

DESCRIPTION

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

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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]

As a result of extensive studies on the above issue, the present inventors found that cyclo-phenylalanyl-phenylalanine (Cyclo(Phe-Phe)), which is a cyclic dipeptide, is effective in reducing amyloid- β production and/or amyloid- β accumulation.

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25 [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 Cyclo(Phe-Phe) or a salt thereof as an active ingredient.

(2) The composition according to (1) above, wherein the composition reduces at least one of amyloid- β

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production or amyloid- β accumulation by inhibiting β -

secretase.

(3) The composition according to (1) or (2) above, wherein the composition reduces amyloid- β accumulation by promoting microglial phagocytosis.

5 (4) The composition according to any one of (1) to (3) above, wherein the composition is for oral use.

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

10 (6) The composition according to any one of (1) to (5) 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",
15 "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 cognitive function", "function suitable for those who are
20 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", "improving executive function", "promoting attention and
25 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 memory", and "maintaining or improving logical thinking".

30 (7) Use of Cyclo(Phe-Phe) or a salt thereof in production of a composition for reducing at least one of amyloid- β production or amyloid- β accumulation.

(8) Use of Cyclo(Phe-Phe) or a salt thereof for
35 reducing at least one of amyloid- β production or amyloid- β accumulation.

- Advantageous Effects of Invention

[0009]

The present invention can provide a composition for
5 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
the like for reducing amyloid- β production and/or amyloid- β
accumulation.

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BRIEF DESCRIPTION OF DRAWINGS

[0010]

FIG. 1 is a graph showing β -secretase (BACE)
inhibitory activity of Cyclo(Phe-Phe) and Cyclo(Leu-Ala).

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FIG. 2 shows a graph of the amount of amyloid- β
monomers in cells of SIM-A9 microglia with 24-hour
Cyclo(Phe-Phe) (CFF) pretreatment, and SIM-A9 microglia
without Cyclo(Phe-Phe) treatment (vehicle control (VC)) (*:
 $p < 0.05$, relative to vehicle control).

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FIG. 3 shows micrographs of BV-2 microglia with A β_{1-42}
oligomer treatment (vehicle control (VC)) and BV-2
microglia with Cyclo(Phe-Phe) and A β_{1-42} oligomer treatment
(CFF) (FIG. 3A, FIG. 3B, and FIG. 3C: vehicle control; FIG.
3D, FIG. 3E, and FIG. 3F: with Cyclo(Phe-Phe) treatment).

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FIG. 4 shows a graph of results from studies on
amyloid- β oligomer phagocytosis by BV-2 microglia with
Cyclo(Phe-Phe) (CFF) treatment, and BV-2 microglia without
Cyclo(Phe-Phe) treatment (vehicle control (VC)) (*: $p <$
 0.05 , relative to vehicle control).

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FIG. 5 is a graph showing the concentration of
amyloid- β_{1-38} in the cell culture supernatant of H4
neuroglioma cells overexpressing wild type amyloid
precursor protein with the Swedish double mutation
K595N/M596L (H4-hAPP cells) (***: $p < 0.001$, relative to
35 vehicle control).

FIG. 6 is a graph showing the concentration of amyloid- β_{1-40} in the cell culture supernatant of H4-hAPP cells (***: $p < 0.001$, relative to vehicle control).

FIG. 7 is a graph showing the concentration of amyloid- β_{1-42} in the cell culture supernatant of H4-hAPP cells (***: $p < 0.001$, relative to vehicle control).

DESCRIPTION OF EMBODIMENTS

[0011]

10 The composition of the present invention for reducing amyloid- β production and/or amyloid- β accumulation contains Cyclo(Phe-Phe) or a salt thereof. The composition of the present invention for reducing amyloid- β production and/or amyloid- β accumulation contains Cyclo(Phe-Phe) or a salt
15 thereof as an active ingredient. Herein, the composition 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
20 amyloid- β production and/or amyloid- β accumulation.

[0012]

Cyclo(Phe-Phe) (cyclo-phenylalanyl-phenylalanine) used in the present invention is a cyclic dipeptide in which two phenylalanine molecules are bonded together by
25 condensation. Herein, Cyclo(Phe-Phe) is sometimes described as CFF.

