

DESCRIPTION

TITLE OF INVENTION: COMPOSITION FOR MINIMIZING PRODUCTION
AND/OR ACCUMULATION OF AMYLOID β

5

TECHNICAL FIELD

[0001]

The present invention relates to, for example, a
composition for reducing amyloid- β production and/or
10 amyloid- β accumulation.

BACKGROUND ART

[0002]

Amyloid- β (A β) is a type of protein produced in the
15 brain. Amyloid- β that has aggregated into oligomers or
fibrils exhibits neuronal cytotoxicity. Such amyloid- β
aggregates are not discharged from the brain but accumulate
therein, which is considered to trigger onset of
Alzheimer's disease. Thus, reducing amyloid- β production
20 or amyloid- β accumulation is considered to be effective in
preventing Alzheimer's disease or suppressing the
progression of the disease.

[0003]

Acetylcholinesterase inhibitors are known as
25 pharmaceutical products that improve symptoms of
Alzheimer's disease. Inhibition of acetylcholinesterase
increases the amount of acetylcholine in the brain, which
is effective in suppressing the progression of decline in
cognitive function. However, inhibition of
30 acetylcholinesterase cannot reduce amyloid- β production or
remove accumulated amyloid- β . There is a demand for a
substance that can be ingested as a food or beverage or the
like on a daily basis and that can reduce amyloid- β
production or amyloid- β accumulation.

35 [0004]

A report has been made that cyclic dipeptides affect the brain function. Patent Literature 1 discloses an agent for reducing a decline in cognitive function and/or improving cognitive function, containing Cyclo(Gly-Pro).

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CITATION LIST

- Patent Literature

[0005]

Patent Literature 1: JP 2020-196686 A

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SUMMARY OF INVENTION

- Technical Problem

[0006]

The present invention aims to provide a composition for reducing amyloid- β production and/or amyloid- β accumulation.

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- Solution to Problem

[0007]

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As a result of extensive studies on the above issue, the present inventors found that cyclo-leucyl-phenylalanine (Cyclo(Leu-Phe)) and cyclo-leucyl-lysine (Cyclo(Leu-Lys)), which are cyclic dipeptides, are effective in reducing amyloid- β production and/or amyloid- β accumulation.

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[0008]

Specifically, although not limited thereto, the present invention relates to, for example, the following composition for reducing amyloid- β production and/or amyloid- β accumulation.

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(1) A composition for reducing at least one of amyloid- β production or amyloid- β accumulation, containing at least one selected from the group consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt thereof as an active ingredient.

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(2) The composition according to (1) above, wherein

the composition reduces at least one of amyloid- β production or amyloid- β accumulation by inhibiting β -secretase.

(3) The composition according to (1) or (2) above,
5 wherein the composition is for oral use.

(4) The composition according to any one of (1) to (3) above, wherein the composition is a food or beverage or a pharmaceutical product.

(5) The composition according to any one of (1) to
10 (4) above, wherein the composition is labeled with one 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",
15 "maintaining good memory", "improving accuracy of memory", "preventing memory disorders", "ameliorating memory disorders", "maintaining memory that is one domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy
20 of memory and/or correctness of judgement that are part of cognitive function", "improving memory retention or consolidation", "sustaining cognitive enhancement", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining
25 or improving orientation", "delaying a decline in aging-related cognitive function", "enhancing short-term and long-term memory", "promoting verbal and visuospatial memory", and "maintaining or improving logical thinking".

(6) Use of at least one selected from the group
30 consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt thereof in production of a composition for reducing at least one of amyloid- β production or amyloid- β accumulation.

(7) Use of at least one selected from the group
35 consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-

Lys), and a salt thereof for reducing at least one of amyloid- β production or amyloid- β accumulation.

- Advantageous Effects of Invention

5 [0009]

The present invention can provide a composition for reducing amyloid- β production and/or amyloid- β accumulation. The composition of the present invention can be used as a food or beverage, a pharmaceutical product, or
10 the like for reducing amyloid- β production and/or amyloid- β accumulation.

BRIEF DESCRIPTION OF DRAWINGS

[0010]

15 FIG. 1 is a graph showing β -secretase (BACE) inhibitory activity of a group to which no test compound is added (control), Cyclo(Leu-Ala) (Comparative Example 1), and Cyclo(Leu-Phe) (Examples 1 to 4).

