, chitins, hyaluronic acid, an oligonucleotide, and a combination thereof.

In still another specific example, F is an immunoglobulin Fc region.

In still another specific example, F is an IgG Fc region.

In still another specific example, L is selected from the group consisting of a peptide, fatty acid, a saccharide, a polymer, a low molecular weight compound, a nucleotide, and a combination thereof.

In still another specific example, L is a polymer selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer, a lipid polymer, chitins, hyaluronic acid, an oligonucleotide, and a combination thereof.

In still another specific example, L is polyethylene glycol.

Still another example of the present disclosure provides a polynucleotide encoding the conjugate, a vector including the polynucleotide, and a transformant including the polynucleotide or vector.

Still another example of the present disclosure provides a composition containing the conjugate.

In a specific example, the composition is a pharmaceutical composition.

In another specific example, the composition is for preventing or treating metabolic syndrome.

In still another specific example, the metabolic syndrome may include impaired glucose tolerance, hypercholesterolemia, dyslipidemia, obesity, diabetes, hypertension, arteriosclerosis due to dyslipidemia, atherosclerosis, arteriosclerosis, or coronary heart disease.

Still another example of the present disclosure provides a method for treating a target disease, which includes administering the conjugate or a composition containing the conjugate to a subject in need thereof.

In a specific example, the disease is metabolic syndrome.

Still another example of the present disclosure provides a use of the conjugate or composition containing the conjugate in the preparation of a medicament.

In a specific example, the medicament is for preventing or treating metabolic syndrome.

[Advantageous Effects of the Invention]

The conjugate of the triple agonist according to the present invention has activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor, and thus can be applied for the treatment of metabolic syndrome.

[Brief Descriptions of the Drawings]

FIG. 1 shows a histogram illustrating the measurement result of the body weight change in an obese animal model (mice) at two-day intervals, during the administration of a long-acting conjugate of a triple agonist via a high-fat diet to the mice once every two days for 28 days ($p < 0.05$, $**p < 0.01$, $***p < 0.001$, vs. vehicle by 1-way ANOVA).

FIG. 2 shows a histogram illustrating the measurement result of the amount of mesenteric fat in an obese animal model (mice) at two-day intervals, during the administration of a long-acting conjugate of a triple agonist via a high-fat diet to the mice once every two days for 28 days ($p < 0.05$, $**p < 0.01$, $***p < 0.001$, vs. vehicle by 1-way ANOVA).