Herein, the term "cyclic dipeptide" refers to a compound consisting of amino acids as structural units and having a diketopiperazine structure generated by
30 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.

[0013]

The salt of Cyclo(Phe-Phe) is not limited as long as
35 it is a pharmacologically or dietary acceptable salt, and

it may be an acid salt or a basic salt. Examples of the acid salt 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 salt include alkali metal salts such as sodium salt and potassium salt; and alkaline earth metal salts such as calcium salt and magnesium salt. A salt of a cyclic dipeptide can be easily prepared by a person skilled in the art by any method known in the relevant field.

[0014]

The source and production method of Cyclo(Phe-Phe) or a salt thereof is not limited. Cyclo(Phe-Phe) or a salt thereof can be produced by a known method. Cyclo(Phe-Phe) or a salt thereof may be one naturally derived or artificially synthesized. It may be produced by the enzyme method or microbial fermentation method. It may be synthesized by dehydration and cyclization of a linear dipeptide. For example, a product of a heat-treated peptide, which is rich in cyclic dipeptides such as Cyclo(Phe-Phe), can be obtained by heating a protein hydrolysate such as a collagen hydrolysate.

A protein hydrolysate or a heat-treated product thereof containing Cyclo(Phe-Phe) or a salt thereof may be used to add Cyclo(Phe-Phe) or a salt thereof to a composition. Alternatively, a concentrate, dry powder, or a purified product of a protein hydrolysate or a heat-treated product thereof containing Cyclo(Phe-Phe) or a salt thereof may be used to add Cyclo(Phe-Phe) or a salt thereof to a composition. For example, the composition of the present invention may contain a protein hydrolysate or a heat-treated product thereof, and Cyclo(Phe-Phe) or a salt thereof may be a part of the protein hydrolysate or the heat-treated product thereof. Cyclo(Phe-Phe) or a salt thereof may be a commercial product.

[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
5 about 40 amino acids. Examples thereof include amyloid- β_{1-42} , amyloid- β_{1-40} , and amyloid- β_{1-38} . 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
10 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, amyloid- β_{1-40} oligomers, and amyloid- β_{1-38} oligomers. The amyloid- β in the present invention includes
15 amyloid- β in the unassociated form and amyloid- β forming an aggregate (e.g., oligomer).

[0016]

Amyloid- β is produced as an amyloid- β monomer by two-step cleavage of an amyloid- β precursor protein (APP) with
20 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.

25 [0017]

Cyclo(Phe-Phe) or a salt thereof has 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.
30 Cyclo(Phe-Phe) or a salt thereof is useful as an active ingredient for inhibiting β -secretase. The present invention also encompasses a composition for inhibiting β -secretase, containing Cyclo(Phe-Phe) or a salt thereof.

[0018]

35 Cyclo(Phe-Phe) or a salt thereof promotes microglial

phagocytosis.

Microglial cells are central nervous system cells in the brain. Microglial cells play an important role for maintenance of the brain environment. One of their actions
5 is phagocytosis of waste products, such as extracellular proteins (e.g., amyloid- β) and damaged cells, in the brain. Promoting microglial phagocytosis promotes removal of amyloid- β . Thus, promoting microglial phagocytosis is effective in reducing amyloid- β accumulation. Cyclo(Phe-
10 Phe) or a salt thereof is useful as an active ingredient for promoting microglial phagocytosis.

[0019]

In one embodiment, the composition of the present invention can be used to reduce amyloid- β accumulation by
15 promoting microglial phagocytosis. The present invention also encompasses a composition for promoting microglial phagocytosis, containing Cyclo(Phe-Phe) or a salt thereof.

[0020]

The composition of the present invention can be used
20 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 inhibiting β -secretase and/or promoting microglial
25 phagocytosis. The composition of the present invention can be used to reduce production and/or accumulation of one or more types of amyloid- β .

[0021]

In one embodiment, the composition of the present
30 invention can be used, for example, to prevent or ameliorate a condition or disease associated with amyloid- β 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
35 involving amyloid- β accumulation or resulting from amyloid-

β accumulation. Example of the condition or disease 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 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, 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, delaying or preventing the progression of a condition or disease, and the like.

