

DESCRIPTION

TITLE OF INVENTION: COMPOSITION FOR SUPPRESSING OR
AMELIORATING DETERIORATION OF COGNITIVE FUNCTION, AND
5 COMPOSITION FOR INHIBITING β -SECRETASE

TECHNICAL FIELD

[0001]

The present invention relates to, for example, a composition for reducing a
10 decline in cognitive function or improving cognitive function, and a composition for
inhibiting β -secretase.

BACKGROUND ART

[0002]

15 Amyloid β ($A\beta$) is a protein produced in the brain. $A\beta$ that has aggregated
into oligomers or fibers exhibits neurotoxicity. Such aggregated $A\beta$ is not discharged
from the brain but accumulates therein, which is considered to trigger the onset of
Alzheimer's disease.

$A\beta$ is generated through the two-stage cleavage of an amyloid precursor protein
20 (APP) by β -secretase (BACE1) and γ -secretase. A report shows an increase in the
activity and expression of BACE1 in the brain of patients with mild cognitive (function)
impairment (MCI) and Alzheimer's disease (Non-Patent Literature 1). Another report
shows that inhibition of BACE1 reduces accumulation of $A\beta$ at an early stage of $A\beta$
accumulation (Non-Patent Literature 2).

25 Thus, inhibiting BACE1 and reducing production or accumulation of $A\beta$ is
considered to be effective in reducing a decline in cognitive function and preventing
Alzheimer's disease, for example.

[0003]

Patent Literature 1 discloses an agent for reducing a decline in cognitive
30 function and/or improving cognitive function of a subject, the agent containing
Cyclo(Gly-Pro).

Patent Literature 2 discloses a composition for preventing neurological
diseases, the composition containing a heat-treated product of an animal- or plant-
derived peptide as an active ingredient.

35 Patent Literature 3 discloses a composition for preventing neurological

diseases, the composition containing, as an active ingredient, a cyclic dipeptide or a salt thereof having an amino acid as a structural unit, with cyclic dipeptides such as cyclolysyllsine [Cyclo(Lys-Lys)] being mentioned as specific examples.

All of these documents are silent about reduction in accumulation of A β and
 5 BACE1 inhibitory activity.

CITATION LIST

- Patent Literature

[0004]

10 Patent Literature 1: JP 2020-196686 A

Patent Literature 2: WO 2017/119476

Patent Literature 3: WO 2017/119481

- Non-Patent Literature

15 [0005]

Non-Patent Literature 1: Cheng et al., Am. J. Pathol. 2014; 184(1):141-147.

Non-Patent Literature 2: Peters et al., Acta Neuropathol. 2018; 135(5):695-710.

SUMMARY OF INVENTION

20 - Technical Problem

[0006]

The present invention aims to provide a novel composition for reducing a decline in cognitive function or improving cognitive function, and a novel composition for inhibiting β -secretase.

25

- Solution to Problem

[0007]

The present inventors performed extensive studies on the above issue to find that Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-
 30 Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys), which are cyclic dipeptides, or salts thereof inhibit β -secretase. The present inventors then found that these cyclic dipeptides or salts thereof reduce production and/or accumulation of amyloid β by inhibiting β -secretase.

[0008]

35 The present inventors also found that Cyclo(Arg-Phe), Cyclo(Arg-Tyr),

Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn), which are cyclic dipeptides, or salts thereof reduce production and/or accumulation of amyloid β by inhibiting β -secretase and are effective in reducing a decline in cognitive function or improving cognitive function, thereby completing the present invention.

5 [0009]

Specifically, although not limited thereto, the present invention relates to, for example, the following composition for reducing a decline in cognitive function or improving cognitive function.

10 (1) A composition for reducing a decline in cognitive function or improving cognitive function, the composition containing: at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) or a salt thereof.

15 (2) The composition according to the clause (1), further containing at least one cyclic dipeptide selected from the group consisting of Cyclo(Hyp-Ala) and Cyclo(Leu-Lys) or a salt thereof.

(3) The composition according to the clause (1) or (2), wherein the composition reduces production and/or accumulation of amyloid β .

20 (4) The composition according to any one of the clauses (1) to (3), wherein the composition reduces production and/or accumulation of amyloid β by inhibiting β -secretase.

(5) A composition for inhibiting β -secretase, containing: at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) or a salt thereof.

25 (6) The composition according to the clause (5), wherein the composition reduces production and/or accumulation of amyloid β by inhibiting β -secretase.

(7) The composition according to any one of the clauses (1) to (6), wherein the composition is an oral composition.

30 (8) The composition according to any one of the clauses (1) to (7), wherein the composition is a food or beverage, or an oral pharmaceutical product.

35 (9) The composition according to any one of the clauses (1) to (8), wherein the composition is provided with one or two or more function claims selected from the group consisting of "enhancing cognitive function", "reducing a decline in cognitive function", "maintaining good cognitive function", "improving memory", "reducing a decline in memory", "maintaining good memory", "improving accuracy of memory",

"preventing memory disorder", "ameliorating memory disorder", "maintaining memory, which is a domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement, which are domains of cognitive function", "improving
5 memory retention or integration", "maintaining enhancement of cognitive function", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining or improving orientation", "delaying an aging-related decline in cognitive function", "enhancing short-term and long-term memory", "promoting verbal and visuospatial memory", and "maintaining or improving logical
10 reasoning".

- Advantageous Effects of Invention

[0010]

The present invention can provide a novel composition for reducing a decline
15 in cognitive function or improving cognitive function, and a novel composition for inhibiting β -secretase.

DESCRIPTION OF EMBODIMENTS

[0011]

20 The composition for reducing a decline in cognitive function or improving cognitive function of the present invention (also referred to as the "composition of the first aspect of the present invention") contains at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) or a salt thereof.

25 The composition of the first aspect of the present invention commonly contains the cyclic dipeptide or a salt thereof as an active ingredient. The composition of the first aspect of the present invention preferably further contains at least one cyclic dipeptide selected from the group consisting of Cyclo(Hyp-Ala) and Cyclo(Leu-Lys) or a salt thereof.