FIG. 2 is a graph showing β -secretase (BACE)
20 inhibitory activity of a group to which no test compound is added (control), Cyclo(Leu-Ala) (Comparative Example 2), and Cyclo(Leu-Lys) (Example 5).

DESCRIPTION OF EMBODIMENTS

25 [0011]

The composition of the present invention for reducing amyloid- β production and/or amyloid- β accumulation contains at least one selected from the group consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt
30 thereof. The composition of the present invention for reducing amyloid- β production and/or amyloid- β accumulation contains at least one selected from the group consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt thereof as an active ingredient. Herein, the composition
35 of the present invention for reducing amyloid- β production

and/or amyloid- β accumulation is sometimes referred to as "the composition of the present invention". The composition of the present invention is used to reduce amyloid- β production and/or amyloid- β accumulation.

5 [0012]

Cyclo(Leu-Phe) (cyclo-leucyl-phenylalanine) used in the present invention is a cyclic dipeptide in which leucine and phenylalanine are bonded together by condensation. Cyclo(Leu-Lys) (cyclo-leucyl-lysine) used in
10 the present invention is a cyclic dipeptide in which leucine and lysine are bonded together by condensation. Herein, Cyclo(Leu-Phe) and Cyclo(Leu-Lys) are sometimes described as CLF and CLK, respectively.

Herein, the term "cyclic dipeptide" refers to a
15 compound consisting of amino acids as structural units and having a diketopiperazine structure generated by dehydration condensation of an amino group of an amino acid at the N-terminal and a carboxy group of an amino acid at the C-terminal. Herein, the description order of amino
20 acids in a cyclic dipeptide is not limited as long as the amino acid structure is the same. For example, Cyclo(Leu-Phe) and Cyclo(Phe-Leu) (cyclo-phenylalanyl-leucine) represent the same cyclic dipeptide. Cyclo(Leu-Lys) and Cyclo(Lys-Leu) (cyclo-lysyl-leucine) represent the same
25 cyclic dipeptide.

[0013]

The salts of CLF and CLK are not limited as long as they are pharmacologically or dietary acceptable salts, and they may be acid salts or basic salts. Examples of the
30 acid salts include inorganic acid salts such as hydrochloride, sulfate, nitrate, and phosphate; and organic acid salts such as acetate, citrate, maleate, malate, oxalate, lactate, succinate, fumarate, and propionate. Examples of the basic salts include alkali metal salts such
35 as sodium salt and potassium salt; and alkaline earth metal

salts such as calcium salt and magnesium salt. Salts of cyclic dipeptides can be easily prepared by a person skilled in the art by any method known in the relevant field.

5 [0014]

The source and production method of CLF, CLK, and salts thereof are not limited. CLF, CLK, and salts thereof can be produced by a known method. CLF, CLK, and salts thereof may be those naturally derived or artificially
10 synthesized. They may be produced by the enzyme method or microbial fermentation method. They may be synthesized by dehydration and cyclization of linear dipeptides. For example, a product of a heat-treated peptide, which is rich in cyclic dipeptides such as CLF or CLK, can be obtained by
15 heating a protein hydrolysate such as a collagen hydrolysate.

A protein hydrolysate or a heat-treated product thereof containing CLF, CLK, and salts thereof may be used to add CLF, CLK, and salts thereof to a composition.
20 Alternatively, a concentrate, dry powder, or a purified product of a protein hydrolysate or a heat-treated product thereof containing CLF, CLK, and salts thereof may be used to add CLF, CLK, and salts thereof to a composition. For example, the composition of the present invention may
25 contain a protein hydrolysate or a heat-treated product thereof, and CLF, CLK, and salts thereof each may be a part of the protein hydrolysate or the heat-treated product thereof. CLF, CLK, and salts thereof may be commercial products.