[0022]

Alzheimer's disease and mild cognitive impairment are known to cause a decline in cognitive function. Examples 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 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 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 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.

[0023]

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 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 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 and/or amyloid- β accumulation. The composition for inhibiting β -secretase can also be referred to as a β -secretase inhibitor. The composition for promoting microglial phagocytosis can also be referred to as an agent for promoting microglial phagocytosis.

[0024]

The composition of the present invention may be for 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, 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 the present invention is not limited and may be in solid
5 (e.g., powder, granule, tablet, etc.), liquid, or paste form, for example.

[0025]

The composition of the present invention contains Cyclo(Phe-Phe) or a salt thereof and may further contain
10 various types of additives, which are accepted as additives to foods, pharmaceutical products, and the like, such as diluents, acidulants, antioxidants, stabilizers, preservatives, flavoring or masking agents, emulsifiers, pigments, seasonings, pH adjusters, and nutrient enhancers.

15 [0026]

For example, when the composition of the present invention is provided as a food or beverage, a component usable in a food or beverage (e.g., a food material or an optional food additive) can be added to Cyclo(Phe-Phe) or a
20 salt thereof to provide various types of foods or beverages. Non-limiting examples of the food or 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
25 foods, the foods with function claims, the foods for specified health uses, dietary supplements, and the like 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.

30 [0027]

When the composition of the present invention is provided as a pharmaceutical or quasi-pharmaceutical product, a pharmacologically acceptable carrier, an optional additive, and the like can be added to Cyclo(Phe-
35 Phe) or a salt thereof to provide various dosage 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
5 excipients, binders, disintegrants, 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
10 injection) administration. When the 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
15 oral 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,
20 adhesive skin patches, suppositories, nasal agents, and transpulmonary agents (inhalants). The pharmaceutical product may be for non-human animals.

[0028]

When the composition of the present invention is
25 provided as feed, Cyclo(Phe-Phe) or 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
30 for animals such as dogs, cats, and birds.

[0029]

For example, when the composition of the present invention is provided as a food or beverage, a
pharmaceutical product, a quasi-pharmaceutical product,
35 feed, or the like, the composition can be produced by a

usual method, using Cyclo(Phe-Phe) or a salt thereof.

[0030]

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.

[0031]

The amount of Cyclo(Phe-Phe) or a salt 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 amount of Cyclo(Phe-Phe) or a salt thereof in the composition of the present invention may be, for example, 1×10^{-6} wt% or more, preferably 1×10^{-5} wt% or more, and may also be 90 wt% or less, 1×10^{-1} wt% or less, or 1×10^{-2} wt% or less. In one embodiment, the amount of Cyclo(Phe-Phe) or a salt thereof in the composition of the present invention may be 1×10^{-6} to 90 wt%, for example. In one embodiment, the amount of Cyclo(Phe-Phe) or a salt thereof in the composition of the present invention may be 1×10^{-6} to 1×10^{-1} wt%, preferably 1×10^{-6} to 1×10^{-2} wt%, more preferably 1×10^{-5} to 1×10^{-2} wt%. In one embodiment, when the composition of the present invention is a liquid composition such as a beverage, the amount of Cyclo(Phe-Phe) or a salt thereof in

the composition is preferably 1×10^{-6} to 1×10^{-1} wt%, more preferably 1×10^{-6} to 1×10^{-2} wt%, still more preferably 1×10^{-5} to 1×10^{-2} wt%.

Cyclo(Phe-Phe) or a salt thereof can be quantified by a known method using liquid chromatography-mass spectrometry (LC/MS), for example.

[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 and/or the microglial phagocytosis promoting effect.

[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 administration amount of Cyclo(Phe-Phe) per day is preferably 1 μ g or more, more preferably 10 μ g or more, still more preferably 150 μ g or more, and is also preferably 1000 μ g or less, more preferably 500 μ g or less, still more preferably 300 μ g or less. In one embodiment, when the composition of the present invention is ingested by or administered to a human (adult), the administration amount as the administration amount of Cyclo(Phe-Phe) per day is preferably 1 to 1000 μ g, more preferably 10 to 500 μ g, still more preferably 150 to 300 μ g.