30 [0012]

The composition for inhibiting β -secretase of the present invention (also referred to as the "composition of the second aspect of the present invention") contains at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn),
35 Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) or a salt thereof.

The composition of the second aspect of the present invention commonly contains the cyclic dipeptide or a salt thereof as an active ingredient.

[0013]

The Cyclo(Arg-Phe) (cycloarginylphenylalanine), Cyclo(Arg-Tyr) (cycloarginyltyrosine), Cyclo(Glu-Leu) (cycloglutamateleucine), Cyclo(His-Phe) (cyclohistidylphenylalanine), Cyclo(Phe-Trp) (cyclophenylalanyltryptophan), Cyclo(Trp-Asn) (cyclotryptophanylasparagine), Cyclo(Hyp-Ala) (cyclohydroxyprolinylalanine), and Cyclo(Leu-Lys) (cycloleucinylllysine) are all cyclic dipeptides. Herein, the “cyclic dipeptide” refers to a compound characteristically having amino acids as structural units and having a diketopiperazine structure formed by dehydration condensation of the amino group of the N-terminal amino acid and the carboxy group of the C-terminal amino acid. Herein, the amino acids may be described in any order as long as the cyclic dipeptide has the same amino acid structure; for example, Cyclo(Arg-Phe) and Cyclo(Phe-Arg) (cyclophenylalanyllarginine) represent the same cyclic dipeptide.

Each cyclic dipeptide may be in the form of a salt.

[0014]

The salt of a cyclic dipeptide may be any pharmacologically acceptable salt or any salt that is acceptable for foods and beverages, and may be either an acidic salt or a basic salt. Examples of the acidic salt include inorganic acidic salts such as hydrochloride, sulfate, nitrate, and phosphate; and organic acidic salts such as acetate, citrate, maleate, malate, oxalate, lactate, succinate, fumarate, and propionate. Examples of the basic salt include alkali metal salts such as sodium salt and potassium salt; and alkaline earth metal salts such as calcium salt and magnesium salt.

[0015]

Each of the cyclic dipeptides or a salt thereof may be derived from any origin and may be produced by any method. Each cyclic dipeptide can be produced by a known method. Each cyclic dipeptide may be derived from a natural origin or may be artificially synthesized, or may be produced by an enzymatic method or a microbial fermentation method, or may be synthesized by dehydrating and cyclizing linear dipeptides. For example, heating a protein hydrolysate such as a collagen hydrolysate can provide a product of a heat treated peptide, which is rich in any of the cyclic dipeptides such as Cyclo(Arg-Phe).

Each cyclic dipeptide may be added to a composition using a protein hydrolysate or a heat-treated product thereof containing the corresponding cyclic

dipeptide, or may be added to a composition using a concentrate, dry powder, or a highly purified product of a protein hydrolysate or a heat-treated product thereof containing the corresponding cyclic dipeptide. For example, the compositions of the first and second aspects of the present invention may each contain a protein hydrolysate or a heat-treated product thereof and any of the cyclic dipeptides may be a portion of the protein hydrolysate or the heat-treated product thereof. Each cyclic dipeptide may be a commercially available product. A salt of the cyclic dipeptide can be easily prepared by a person skilled in the art by any method known in the art.

[0016]

The compositions of the first and second aspects of the present invention preferably reduce production and/or accumulation of amyloid β .

$A\beta$ is a peptide also referred to as amyloid β protein, amyloid β peptide, or β -amyloid. $A\beta$ is a peptide commonly consisting of about 40 amino acids. Examples thereof include amyloid β_{1-38} , amyloid β_{1-40} , and amyloid β_{1-42} .

[0017]

Unassociated $A\beta$ is also referred to as an amyloid β monomer. Two or more $A\beta$ monomers may gather to form an aggregate. For example, two or more (e.g., 2 to 50) $A\beta$ monomers may gather to form a soluble aggregate (amyloid β oligomer). Examples of the amyloid β oligomer include an amyloid β_{1-38} oligomer, an amyloid β_{1-40} oligomer, and an amyloid β_{1-42} oligomer. The $A\beta$ in the present invention includes unassociated $A\beta$ and aggregate-forming (e.g., oligomer-forming) $A\beta$.

[0018]

The amyloid β is preferably at least one selected from the group consisting of amyloid β_{1-38} , amyloid β_{1-40} , and amyloid β_{1-42} .

[0019]

The compositions of the first and second aspects of the present invention can be used to reduce production and/or accumulation of amyloid β in the brain.

[0020]

In an embodiment, the compositions of the first and second aspects of the present invention can be used to prevent or ameliorate a condition or disease associated with production and/or accumulation of amyloid β , preferably a condition or disease associated with production and/or accumulation of amyloid β in the brain. Examples of this condition or disease include a condition or disease involving production and/or accumulation of amyloid β or resulting from production and/or accumulation of amyloid β . Examples of a condition or disease involving production and/or accumulation of

amyloid β or resulting from production and/or accumulation of amyloid β in the brain include Alzheimer's disease (including Alzheimer's type dementia), mild cognitive (function) impairment, and aging-related cognitive (function) impairment. The compositions of the first and second aspects of the present invention can be used to prevent or ameliorate these conditions or diseases.

Herein, preventing a condition or disease encompasses preventing the onset, delaying the onset, reducing the incidence, reducing the onset risk, and the like; and ameliorating a condition or disease encompasses helping a subject recover from a condition or disease, alleviating a symptom of a condition or disease, changing a symptom of a condition or disease for the better, delaying or preventing the progress of a condition or disease, and the like. The recovery includes partial recovery.

[0021]

Alzheimer's disease, mild cognitive (function) impairment, and aging-related cognitive (function) impairment are known to cause a decline in cognitive function.