30 [0015]

Amyloid- β is a peptide that is sometimes also referred to as "amyloid- β protein", "amyloid- β peptide", or " β -amyloid". Amyloid- β is a peptide usually consisting of about 40 amino acids. Examples thereof include amyloid- β_{1-42} and amyloid- β_{1-40} .
35

Amyloid- β in the unassociated form is sometimes referred to as "amyloid- β monomer". Two or more amyloid- β monomers may form an aggregate. For example, amyloid- β monomers may form a soluble aggregate (amyloid- β oligomer) in which two or more (e.g., 2 to 50) amyloid- β monomers are aggregated. Examples of the amyloid- β oligomer include amyloid- β_{1-42} oligomers and amyloid- β_{1-40} oligomers. The amyloid- β in the present invention includes amyloid- β in the unassociated form and amyloid- β forming an aggregate (e.g., oligomer).
[0016]

Amyloid- β is produced from an amyloid- β precursor protein (APP) by two-step cleavage with β -secretase and γ -secretase. β -Secretase is a rate-determining enzyme for amyloid- β production, and its cleavage regulates the amount of total amyloid- β production. Thus, inhibiting β -secretase can reduce amyloid- β production and can reduce amyloid- β accumulation.
[0017]

CLF, CLK, and salts thereof have β -secretase inhibitory action. The composition of the present invention can be used to reduce amyloid- β production and/or amyloid- β accumulation by inhibiting β -secretase. CLF, CLK, and salts thereof are useful as active ingredients for inhibiting β -secretase. The present invention also encompasses a composition for inhibiting β -secretase, containing at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof.

In one embodiment, the composition of the present invention may contain Cyclo(Leu-Phe) or a salt thereof as an active ingredient. In another embodiment, the composition of the present invention may contain Cyclo(Leu-Lys) or a salt thereof as an active ingredient.
[0018]

The composition of the present invention can be used

to reduce amyloid- β production and/or amyloid- β accumulation in the brain. In one embodiment, the composition of the present invention can be used to reduce amyloid- β production and/or amyloid- β accumulation by
5 inhibiting β -secretase.

[0019]

In one embodiment, the composition of the present invention can be used, for example, to prevent or ameliorate a condition or disease associated with amyloid- β
10 accumulation, preferably a condition or disease associated with amyloid- β accumulation in the brain. Examples of such a condition or disease include a condition or disease involving amyloid- β accumulation or resulting from amyloid- β accumulation. Example of the condition or disease
15 involving amyloid- β accumulation or resulting from amyloid- β accumulation in the brain include Alzheimer's disease (including Alzheimer's dementia) and mild cognitive impairment.

The composition of the present invention can be used
20 to prevent or ameliorate the condition or disease and can be suitably used to prevent the condition or disease.

Herein, the expression "to prevent a condition or disease" or its similar expression encompasses preventing the onset, delaying the onset, reducing the incidence rate,
25 reducing the onset risk, and the like. The expression "to ameliorate a condition or disease" or its similar expression encompasses helping a subject recover from a condition or disease, alleviating a condition or disease symptoms, turning around a condition or disease symptoms,
30 delaying or preventing the progression of a condition or disease, and the like.

[0020]

Alzheimer's disease and mild cognitive impairment are known to cause a decline in cognitive function. Examples
35 of symptoms of the decline in cognitive function caused by

Alzheimer's disease and mild cognitive impairment include a decline in memory, memory disorders (forgetfulness), aphasia (inability to formulate words), apraxia, agnosia (e.g., getting lost in a familiar place), decline in
5 orientation (ability to correctly recognize one's situation, such as a place, time, and people's names), decline in verbal and non-verbal learning abilities, a decline in auditory and visual processing, a decline in executive function, executive dysfunction (an inability to
10 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, and a decline in information processing speed. Reducing amyloid- β production or amyloid- β accumulation is expected
15 to produce an effect of preventing a decline in cognitive function or improving cognitive function, for example, an effect of preventing or ameliorating any of the symptoms described above.

[0021]

20 The composition of the present invention is applicable for therapeutic use (medical use) and non-therapeutic use (non-medical use). The term "non-therapeutic" is a concept that does not include medical activities, i.e., a concept that does not include methods
25 of surgery, therapy, or diagnosis of humans.

For example, the composition of the present invention for reducing amyloid- β production and/or amyloid- β accumulation may be provided as an agent or the like, but it is not limited thereto. The agent can be directly
30 provided as a composition or can be provided as a composition containing the agent. In one embodiment, the composition of the present invention for reducing amyloid- β production and/or amyloid- β accumulation can also be referred to as an agent for reducing amyloid- β production
35 and/or amyloid- β accumulation. The composition for

inhibiting β -secretase can also be referred to as a β -secretase inhibitor.