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 Cyclo(Phe-Phe) or a salt thereof is orally ingested or administered. In the case of a human (adult), preferably, the above amount of Cyclo(Phe-Phe) or a salt thereof is ingested or administered per 60 kg body weight per day. In one embodiment, the composition of the present invention may be an oral composition for allowing the above amount of Cyclo(Phe-Phe) or a salt thereof to be ingested by or administered to a human per 60 kg body weight per day. [0034]

Preferably, the composition of the present invention is continuously ingested or administered. Continuous ingestion or administration of Cyclo(Phe-Phe) or 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.

Cyclo(Phe-Phe) or salt thereof can be ingested as a food or beverage 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
5 preferred subjects among the middle-aged and older people. 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
10 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.

15 [0036]

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
20 is not limited. Examples of such labels include "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",
25 "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 of memory and/or correctness of judgement that are part of
30 cognitive function", "improving memory retention or consolidation", "sustaining cognitive enhancement", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining or improving orientation", "delaying a decline in aging-
35 related cognitive function", "enhancing short-term and

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.

5 In one embodiment of the present invention, 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.
10 The label may be directly attached to the composition or may be attached to a container or package of the composition.

[0037]

 The present invention also encompasses the following
15 methods and uses:

 a method of reducing amyloid- β production and/or amyloid- β accumulation, the method including administering Cyclo(Phe-Phe) or a salt thereof;

 a method of inhibiting β -secretase, the method
20 including administering Cyclo(Phe-Phe) or a salt thereof;

 a method of promoting microglial phagocytosis, the method including administering Cyclo(Phe-Phe) or a salt thereof;

 use of Cyclo(Phe-Phe) or a salt thereof for reducing
25 amyloid- β production and/or amyloid- β accumulation;

 use of Cyclo(Phe-Phe) or a salt thereof for inhibiting β -secretase; and

 use of Cyclo(Phe-Phe) or a salt thereof for promoting microglial phagocytosis.

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

 Cyclo(Phe-Phe) or a salt thereof can be used to reduce production and/or accumulation of one or more types
35 of amyloid- β .

[0038]

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

[0039]

In the above methods and uses, preferably, Cyclo(Phe-Phe) or a salt thereof is ingested by or administered to a
10 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, Cyclo(Phe-Phe)
15 or a salt thereof can be used to prevent or ameliorate a condition or disease associated with amyloid- β accumulation by inhibiting β -secretase and/or promoting microglial phagocytosis. The present invention also encompasses a method of preventing or ameliorating a condition or disease
20 associated with amyloid- β accumulation, the method including administering Cyclo(Phe-Phe) or a salt thereof.

[0040]

In the above methods and uses, Cyclo(Phe-Phe) or a salt thereof may be used in an amount (effective amount)
25 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 and/or the microglial phagocytosis promoting effect. Preferred administration amount,
30 administration method, administration subject, and the like of Cyclo(Phe-Phe) or a salt thereof are the same as those of the composition of the present invention described above. Cyclo(Phe-Phe) or a salt thereof may be ingested or administered as-is or may be ingested or administered in
35 the form of a composition containing the same. For

example, the composition of the present invention may be ingested or administered.

[0041]

Cyclo(Phe-Phe) or a salt thereof can be used to
5 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
also encompasses use of Cyclo(Phe-Phe) or a salt thereof in
10 production of a composition for reducing amyloid- β
production and/or amyloid- β accumulation. The present
invention also encompasses use of Cyclo(Phe-Phe) or a salt
thereof in production of a composition for inhibiting β -
secretase or a composition for promoting microglial
15 phagocytosis.

Cyclo(Phe-Phe) or a salt thereof can be used to
produce a composition for preventing or ameliorating a
condition or disease associated with amyloid- β
accumulation.

20 [0042]

The numerical range defined by the lower limit and
the upper limit herein, i.e., "the lower limit to the upper
limit", includes the lower limit and the upper limit. For
example, the range defined by "1 to 2" means 1 or more and
25 2 or less, with 1 and 2 being inclusive. Herein, the range
may be any combination of any upper limit and any lower
limit.