Examples of symptoms of a decline in cognitive function caused by Alzheimer's disease, mild cognitive (function) impairment, and aging-related cognitive (function) impairment include a decline in memory, memory disorder (forgetfulness), aphasia (difficulty in naming objects), apraxia, agnosia (e.g., getting lost in familiar places), a decline in orientation (ability to correctly recognize one's situation, such as a place, time, and people's names), a decline in verbal and non-verbal learning abilities, a decline in auditory and visual processing, a decline in executive function, executive dysfunction (inability to formulate and carry out plans), a decline in concentration, a decline in attention, a decline in judgement, a decline in spatial awareness, a decline in cognitive flexibility, a decline in information processing speed, and a decline in logical reasoning. Reducing production and/or accumulation of amyloid β can presumably lead to an effect of reducing a decline in cognitive function or improving cognitive function, such as an effect of reducing or ameliorating any of the above symptoms. In an embodiment, the compositions of the first and second aspects of the present invention can be used to prevent or ameliorate a condition or disease associated with a decline in cognitive function.

[0022]

The composition of the first aspect of the present invention reduces a decline in cognitive function or improves cognitive function. The composition of the first aspect of the present invention preferably reduces a decline in cognitive function or improves cognitive function resulting from Alzheimer's disease, mild cognitive (function)

impairment, or aging-related cognitive (function) impairment.

Herein, reducing a decline in cognitive function or improving cognitive function encompasses, with regard to “cognitive function”, an action that reduces a further decline in (worsening of), i.e., maintains a symptom, an action that delays a decline in (worsening of) cognitive function, and an action that ameliorates cognitive function after a decline (worsening). Also, the “cognitive function” means brain functions such as memory function, learning ability, consolidation of memory, executive function, attention (attention function), and concentration. The cognitive (function) impairment means loss of part or all of the above cognitive functions.

10 [0023]

The β -secretase (BACE1) inhibited by the composition for inhibiting β -secretase of the second aspect of the present invention is an aspartyl protease that plays a major role in generating an amyloid beta ($A\beta$) peptide from an amyloid precursor protein (APP). Thus, inhibiting the action of β -secretase can reduce production and/or accumulation of amyloid β .

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The composition of the second aspect of the present invention can be used to inhibit β -secretase in the brain. The composition of the first aspect of the present invention can also be used to inhibit β -secretase in the brain.

[0024]

20

Preferably, the compositions of the first and second aspects of the present invention reduce production and/or accumulation of amyloid β by inhibiting β -secretase.

The compositions of the first and second aspects of the present invention can be used to reduce production and/or accumulation of amyloid β by inhibiting β -secretase in the brain.

25

Preferably, the compositions of the first and second aspects of the present invention reduce production and/or accumulation of amyloid β by inhibiting β -secretase to reduce a decline in cognitive function or improve cognitive function.

The compositions of the first and second aspects of the present invention can be used to reduce production and/or accumulation of amyloid β by inhibiting β -secretase in the brain to reduce a decline in cognitive function or improve cognitive function.

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[0025]

The compositions of the first and second aspects of the present invention are applicable for both therapeutic use (medical use) and non-therapeutic use (non-medical use). The term “non-therapeutic” is a concept that does not include medical activities,

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i.e., a concept that does not include surgery, therapy, or diagnosis of humans.

The compositions of the first and second aspects of the present invention can each be provided in the form of an agent, for example, but not limited thereto. The agent can be directly provided as a composition, or can be provided as a composition
5 containing the agent. In an embodiment, the composition of the first aspect of the present invention can also be referred to as an agent for reducing a decline in cognitive function or an agent for ameliorating a decline in cognitive function. The composition of the second aspect of the present invention can also be referred to as an agent for inhibiting β -secretase. The compositions of the first and second aspects of the present
10 invention can each also be referred to as an agent for reducing production of amyloid β or an agent for reducing accumulation of amyloid β .

[0026]

The compositions of the first and second aspects of the present invention may be either for oral administration or for parenteral administration. Preferably, the
15 compositions of the first and second aspects of the present invention are oral compositions. The compositions of the first and second aspects of the present invention can each be provided in the form of a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, with a food or beverage or a pharmaceutical product being preferred, and with a food or beverage or an oral
20 pharmaceutical product being more preferred. The compositions of the first and second aspects of the present invention can also be used in admixture with a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like. The compositions of the first and second aspects of the present invention may be in any form such as solid (e.g., powder, granules, or tablet), liquid, or paste, for example.

25 [0027]

The compositions of the first and second aspects of the present invention may each further contain a variety of additives accepted as those for food and beverages, pharmaceutical products, or the like, such as diluents, acidulants, antioxidants, stabilizers, preservatives, flavorings, emulsifiers, colorants, seasonings, pH adjusters,
30 and nutrient enhancers.

[0028]

For example, in the case where the compositions of the first and second aspects of the present invention are each provided as a food or beverage, the cyclic dipeptide or a salt thereof contained in the compositions of the first and second aspects of the present
35 invention may be blended with components usable in a food or beverage (e.g., food

material and optional food additives), so that a variety of foods and beverages can be provided. Non-limiting examples of the foods and beverages include general foods and beverages, health foods, foods with function claims, foods for specified health uses, dietary supplements, and foods for the sick. The health foods, the foods with function
5 claims, the foods for specified health uses, the dietary supplements, and the like can be used, for example, in various forms of preparations such as liquid, fine granules, tablet, granules, powder, capsule, chewable tablet, dry syrup, and liquid food.

[0029]

In the case where the compositions of the first and second aspects of the
10 present invention are each provided as a pharmaceutical product or a quasi-pharmaceutical product, the cyclic dipeptide or a salt thereof contained in the compositions of the first and second aspects of the present invention may be blended with pharmacologically acceptable carriers, optional additives, and the like, so that a variety of dosage forms of pharmaceutical or quasi-pharmaceutical products can be
15 provided. These carriers, additives, and the like are not limited as long as they are pharmacologically acceptable and can be used in pharmaceutical or quasi-pharmaceutical products. For example, one or two or more of excipients, binders, disintegrants, lubricants, antioxidants, colorants, and the like may be used. Examples of the administration (intake) form of pharmaceutical or quasi-pharmaceutical products
20 include oral administration and parenteral administration (e.g., transdermal, transmucosal, enteral, and injection).