[0022]

The composition of the present invention may be for
5 oral use or parenteral use. Preferably, the composition of
the present invention is a composition for oral use. The
composition of the present invention can be provided, for
example, in the form of a food or beverage, a
pharmaceutical product, a quasi-pharmaceutical product,
10 feed, or the like. A food or beverage or a pharmaceutical
product is preferred. The composition of the present
invention can also be used by being added to a food or
beverage, a pharmaceutical product, a quasi-pharmaceutical
product, feed, or the like. The form of the composition of
15 the present invention is not limited and may be in solid
(e.g., powder, granule, tablet, etc.), liquid, or paste
form, for example.

[0023]

The composition of the present invention contains at
20 least one selected from the group consisting of CLF, a salt
thereof, CLK, and a salt thereof and may further contain
various types of additives, which are accepted as additives
to foods, pharmaceutical products, and the like, such as
diluents, acidulants, antioxidants, stabilizers,
25 preservatives, flavoring or masking agents, emulsifiers,
pigments, seasonings, pH adjusters, and nutrient enhancers.

[0024]

For example, when the composition of the present
invention is provided as a food or beverage, a component
30 usable in a food or beverage (e.g., a food material or an
optional food additive) can be added to at least one
selected from the group consisting of CLF, a salt thereof,
CLK, and a salt thereof to provide various types of foods
or beverages. Non-limiting examples of the food or
35 beverage 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 claims, the foods for specified health uses, dietary supplements, and the like
5 can be used, for example, in various forms of preparations such as liquids, fine granules, tablets, granules, powders, capsules, chewable tablets, dry syrups, and liquid foods.
[0025]

When the composition of the present invention is
10 provided as a pharmaceutical or quasi-pharmaceutical product, a pharmacologically acceptable carrier, an optional additive, and the like can be added to at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof to provide various dosage
15 forms of pharmaceutical or quasi-pharmaceutical products. Such a carrier, additive, or the like may be any pharmacologically acceptable one that can be used in pharmaceutical or quasi-pharmaceutical products. Examples thereof include excipients, binders, disintegrants,
20 lubricants, antioxidants, and colorants. One or more of these can be used. The form of administration (ingestion) of the pharmaceutical or quasi-pharmaceutical product may be oral or parenteral (e.g., transdermal, transmucosal, enteral, or injection) administration. When the
25 composition of the present invention is provided as a pharmaceutical or quasi-pharmaceutical product, a pharmaceutical product for oral administration or a quasi-pharmaceutical product for oral administration is preferred. Examples of dosage forms for oral
30 administration include liquids, tablets, powders, fine granules, granules, sugar-coated tablets, capsules, suspensions, emulsions, and chewable tablets. Examples of dosage forms for parenteral administration include injections, intravenous agents, ointments, lotions,
35 adhesive skin patches, suppositories, nasal agents, and

transpulmonary agents (inhalants). The pharmaceutical product may be for non-human animals.

[0026]

When the composition of the present invention is provided as feed, at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof 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.

[0027]

For example, when the composition of the present invention is provided as a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, the composition can be produced by a usual method, using at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof.

[0028]

In one embodiment, the composition of the present invention is preferably a liquid composition, more preferably a beverage. The beverage may be a functional beverage. The beverage may be in any form, and it may be a beverage in a sealed container. The container of the beverage in a sealed container is not limited. The container may be in any form and made of any material. For example, any of the following commonly used containers can be used: metal containers such as aluminum cans and steel cans; resin containers such as plastic bottles; paper containers such as drink cartons; glass containers such as glass bottles; and wooden containers such as barrels. Any of these containers is filled with the beverage and sealed, whereby the beverage in a sealed container can be obtained.

[0029]

The total amount of CLF, CLK, and salts thereof in

the composition of the present invention is not limited and can be set according to the form of the composition or the like.

In one embodiment, the total amount of CLF, CLK, and salts thereof in the composition of the present invention may be, for example, 0.00001 wt% or more, preferably 0.0001 wt% or more, more preferably 0.001 wt% or more, still more preferably 0.01 wt% or more, particularly preferably 0.1 wt% or more, and is also preferably 50 wt% or less. In one embodiment, for example, the total amount of CLF, CLK, and salts thereof in the composition of the present invention may be 0.00001 to 25 wt%, preferably 0.0001 to 10 wt%, more preferably 0.001 to 5 wt%, still more preferably 0.01 to 5 wt%, particularly preferably 0.1 to 1 wt%.