EXAMPLES

30 [0043]

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

[0044]

35 <Examples 1 to 4 and Comparative Example 1>

(Screening for compound having β -secretase inhibitory activity)

β -Secretase inhibitory activity of test compound was examined. The test compounds were cyclo-phenylalanyl-phenylalanine (Cyclo(Phe-Phe)) (Examples 1 to 4) and cyclo-leucyl-alanine (Cyclo(Leu-Ala)) (Comparative Example 1) (both available from Bachem).

A commercial kit "Beta Secretase (BACE1) Activity Assay Kit (Abcam)" was used for evaluation of β -secretase 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), 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" (Molecular Devices) at every five minutes from 0 to 60 minutes after the start of the reaction. The reaction was performed with n = 2. 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	—
Example 1	Cyclo(Phe-Phe)	4.4
Example 2	Cyclo(Phe-Phe)	2.2
Example 3	Cyclo(Phe-Phe)	1.1
Example 4	Cyclo(Phe-Phe)	0.55
Comparative Example 1	Cyclo(Leu-Ala)	20

[0046]

The enzyme activity was evaluated using Δ fluorescence intensity of the reaction solution ((fluorescence intensity at end of reaction (at minute 60)) - (fluorescence intensity before start of reaction (at minute 0))). A group to which no test compound was used as control. An inhibitor included in the kit was used as positive control.

[0047]

FIG. 1 is a graph 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 FIG. 1 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 the control reaction solution), which was taken as 1.

A reduction in the enzyme activity of β -secretase was observed in Cyclo(Phe-Phe). It became clear that Cyclo(Phe-Phe), which is a cyclic dipeptide, has β -secretase inhibitory activity.

[0048]

<Example 5>

(Measurement of phagocytosis)

Objective

The effect of Cyclo(Phe-Phe) (CFF) in enhancement of amyloid- β ($A\beta$)₁₋₄₂ (monomers and oligomers) phagocytosis by brain microglia was studied using *in vitro* assay.

[0049]

Materials and method

A. Reagent

Gibco Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 Ham 1:1 (DMEM/F12), sodium pyruvate, fetal bovine serum (FBS), horse serum (HS), penicillin/streptomycin (P/S), a poly-D-lysine (PDL) coated

24-well plate, Hanks' Balanced Salt Solution (HBSS), Dulbecco's phosphate-buffered saline (DPBS), BlockAid™ Blocking Solution, IBA1 polyclonal antibody (IBA1), and Hoechst 33342, Trihydrochloride Trihydrate were obtained
 5 from ThermoFischer Scientific.

β -Amyloid(1-42), HiLyte™ Fluor 488 ($A\beta_{1-42}$), and 1% NH_4OH solution were purchased from Anaspec. Triton® X-100 and paraformaldehyde (PFA) were obtained from Sigma Aldrich. Cyclo-phenylalanine-phenylalanine (Cyclo(Phe-
 10 Phe): CFF) was purchased from Bachem.
 [0050]

B. $A\beta_{1-42}$ preparation

A soluble species of $A\beta_{1-42}$ peptides ($A\beta_{1-42}$ monomers) was prepared according to manufacturer's (Anaspec)
 15 recommendations. Lyophilized $A\beta_{1-42}$ peptide was dissolved in a 1% NH_4OH solution to a concentration of 2 mg/mL. The peptide solution was further diluted with DPBS to a final concentration of 1 mg/mL of stock solution. Reconstituted peptide was aliquoted into several freezer vials and stored
 20 at $-20^{\circ}C$.