In the case where the compositions of the first and second aspects of the present invention are each provided as a pharmaceutical product or a quasi-pharmaceutical product, the composition is preferably prepared in the form of a
25 pharmaceutical product for oral administration or a quasi-pharmaceutical product for oral administration. Examples of dosage forms for oral administration include liquid, tablet, powder, fine granules, granules, sugar-coated tablet, capsule, suspension, emulsion, and chewable tablet. Examples of dosage forms for parenteral administration include injection, intravenous agent, ointment, lotion, adhesive skin
30 patch, suppository, nasal agent, and transpulmonary agent (inhalant). The pharmaceutical product may be a pharmaceutical for non-human animals.

[0030]

In the case where the compositions of the first and second aspects of the present invention are provided as feed, the cyclic dipeptide or a salt thereof contained in
35 the compositions of the first and second aspects of the present invention may be simply

added to feed. The feed includes feed additives. Examples of the feed include livestock feed for animals such as cows, pigs, chickens, sheep, and horses; feed for small animals such as rabbits, rats, and mice; and pet food for animals such as dogs, cats, and birds.

5 [0031]

For example, in the case where the compositions of the first and second aspects of the present invention are each provided as a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, the compositions can be produced by any common method using the cyclic dipeptide or a salt thereof contained
10 in the compositions of the first and second aspects of the present invention.

[0032]

In an embodiment, the compositions of the first and second aspects of the present invention are each preferably a liquid composition, more preferably a beverage. The beverage may be a functional beverage, for example. The beverage may be in any
15 form, and may be a packed beverage. The package of the packed beverage is not limited and may be a package in any form and of any material. For example, any of the following commonly used packages can be used: metal packages such as aluminum cans and steel cans; resin packages such as plastic bottles; paper packages such as drink cartons; glass packages such as glass bottles; and wooden packages such as barrels.
20 Filling a package with a beverage and sealing the package can provide a packed beverage.

[0033]

The total amount of at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe),
25 Cyclo(Phe-Trp), and Cyclo(Trp-Asn) as well as a salt thereof in the composition of the first aspect of the present invention is not limited and can be set according to the form of the composition, for example.

[0034]

In the case where the composition of the first aspect of the present invention
30 further contains at least one cyclic dipeptide selected from the group consisting of Cyclo(Hyp-Ala) and Cyclo(Leu-Lys) or a salt thereof, the total amount of the cyclic dipeptides and salts thereof in the composition is not limited and can be set according to the form of the composition, for example.

[0035]

35 The total amount of at least one cyclic dipeptide selected from the group

consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) as well as a salt thereof in the composition of the second aspect of the present invention is not limited and can be set according to the form of the composition, for example.

5 [0036]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(Arg-Phe) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

10 In an embodiment where the compositions of the first and second aspects of the present invention each contain Cyclo(Arg-Phe) or a salt thereof, the amount of Cyclo(Arg-Phe) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.001% by weight or more, preferably 0.005% by weight or more, more preferably 0.01% by weight or more, while preferably 50% by weight or less, more
15 preferably 20% by weight or less, still more preferably 10% by weight or less, further preferably 5% by weight or less. In an embodiment, the amount of Cyclo(Arg-Phe) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.001 to 50% by weight, more preferably 0.005 to 20% by weight, still more preferably 0.01 to 10% by weight, for
20 example.

[0037]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(Arg-Tyr) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

25 In an embodiment where the compositions of the first and second aspects of the present invention each contain Cyclo(Arg-Tyr) or a salt thereof, the amount of Cyclo(Arg-Tyr) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.0005% by weight or more, preferably 0.001% by weight or more, more
30 preferably 0.005% by weight or more, while preferably 5% by weight or less, more preferably 1% by weight or less, still more preferably 0.5% by weight or less. In an embodiment, the amount of Cyclo(Arg-Tyr) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.0005 to 5% by weight, more preferably 0.001 to 1% by weight,
35 still more preferably 0.005 to 0.5% by weight, for example.

[0038]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(Glu-Leu) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

5 In an embodiment where the compositions of the first and second aspects of the present invention each contain Cyclo(Glu-Leu) or a salt thereof, the amount of Cyclo(Glu-Leu) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.001% by weight or more, preferably 0.005% by weight or more, more
10 preferably 0.01% by weight or more, while preferably 20% by weight or less, more preferably 10% by weight or less, still more preferably 5% by weight or less. In an embodiment, the amount of Cyclo(Glu-Leu) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.001 to 20% by weight, more preferably 0.005 to 10% by
15 weight, still more preferably 0.01 to 5% by weight, for example.

[0039]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(His-Phe) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

20 In an embodiment where the compositions of the first and second aspects of the present invention each contain Cyclo(His-Phe) or a salt thereof, the amount of Cyclo(His-Phe) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.0005% by weight or more, preferably 0.001% by weight or more, more
25 preferably 0.005% by weight or more, while preferably 5% by weight or less, more preferably 1% by weight or less, still more preferably 0.5% by weight or less. In an embodiment, the amount of Cyclo(His-Phe) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.0005 to 5% by weight, more preferably 0.001 to 1% by weight,
30 still more preferably 0.005 to 0.5% by weight, for example.

[0040]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(Phe-Trp) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

35 In an embodiment where the compositions of the first and second aspects of the

present invention each contain Cyclo(Phe-Trp) or a salt thereof, the amount of Cyclo(Phe-Trp) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.0001% by weight or more, preferably 0.0005% by weight or more, more preferably 0.001% by weight or more, while preferably 5% by weight or less, more preferably 1% by weight or less, still more preferably 0.1% by weight or less. In an embodiment, the amount of Cyclo(Phe-Trp) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.0001 to 5% by weight, more preferably 0.0005 to 1% by weight, still more preferably 0.001 to 0.1% by weight, for example.