CLF, CLK, and salts thereof can be quantified by a known method using liquid chromatography-mass spectrometry (LC/MS), for example.

[0030]

When the composition of the present invention contains CLF or a salt thereof, the amount is not limited but is preferably 0.000001 to 20 wt%, for example. The amount is more preferably 0.00001 to 10 wt%, still more preferably 0.0001 to 5 wt%, yet still more preferably 0.001 to 2 wt%, particularly preferably 0.01 to 1 wt%.

[0031]

When the composition of the present invention contains CLK or a salt thereof, the amount is not limited but is preferably 0.00001 to 20 wt%, for example. The amount is more preferably 0.0001 to 10 wt%, still more preferably 0.001 to 5 wt%, yet still more preferably 0.01 to 2 wt%, particularly preferably 0.01 to 1 wt%.

[0032]

Preferably, the composition of the present invention is orally ingested (orally administered). The administration amount (intake) of the composition of the

present invention is not limited. The administration amount of the composition of the present invention is not limited as long as it is an amount that achieves the effect of reducing amyloid- β production and/or amyloid- β accumulation and may be suitably set according to the administration form, administration method, body weight of a subject, and the like. Preferably, the administration amount of the composition of the present invention is an amount that achieves the β -secretase inhibitory effect.

10 [0033]

In one embodiment, when the composition of the present invention is ingested by or administered to a human (adult) as a subject, the administration amount as the total administration amount of CLF, CLK, and salts thereof (in terms of 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 also preferably 5000 mg or less, more preferably 1000 mg or less, still more preferably 100 mg or less. In one embodiment, when the composition of the present invention is ingested by or administered to a human (adult), the total administration amount of CLF, CLK, and salts thereof per day is preferably 0.001 to 5000 mg, more preferably 0.01 to 1000 mg, still more preferably 0.1 to 100 mg.

25 Preferably, the above amount is ingested or administered in one or more portions per day, for example, one to several portions (e.g., two to three portions) per day. In one embodiment, preferably, the above amount of at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof is orally ingested or administered. In the case of a human (adult), preferably, the above amount of at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof is ingested or administered per 60 kg body weight per day.

35 In one embodiment, the composition of the present invention

may be an oral composition for allowing the above amount of at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof to be ingested by or administered to a human per 60 kg body weight per day.

5 [0034]

Preferably, the composition of the present invention is continuously ingested or administered. Continuous ingestion or administration of at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof is expected to enhance the effects. In one embodiment, the composition of the present invention is continuously ingested or administered preferably for at least one week, more preferably for at least four weeks, still more preferably for at least eight weeks.

15 CLF, CLK, and salts thereof can be ingested as food or beverages or the like. For example, long-term ingestion is considered to be low risk in terms of safety.

[0035]

The composition of the present invention may be ingested by or administered to any subject (administration subject). The administration subject is preferably a human or non-human mammal, more preferably a human.

In one embodiment, examples of the administration subject of the composition of the present invention include a subject needing or wanting to reduce amyloid- β production and/or amyloid- β accumulation and a subject needing or wanting to prevent or ameliorate a condition or disease associated with amyloid- β accumulation. In one embodiment, the administration subject in the present invention may include middle-aged and older people. The term "middle-aged and older people" includes the elderly. The middle-aged and older people may be those who are 40 years old and older, for example. In one embodiment, the elderly are preferred subjects among the middle-aged and older people.

35 The elderly may be people who are 60 years old and older or

65 years old and older, for example. In one embodiment, the administration subject in the composition of the present invention may be a healthy person. For example, the composition can also be used on a healthy person for purposes such as reducing amyloid- β production and/or amyloid- β accumulation in the brain, preventing amyloid- β accumulation in the brain, and preventing Alzheimer's disease or mild cognitive impairment.