Rodamin B-labeled synthetic $A\beta_{1-42}$ peptide (GL Biochem, Shanghai, China) was dissolved in cold hexafluoroisopropanal (HFIP) to a concentration of 1 mM. The solution was incubated at room temperature for one hour
 25 and dried by a termovap sample concentrator. The residues were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 5 mM and further diluted with minimum essential medium (MEM) to a final concentration of 200 μM stock solution. The diluted $A\beta_{1-42}$ was incubated at $4^{\circ}C$ for
 30 24 hours to obtain oligomeric $A\beta_{1-42}$ ($A\beta_{1-42}$ oligomer).
 [0051]

C. Cell culture and Treatments

SIM-A9 murine microglial cells (ATCC CRL-3265) were maintained in DMEM/F12 supplemented with 10% FBS, 5% HS and
 35 1% P/S. For phagocytosis assay, SIM-A9 cells were detached

from a culture flask, pelleted, and resuspended in serum-free DMEM/F12. The cells were seeded in a PDL-coated 24-well plate at a density of 200000 cells/well and pretreated with Cyclo(Phe-Phe) for 24 hours at 37°C in a humidified gas chamber containing 5% CO₂. The final treatment concentration of Cyclo(Phe-Phe) was 2.2 µg/mL.

[0052]

BV-2 murine microglial cells were maintained in MEM supplemented with 10% FBS, 1% sodium pyruvate and 1% P/S. The cells were co-treated with 11.05 µg/mL Cyclo(Phe-Phe) and 2 µM Aβ₁₋₄₂ oligomer for 16 hours at 37°C in a humidified gas chamber containing 5% CO₂. Cyclo (Phe-Phe) was dissolved in hydrochloric acid (HCl) and added to the MEM.

[0053]

D. Phagocytosis assay

Adherent SIM-A9 microglia were pretreated with Cyclo(Phe-Phe) (2.2 µg/mL) for 24 hours and then rinsed once with serum-free DMEM/F12, followed by addition of Aβ₁₋₄₂ (monomer) at a concentration of 4.51 µg/mL in serum-free DMEM/F12 and incubation at 37°C for six hours. At the end of the incubation period, Aβ₁₋₄₂-containing medium was removed, and the cells were gently rinsed thrice with HBSS and twice with DPBS to remove extracellular Aβ₁₋₄₂. The cells were then fixed with 4% PFA, permeabilized with 0.2% Triton® X-100 and blocked with BlockAid™ Blocking Solution for one hour at room temperature. IBA1 antibody was used to visualize the cell shape, and Hoechst 33342 was used to stain the nuclei. Five sets of images were acquired per well for analysis and cells containing intracellular Aβ₁₋₄₂ were determined by co-localization of Aβ₁₋₄₂/IBA1/nuclei signals. The values are represented as the average number of phagocytic cells normalized to vehicle group (cells without Cyclo(Phe-Phe) treatment, vehicle control).

[0054]

BV-2 cells co-treated with Cyclo(Phe-Phe) and A β ₁₋₄₂ oligomer were fixed with 4% PFA and taken for microscope photography. For flow cytometry analysis, cells were washed twice with 2% FBS, centrifuged at 1000 rpm for five minutes, and blocked with Fc-Block (BD Bioscience) for 30 minutes. Fluorescence data was analyzed using FlowJo software (Tree Star, Ashland, OR).

[0055]

E. Statistical analyses

Statistical analysis was conducted using GraphPad Prism version 5.0 (San Diego, California, USA). All results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed by Student's t-test. Data was considered to be significant when P-value, $p < 0.05$.

[0056]

(Results)

To assess the effect of Cyclo(Phe-Phe) in enhancement of amyloid- β ₁₋₄₂ (monomers and oligomers) phagocytosis by brain microglia cells, a comparison was made between the cells without Cyclo(Phe-Phe) treatment (vehicle control) and the Cyclo(Phe-Phe)-treated cells.

[0057]

Effect of Cyclo(Phe-Phe) in enhancement of amyloid- β ₁₋₄₂ monomer phagocytosis

FIG. 2 shows a graph of the amount of amyloid- β monomers in cells of SIM-A9 microglia with 24-hour Cyclo(Phe-Phe) (CFF) pretreatment, and SIM-A9 microglia without Cyclo(Phe-Phe) (vehicle control (VC)) treatment. "*" indicates significance relative to the vehicle control (VC) ($p < 0.05$). The amount of amyloid- β in microglia was indicated by a relative value (%VC), with the amount of amyloid- β in vehicle control taken as 100 (mean $\pm$ SD, $n = 3$).