[0041]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(Trp-Asn) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

In an embodiment where the compositions of the first and second aspects of the present invention each contain Cyclo(Trp-Asn) or a salt thereof, the amount of Cyclo(Trp-Asn) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.0001% by weight or more, preferably 0.0005% by weight or more, more preferably 0.001% by weight or more, and while preferably 5% by weight or less, more preferably 1% by weight or less, still more preferably 0.1% by weight or less. In an embodiment, the amount of Cyclo(Trp-Asn) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.0001 to 5% by weight, more preferably 0.0005 to 1% by weight, still more preferably 0.001 to 0.1% by weight, for example.

[0042]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(Hyp-Ala) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

In an embodiment where the compositions of the first and second aspects of the present invention each contain Cyclo(Hyp-Ala) or a salt thereof, the amount of Cyclo(Hyp-Ala) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.0001% by weight or more, preferably 0.0005% by weight or more, more preferably 0.001% by weight or more, while preferably 5% by weight or less, more

preferably 1% by weight or less, still more preferably 0.1% by weight or less. In an embodiment, the amount of Cyclo(Hyp-Ala) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.0001 to 5% by weight, more preferably 0.0005 to 1% by weight, still more preferably 0.001 to 0.1% by weight, for example.
[0043]

In the case where the compositions of the first and second aspects of the present invention each contain Cyclo(Leu-Lys) or a salt thereof, the amount thereof is not limited and can be set according to the form of the composition, for example.

10 In an embodiment where the compositions of the first and second aspects of the present invention each contain Cyclo(Leu-Lys) or a salt thereof, the amount of Cyclo(Leu-Lys) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention may be, for example, 0.001% by weight or more, preferably 0.005% by weight or more, more
15 preferably 0.01% by weight or more, while preferably 20% by weight or less, more preferably 10% by weight or less, still more preferably 5% by weight or less. In an embodiment, the amount of Cyclo(Leu-Lys) or a salt thereof in terms of free cyclic dipeptide in each of the compositions of the first and second aspects of the present invention is preferably 0.001 to 20% by weight, more preferably 0.005 to 10% by
20 weight, still more preferably 0.01 to 5% by weight, for example.
[0044]

The total amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) as well as salts thereof in the composition of the first aspect of the present invention is not limited and can be set
25 according to the form of the composition, for example.

In an embodiment, the total amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp) and Cyclo(Trp-Asn) as well as salts thereof in terms of free cyclic dipeptide in the composition of the first aspect of the present invention may be, for example, 0.0001% by weight or more, preferably
30 0.0005% by weight or more, more preferably 0.001% by weight or more, still more preferably 0.01% by weight or more, while preferably 50% by weight or less, more preferably 30% by weight or less, still more preferably 20% by weight or less, further preferably 10% by weight or less. In an embodiment, the total amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and
35 Cyclo(Trp-Asn) as well as salts thereof in terms of free cyclic dipeptide in the

composition of the first aspect of the present invention may be, for example, 0.0001 to 50% by weight, preferably 0.0005 to 30% by weight, more preferably 0.001 to 20% by weight, still more preferably 0.01 to 10% by weight.

[0045]

5 In the case where the composition of the first aspect of the present invention further contains at least one cyclic dipeptide selected from the group consisting of Cyclo(Hyp-Ala) and Cyclo(Leu-Lys) or a salt thereof, the total amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) as well as salts thereof in each of the
10 compositions of the first and second aspects of the present invention is not limited and can be set according to the form of the composition, for example.

 In an embodiment, the total amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) as well as salts thereof in terms of free cyclic dipeptide in each of
15 the compositions of the first and second aspects of the present invention may be, for example, 0.0001% by weight or more, preferably 0.0005% by weight or more, more preferably 0.001% by weight or more, still more preferably 0.01% by weight or more, while preferably 50% by weight or less, more preferably 30% by weight or less, still more preferably 20% by weight or less, further preferably 10% by weight or less. In
20 an embodiment, the total amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) as well as salts thereof in terms of free cyclic dipeptide in the compositions of the first and second aspects of the present invention may be, for example, 0.0001 to 50% by weight, preferably 0.0005 to 30% by weight, more preferably 0.001 to 20% by weight,
25 still more preferably 0.01 to 10% by weight.

[0046]

 Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), Cyclo(Leu-Lys), and salts thereof can be quantified by a known method using liquid chromatography mass spectroscopy
30 (LC-MS/MS), for example. In the case where the composition contains two or more cyclic dipeptides or salts thereof, the total amount of the cyclic dipeptide or a salt thereof refers to the sum of the amounts of these cyclic dipeptides or salts thereof.

 The LC-MS/MS measurement can be performed under the following conditions.

35 The device may be Agilent 1290 binary LC coupled with AB Sciex 5600+

TripleTOF system, for example.

LC column: CORTECS T3 Column, 120 Å, 1.6 µm, 2.1 mm × 100 mm (Waters Corporation)

Amount of sample injected: 5 µL

5 Column temperature: 40°C

(Conditions for LC chromatography)

Mobile phase:

Solution A: water with 0.1% formic acid

Solution B: acetonitrile with 0.1% formic acid

10 Gradient (Concentration of solution B):

0 to 1 min: Solution B, 1%

1 to 8.5 min: Solution B, 1% → 40%

8.5 to 13 min: Solution B, 40% → 90%

13 to 15.5 min: Solution B, 90% → 100%

15 15.5 to 17.8 min: Solution B, 100%

17.8 to 18 min: Solution B, 100% → 1%

18 to 20 min: Solution B, 1%

[0047]

20 The compositions of the first and second aspects of the present invention are preferably orally ingested (orally administered). The administration amount (intake) of each of the compositions of the first and second aspects of the present invention is not limited. The administration amount of the composition of the first aspect of the present invention is any amount that provides an effect of reducing a decline in cognitive function or improving cognitive function. For example, the amount is any amount that provides an effect of reducing a decline in cognitive function or improving cognitive function by reducing production and/or accumulation of amyloid β.