[0036]

10 The composition of the present invention may be labeled with a function claim based on reduction of amyloid- β production and/or amyloid- β accumulation. Such a label is also referred to as a function claim. The label is not limited. Examples of such labels include "enhancing
15 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 disorders", "ameliorating memory
20 disorders", "maintaining memory that is one 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 that are part of cognitive function", "improving memory retention or
25 consolidation", "sustaining cognitive enhancement", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining or improving orientation", "delaying a decline in aging-related cognitive function", "enhancing short-term and
30 long-term memory", "promoting verbal and visuospatial memory", and "maintaining or improving logical thinking", as well as labels or function claims considered to be equated to these labels.

 In one embodiment of the present invention,
35 preferably, the composition of the present invention is a

food or beverage labeled with one or more function claims described above. The label may be one indicating use of the composition for obtaining any of the above functions. The label may be directly attached to the composition or
5 may be attached to a container or package of the composition.

[0037]

The present invention also encompasses the following methods and uses:

10 a method of reducing amyloid- β production and/or amyloid- β accumulation, the method including administering at least one selected from the group consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt thereof;

15 a method of inhibiting β -secretase, the method including administering at least one selected from the group consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt thereof;

use of at least one selected from the group
20 consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt thereof for reducing amyloid- β production and/or amyloid- β accumulation; and

use of at least one selected from the group
consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-
25 Lys), and a salt thereof for inhibiting β -secretase.

Each method may be a therapeutic or non-therapeutic method. Each use may be a therapeutic or non-therapeutic use.

[0038]

30 In one embodiment, Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), or a salt thereof can be used to reduce amyloid- β production and/or amyloid- β accumulation by inhibiting β -secretase.

[0039]

35 In the above methods and uses, preferably, at least

one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof is ingested by or administered to a subject at least once a day, for example, once to several times (for example, two or three times) per day. The above uses are preferably for humans or non-human mammals, more preferably for humans. In one embodiment, at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof can be used to prevent or ameliorate a condition or disease associated with amyloid- β accumulation by inhibiting β -secretase. The present invention also encompasses a method of preventing or ameliorating a condition or disease associated with amyloid- β accumulation, the method including administering at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof.

[0040]

In the above methods and uses, at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof may be used in an amount (effective amount) that achieves the effect of reducing amyloid- β production and/or amyloid- β accumulation. Preferably, the effective amount is an amount that can achieve the β -secretase inhibitory effect. Preferred administration amount, administration method, administration subject, and the like of at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof are the same as those of the composition of the present invention described above. CLF, a salt thereof, CLK, or a salt thereof may be ingested or administered as-is or may be ingested or administered in the form of a composition containing the same. For example, the composition of the present invention may be ingested or administered.

[0041]

At least one selected from the group consisting of CLF, a salt thereof, CLK, and 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 amyloid- β production and/or amyloid- β accumulation. In one embodiment, the present invention
5 also encompasses use of at least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof in production of a composition for reducing amyloid- β production and/or amyloid- β accumulation. The present invention also encompasses use of CLF, a salt
10 thereof, CLK, or a salt thereof in production of a composition for inhibiting β -secretase.

At least one selected from the group consisting of CLF, a salt thereof, CLK, and a salt thereof can be used to produce a composition for preventing or ameliorating a
15 condition or disease associated with amyloid- β accumulation.

[0042]

The numerical range defined by the lower limit and the upper limit herein, i.e., "the lower limit to the upper
20 limit", includes the lower limit and the upper limit. For example, the range defined 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.

25

EXAMPLES

[0043]

The present invention is described in further detail below with reference to Examples, which are not intended to
30 limit the scope of the present invention.

[0044]

<Examples 1 to 5 and Comparative Example 1>

(Evaluation of compound having β -secretase inhibitory activity)

35 Materials and methods

β -Secretase inhibitory activity of cyclo-leucyl-alanine (Cyclo(Leu-Ala)) (hereinafter also referred to as "CLA"), cyclo-leucyl-phenylalanine (Cyclo(Leu-Phe)), and cyclo-leucyl-lysine (Cyclo(Leu-Lys)) was examined.

5 Cyclo(Leu-Ala) and Cyclo(Leu-Phe) as test compounds were purchased from Bachem. Cyclo(Leu-Lys) as a test compound was purchased from Peptide Institute, Inc.

A commercial kit "Beta Secretase (BACE1) Activity Assay Kit (Abcam)" was used for evaluation of β -secretase
10 inhibitory activity. The β -secretase enzyme activity inhibitory effect was evaluated according to the method described in the kit.