25 Alternatively, for example, the amount is any amount that provides an effect of reducing a decline in cognitive function or improving cognitive function by inhibiting β-secretase and thus reducing production and/or accumulation of amyloid β. The administration amount is set as appropriate according to the dosage form, administration method, body weight of a subject, and the like as long as the amount provides any of these effects.

30 The administration amount of the composition of the second aspect of the present invention is any amount that provides an effect of inhibiting β-secretase. For example, the amount is any amount that provides an effect of reducing production and/or accumulation of amyloid β by inhibiting β-secretase. Alternatively, for

35

example, the amount may be an amount that provides an effect of reducing a decline in cognitive function or improving cognitive function by inhibiting β -secretase and thus reducing production and/or accumulation of amyloid β . The administration amount is set as appropriate according to the dosage form, administration method, body weight of a subject, and the like as long as the amount provides any of these effects.

[0048]

In an embodiment where the composition of the first aspect of the present invention is ingested by or administered to a human (adult), the administration amount as the total administration amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) as well as salts thereof (in terms of free cyclic dipeptide) per day is preferably 0.001 mg or more, more preferably 0.01 mg or more, still more preferably 0.1 mg or more, and is preferably 5000 mg or less, more preferably 3000 mg or less, still more preferably 2000 mg or less. In an embodiment where the composition of the first aspect of the present invention is ingested by or administered to a human (adult), the total administration amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) as well as salts thereof (in terms of free cyclic dipeptide) per day is preferably 0.001 to 5000 mg, more preferably 0.01 to 3000 mg, still more preferably 0.1 to 2000 mg. In the case where the composition of the first aspect of the present invention further contains at least one cyclic dipeptide selected from the group consisting of Cyclo(Hyp-Ala) and Cyclo(Leu-Lys) or a salt thereof, the administration amount of the composition of the first aspect of the present invention for ingestion by or administration to a human (adult) can be set as follows.

[0049]

In an embodiment where the compositions of the first and second aspects of the present invention are each ingested by or administered to a human (adult), the administration amount as the total administration amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) as well as salts thereof (in terms of free cyclic dipeptide) per day is preferably 0.001 mg or more, more preferably 0.01 mg or more, still more preferably 0.1 mg or more, and is preferably 5000 mg or less, more preferably 3000 mg or less, still more preferably 2000 mg or less. In an embodiment where the compositions of the first and second aspects of the present invention are each ingested by or administered to a human (adult), the total administration amount of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-

Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) as well as salts thereof per day is preferably 0.001 to 5000 mg, more preferably 0.01 to 3000 mg, still more preferably 0.1 to 2000 mg.

Preferably, the above amount is ingested or administered in one or more
5 portions per day, for example, one to several portions (e.g., two or three portions) per day. In an embodiment, preferably, the above amount of the cyclic dipeptide or a salt thereof is orally ingested or administered. In the case of a human (adult), preferably, the above amount of the cyclic dipeptide or a salt thereof is ingested or administered per 60 kg body weight per day. In an embodiment, the compositions of the first and
10 second aspects of the present invention may be an oral composition that allows the above amount of the cyclic dipeptide or a salt thereof to be ingested by or administered to a person per 60 kg body weight per day.

[0050]

The compositions of the first and second aspects of the present invention are
15 preferably each ingested or administered continuously. In the first aspect of the present invention, continuous ingestion or administration of at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) or a salt thereof is expected to enhance the effect. In the second aspect of the present invention, continuous
20 ingestion or administration of at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) or a salt thereof is expected to enhance the effect.

In an embodiment, preferably, the compositions of the first and second aspects
25 of the present invention are each continuously ingested or administered for preferably one week or longer, more preferably four weeks or longer, still more preferably eight weeks or longer.

The cyclic dipeptide or a salt thereof can be ingested in the form of a food or beverage, for example, so that long-term ingestion thereof, for example, is less likely to
30 cause issues in terms of safety.

[0051]

The compositions of the first and second aspects of the present invention may each be ingested by or administered to any subject (also referred to as an administration subject). The subject is preferably a human or non-human mammal, more preferably a
35 human.

In an embodiment, the administration subject of the compositions of the first and second aspects of the present invention may be, for example, a subject in need of or desiring reduction in a decline in cognitive function or improvement of cognitive function, a subject in need of or desiring prevention or amelioration of a condition or disease associated with a decline in cognitive function, a subject in need of or desiring inhibition of β -secretase, a subject in need of or desiring prevention or amelioration of a condition or disease associated with β -secretase production, a subject in need of or desiring reduction in production and/or accumulation of amyloid β , and a subject in need of or desiring prevention or amelioration of a condition or disease associated with production and/or accumulation of amyloid β . In an embodiment, examples of the administration subject in the first and second aspects of the present invention include middle-aged and older people. The middle-aged and older people include the elderly. The middle-aged and older people may be those who are 40 years old or older, for example. In an embodiment, the elderly are preferred subjects among the middle-aged and older people. The elderly may be those who are 60 years old or older or 65 years old or older. In an embodiment, the administration subject of the composition of the first aspect of the present invention may be a healthy person. For example, the composition may be used on a healthy person for purposes such as reducing a decline in cognitive function or improving cognitive function, reducing production and/or accumulation of amyloid β in the brain, preventing production and/or accumulation of amyloid β in the brain, preventing Alzheimer's disease, mild cognitive (function) impairment, or aging-related cognitive (function) impairment, and inhibiting β -secretase in the brain. In an embodiment, the administration subject of the composition of the second aspect of the present invention may be a healthy person. For example, the composition may be used on a healthy person for purposes such as inhibiting β -secretase in the brain, reducing production and/or accumulation of amyloid β in the brain, preventing production and/or accumulation of amyloid β in the brain, and preventing Alzheimer's disease, mild cognitive (function) impairment, or aging-related cognitive (function) impairment.

[0052]

The composition of the first aspect of the present invention may be provided with an indication of function exhibited owing to reduction in a decline in cognitive function or improvement of cognitive function. The composition of the second aspect of the present invention may be provided with an indication of function exhibited owing to inhibition of β -secretase. These indications are also referred to as function claims.

The indications that may be attached to the compositions of the first and second aspects of the present invention are not limited. Examples of the indications include "enhancing cognitive function", "reducing a decline in cognitive function", "maintaining good cognitive function", "improving memory", "reducing a decline in memory",
 5 "maintaining good memory", "improving accuracy of memory", "preventing memory disorder", "ameliorating memory disorder", "maintaining memory, which is a domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement, which are domains of cognitive function", "improving memory retention or integration",
 10 "maintaining enhancement of cognitive function", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining or improving orientation", "delaying an aging-related decline in cognitive function", "enhancing short-term and long-term memory", "promoting verbal and visuospatial memory", and "maintaining or improving logical reasoning", and indications or function
 15 claims considered to be equated thereto.

In an embodiment of the present invention, the compositions of the first and second aspects of the present invention are each preferably a food or beverage provided with one or two or more of the indications. The indications may each be an indication of using the compositions for achieving the corresponding function. The indication
 20 may be directly provided to the compositions or may be attached to containers or packages of the compositions.

[0053]

The present invention also encompasses the following methods and uses.

A method for reducing a decline in cognitive function or improving cognitive
 25 function, the method including administering at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) or a salt thereof.

Use of at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp),
 30 and Cyclo(Trp-Asn) or a salt thereof for reducing a decline in cognitive function or improving cognitive function.

A method for inhibiting β -secretase, the method including administering at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn),
 35 Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) or a salt thereof.

Use of at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) or a salt thereof for inhibiting β -secretase.

5 The above methods may be therapeutic or non-therapeutic. The above uses may be therapeutic or non-therapeutic.

[0054]

10 In the above methods and uses, preferably, the cyclic dipeptide or a salt thereof is ingested by or administered to a subject in one or more portions per day, for example, one to several portions (e.g., , two or three portions) per day. The above uses are each preferably for humans or non-human mammals, more preferably for humans. In an embodiment, Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), or a salt thereof can be used to prevent or ameliorate a condition or disease associated with a decline in cognitive function. Also, Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), Cyclo(Leu-Lys), or a salt thereof can be used to prevent or ameliorate a condition or disease that can be effectively prevented or ameliorated by inhibiting β -secretase. Also, Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), Cyclo(Leu-Lys), or a salt thereof can be used to prevent or ameliorate a condition or disease associated with production and/or accumulation of amyloid β . The present invention also encompasses a method for preventing or ameliorating a condition or disease associated with a decline in cognitive function; a method for preventing or ameliorating a condition or disease that can be effectively prevented or ameliorated by inhibiting β -secretase; and a method for preventing or ameliorating a condition or disease associated with production and/or accumulation of amyloid β , the methods each including administering any of the cyclic dipeptides or salts thereof.

[0055]

30 In the above methods and uses, the cyclic dipeptide or a salt thereof is used in an amount (also referred to as an effective amount) that provides an effect of reducing a decline in cognitive function or improving cognitive function, an effect of inhibiting β -secretase, or an effect of reducing production and/or accumulation of amyloid β . Preferred administration amounts, administration methods, administration subjects, and the like of the cyclic dipeptide or a salt thereof are as described above for the compositions of the first and second aspects of the present invention. The cyclic

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dipeptide or a salt thereof may be directly ingested or administered, or may be ingested or administered in the form of a composition containing the same. For example, the composition of the first or second aspect of the present invention may be ingested or administered.

5 [0056]

Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn) or a salt thereof can be used to produce a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like for use in reducing a decline in cognitive function or improving cognitive function.

10 In an embodiment, the present invention also encompasses use of at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) or a salt thereof in production of a composition for reducing a decline in cognitive function or improving cognitive function.

15 Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), Cyclo(Leu-Lys), or a salt thereof can be used to produce a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like for use in inhibiting β -secretase or reducing production and/or accumulation of amyloid β .

20 In an embodiment, the present invention also encompasses use of at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) or a salt thereof in production of a composition for inhibiting β -secretase or a composition for reducing production and/or accumulation of amyloid β .

25 [0057]

Herein, any numerical range expressed by a lower limit and an upper limit, i.e., "a lower limit to an upper limit" includes the lower limit and the upper limit. For example, a range expressed by "1 to 2" means 1 or more and 2 or less, with 1 and 2 being inclusive. Herein, the range may be any combination of any upper limit and any lower limit.

30

All the academic literature and patent literature described herein are incorporated herein by reference.

EXAMPLES

35 [0058]

The present invention is described in more detail below with reference to examples, but these examples are not intended to limit the scope of the present invention.

[0059]

5 <Example 1>

Each cyclic dipeptide was evaluated in terms of β -secretase (BACE1) inhibitory activity.

[0060]

(Materials and methods)

10 A. Reagents

BACE1 Inhibitor Screening Kit (Fluorometric) was obtained from Abcam.

The cyclic dipeptides (Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys)) were purchased from Axil Scientific Pte.

15 The other reagents were purchased from Sigma-Aldrich.

[0061]

B. BACE1 inhibitory assay

BACE1 Inhibitor Screening Kit (Fluorometric) was used to evaluate the BACE1 inhibitory activity of each cyclic dipeptide.

20 The BACE1 inhibitory assay was performed according to the kit manufacturer's protocol. The cyclic dipeptides were each individually dissolved in dimethyl sulfoxide (DMSO) at a high, 100-fold (100 $\times$) stock concentration and then diluted with assay buffer to twice (2 $\times$) the assay concentration. The resulting dilutions of the respective cyclic dipeptides were used in the following experiment.