A sample solution (test compound solution) was mixed with an enzyme solution on a 96-well plate (Corning),
15 followed by incubation at 37°C for 10 minutes. Subsequently, a substrate solution was added for reaction at 37°C for 60 minutes. The fluorescence intensity (RFU) (Ex/Em = 335 nm/495 nm) of the reaction solution was measured using a microplate reader "FlexStation 3"
20 (Molecular Devices) at every five minutes from 0 to 60 minutes after the start of the reaction. The reaction was performed with n = 2, and the average fluorescence intensity of the reaction solution was regarded as the measured value.

25 The concentration (final concentration) of each test compound in the evaluation was set as shown in Table 1.

[0045]

[Table 1]

	Compound	Concentration ($\mu\text{g/mL}$)
Control	Not added	—
Comparative Example 1	Cyclo(Leu-Ala)	20
Example 1	Cyclo(Leu-Phe)	19
Example 2	Cyclo(Leu-Phe)	9.5
Example 3	Cyclo(Leu-Phe)	4.75
Example 4	Cyclo(Leu-Phe)	2.375
Example 5	Cyclo(Leu-Lys)	17

[0046]

The enzyme activity was evaluated using Δ fluorescence intensity of the reaction solution ((fluorescence intensity at end of reaction (minutes 60)) - (fluorescence intensity before start of reaction (minutes 0))). A group to which no test compound added was used as control. The inhibitory activity of each test compound was evaluated, with the enzyme activity (Δ fluorescence intensity) of control taken as 1.

[0047]

Results

FIGS. 1 and 2 are graphs each showing β -secretase (BACE) inhibitory activity of each test compound.

The results were represented as means $\pm$ standard deviation ($n = 2$). BACE activity shown in each of FIGS. 1 and 2 is a value of BACE activity of each test compound (Δ fluorescence intensity of each reaction solution to which the test compound was added) relative to BACE activity of control (Δ fluorescence intensity of control), which was taken as 1.

The inhibitory activity was not observed in cyclo-leucyl-alanine (Cyclo(Leu-Ala)) measured as Comparative Example 1. A reduction in the enzyme activity of β -secretase was observed in cyclo-leucyl-phenylalanine (Cyclo(Leu-Phe)) (Examples 1 to 4) and cyclo-leucyl-lysine

(Cyclo(Leu-Lys)) (Example 5).

The above results show that Cyclo(Leu-Phe) and Cyclo(Leu-Lys), which are cyclic dipeptides, have β -secretase inhibitory activity.

CLAIMS

Claim 1. A composition for reducing at least one of amyloid- β production or amyloid- β accumulation, comprising:
5 at least one selected from the group consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-Lys), and a salt thereof as an active ingredient.

Claim 2. The composition according to claim 1,
10 wherein the composition reduces at least one of amyloid- β production or amyloid- β accumulation by inhibiting β -secretase.

Claim 3. The composition according to claim 1 or 2,
15 wherein the composition is for oral use.

Claim 4. The composition according to claim 1 or 2,
wherein the composition is a food or beverage or a pharmaceutical product.

20 Claim 5. The composition according to claim 1 or 2,
wherein the composition is labeled with one or more function claims selected from the group consisting of "enhancing cognitive function", "reducing a decline in
25 cognitive function", "maintaining good cognitive function", "improving memory", "reducing a decline in memory", "maintaining good memory", "improving accuracy of memory", "preventing memory disorders", "ameliorating memory disorders", "maintaining memory that is one domain of
30 cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement that are part of cognitive function", "improving memory retention or consolidation", "sustaining cognitive enhancement",
35 "improving executive function", "promoting attention and

concentration", "improving learning ability", "maintaining
or improving orientation", "delaying a decline in aging-
related cognitive function", "enhancing short-term and
long-term memory", "promoting verbal and visuospatial
5 memory", and "maintaining or improving logical thinking".

Claim 6. Use of at least one selected from the group
consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-
Lys), and a salt thereof in production of a composition for
10 reducing at least one of amyloid- β production or amyloid- β
accumulation.

Claim 7. Use of at least one selected from the group
consisting of Cyclo(Leu-Phe), a salt thereof, Cyclo(Leu-
15 Lys), and a salt thereof for reducing at least one of
amyloid- β production or amyloid- β accumulation.