25 BACE1 enzyme assay mix was prepared by diluting an enzyme stock solution (25 $\times$) with assay buffer. A 50- μ L portion of the resulting diluted enzyme solution was mixed with 50 μ L of each of the diluted solutions of the respective cyclic dipeptides. The resulting mixtures were transferred into wells of a flat-bottom 96-well black plate, whereby reaction mixes were prepared. A Tecan Spark[®] multimode microplate reader
30 was used to monitor the fluorescence ($\lambda_{Ex}/\lambda_{Em}$ = 345/500 nm) readout of the assay plate for 30 minutes at 37°C. The relative inhibition (%) of each sample was calculated from the measured fluorescence value according to the manufacturer's protocol.

[0062]

35 (Statistical analysis)

Statistical analysis was performed using GraphPad Prism version 7.0 (San Diego, California, USA). The best-fit IC₅₀ value (hereinafter, referred to as “IC₅₀”) of each cyclic dipeptide was determined by fitting the corresponding concentration and relative inhibition into the equation of log (cyclic dipeptide) vs. normalized inhibition.

5 [0063]

(Results)

Each cyclic dipeptide had BACE1 inhibitory action. Table 1 shows IC₅₀ (μg/mL) of each cyclic dipeptide.

[0064]

10 [Table 1]

Cyclic dipeptide	IC ₅₀ (μg/mL)
Cyclo(Arg-Phe)	28.2
Cyclo(Arg-Tyr)	430.8
Cyclo(Glu-Leu)	50.0
Cyclo(His-Phe)	370.7
Cyclo(Hyp-Ala)	109.4
Cyclo(Leu-Lys)	272.7
Cyclo(Phe-Trp)	126.4
Cyclo(Trp-Asn)	36.2

[0065]

Table 2 shows the percent relative inhibition of each cyclic dipeptide tested at various concentrations. Table 2 shows the average (%) of the BACE1 inhibition values (n = 2). The concentration and relative inhibition value (%) of each cyclic dipeptide were fitted into the equation of log (cyclic dipeptide) vs. normalized inhibition to determine the experimental IC₅₀ values (Table 1).

15

[0066]

[Table 2]

Concentration (mg/mL)	BACE1 inhibition (%)									
4.5	99.4	92.5		98	97.7					
1.5	98.6	77.7		82.5	92					
0.5	95.7	53.2		59.5	79.9					
0.166665	88.1	27.3		25.4	55.5					
0.09			68.7			32.5	47.1		61.5	
0.0555555	68.4	12.6		14.2	40.6					
0.03			32.9			19.4	23.3		48.3	
0.015	32.8	4.52		1.54	10.9					
0.01			12.6			10	21.2		33	
Cyclic dipeptide	Cyclo (Arg-Phe)	Cyclo (Arg-Tyr)	Cyclo (Glu-Leu)	Cyclo (His-Phe)	Cyclo (Hyp-Ala)	Cyclo (Leu-Lys)	Cyclo (Phe-Trp)	Cyclo (Trp-Asn)		

[0067]

BACE1 is an aspartyl protease that plays a major role in generation of amyloid beta (A β) peptides from amyloid precursor protein. A strongly positive correlation is

present between the A β peptide concentration and cognitive function (Reference 1); this strongly positive correlation, coupled with the evidence of increased BACE1 activity in Alzheimer's disease models (Reference 2), suggests that BACE1 inhibition is a promising strategy for delaying a decline in cognitive function (Reference 3).

5 The results of the BACE1 inhibition assay demonstrate that each cyclic dipeptide successfully inhibited BACE1 with IC₅₀ ranging from 28.2 to 430.8 μ g/mL (Table 1).

Reference 1: Naslund et al., JAMA 2000, 283(12), 1571-1577

Reference 2: Fukumoto et al., Arch. Neurol. 2002, 59(9), 1381-9.

10 Reference 3: McDade et al., Nature Reviews Neurology 2021, 17(11), 703-714.

CLAIMS

[Claim 1] A composition for reducing a decline in cognitive function or improving cognitive function, the composition comprising:

5 at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), and Cyclo(Trp-Asn) or a salt thereof.

10 [Claim 2] The composition according to claim 1, further comprising at least one cyclic dipeptide selected from the group consisting of Cyclo(Hyp-Ala) and Cyclo(Leu-Lys) or a salt thereof.

15 [Claim 3] The composition according to claim 1, wherein the composition reduces production and/or accumulation of amyloid β .

 [Claim 4] The composition according to claim 1, wherein the composition reduces production and/or accumulation of amyloid β by inhibiting β -secretase.

20 [Claim 5] A composition for inhibiting β -secretase, comprising: at least one cyclic dipeptide selected from the group consisting of Cyclo(Arg-Phe), Cyclo(Arg-Tyr), Cyclo(Glu-Leu), Cyclo(His-Phe), Cyclo(Phe-Trp), Cyclo(Trp-Asn), Cyclo(Hyp-Ala), and Cyclo(Leu-Lys) or a salt thereof.

25 [Claim 6] The composition according to claim 5, wherein the composition reduces production and/or accumulation of amyloid β by inhibiting β -secretase.

30 [Claim 7] The composition according to any one of claims 1 to 6, wherein the composition is an oral composition.

 [Claim 8] The composition according to any one of claims 1 to 6, wherein the composition is a food or beverage, or an oral pharmaceutical product.

35

[Claim 9] The composition according to any one of claims 1 to 6,
wherein the composition is provided with one or two or more function claims
selected from the group consisting of "enhancing cognitive function", "reducing a
decline in cognitive function", "maintaining good cognitive function", "improving
5 memory", "reducing a decline in memory", "maintaining good memory", "improving
accuracy of memory", "preventing memory disorder", "ameliorating memory disorder",
"maintaining memory, which is a domain of cognitive function", "function suitable for
those who are concerned about a decline in memory", "improving accuracy of memory
and/or correctness of judgement, which are domains of cognitive function", "improving
10 memory retention or integration", "maintaining enhancement of cognitive function",
"improving executive function", "promoting attention and concentration", "improving
learning ability", "maintaining or improving orientation", "delaying an aging-related
decline in cognitive function", "enhancing short-term and long-term memory",
"promoting verbal and visuospatial memory", and "maintaining or improving logical
15 reasoning".