As shown in FIG. 2, Cyclo(Phe-Phe) significantly increased phagocytosis of amyloid- β_{1-42} monomers, compared to untreated cells (vehicle control).

[0058]

5 B. Effect of Cyclo(Phe-Phe) in enhancement of amyloid- β_{1-42} oligomer phagocytosis

FIG. 3 and FIG. 4 show the results.

FIG. 3 shows micrographs of BV-2 microglia with A β_{1-42} oligomer treatment (vehicle control (VC)) and BV-2
10 microglia with Cyclo(Phe-Phe) and A β_{1-42} oligomer treatment (CFF) (FIG. 3A, FIG. 3B, and FIG. 3C: vehicle control; FIG. 3D, FIG. 3E, and FIG. 3F: with Cyclo(Phe-Phe) treatment). In the Cyclo(Phe-Phe)-treated group (CFF), BV-2 microglia were treated with CFF and A β_{1-42} oligomer for 16 hours.
15 FIG. 3A and FIG. 3D show the cell density observed under brightfield microscopy; FIG. 3B and FIG. 3E show the presence of Rhodamine B-stained A β_{1-42} peptide; and FIG. 3C and FIG. 3F are merged images of cells and Rhodamine B-stained A β_{1-42} peptide taken up by the cells.

20 [0059]

FIG. 4 shows a graph of results from studies on amyloid- β oligomer phagocytosis by BV-2 microglia with Cyclo(Phe-Phe) (CFF) treatment, and BV-2 microglia without Cyclo(Phe-Phe) treatment (vehicle control (VC)). The
25 vertical axis shows the amyloid- β_{1-42} density per microglia (mean $\pm$ SD, n = 3). "*" indicates significance relative to the vehicle control (VC) (p < 0.05).

[0060]

It was found that CFF administration was able to
30 significantly promote phagocytic activity in BV-2 cells incubated with A β_{1-42} oligomeric peptide, as characterized by the increased frequencies of fluorescent cells and enhanced mean fluorescence intensity (MFI) levels of microglia when compared with the untreated control cells
35 (VC) (FIG. 3 and FIG. 4).

[0061]

As described above, Cyclo(Phe-Phe) was able to enhance phagocytosis of amyloid- β_{1-42} monomers by SIM-A9 microglial cells and phagocytosis of amyloid- β_{1-42} oligomers by BV-2 microglial cells, respectively.

[0062]

<Example 6>

Amyloid precursor protein (APP) overexpressing cells assay

In vitro assays were conducted to study the effect of Cyclo(Phe-Phe) (CFF) on reduction of amyloid- β accumulation. H4 neuroglioma cells overexpressing wild type amyloid precursor protein with the Swedish double mutation K595N/M596L (H4-hAPP cells) were used in the assay.

A comparison was made between the cells not treated with CFF (vehicle control) and the CFF-treated cells to evaluate the effects of CFF on reduction of amyloid- β accumulation by H4-hAPP cells.

[0063]

Materials and methods

A. Reagents and cells

The following reagents and cells were used.

Gibco Opti-MEM™ I Reduced Serum Medium (Opti-MEM) (cell culture medium), Fetal calf serum (FCS), Penicillin/Streptomycin (P/S), Hygromycin B, and Blasticidin S HCl available from ThermoFischer Scientific were used.

MSD® 96-well MULTISPOT® 6E10 Abeta Triplex Assay kit (amyloid- β detection kit) available from Meso Scale Discovery was used.

N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester (DAPT, γ -secretase inhibitor) available from Calbiochem was used. Cyclo(Phe-Phe) (CFF) available from Bachem was used.

H4-hAPP cells were obtained by stable transfection of the human H4 neuroglioma cell line with pAG3 vector containing human amyloid precursor protein 695 (APP695) with the Swedish double mutation K595N/M596L. The pAG3
5 vector is a modified pcDNA3 plasmid containing hygromycin-B resistance cassette with the transgene under the control of a fused CMV and chicken β -actin promoter.

[0064]

B. Cell culture and treatments

10 H4-hAPP cells were maintained in Opti-MEM supplemented with 10% FCS, 1% P/S, 200 μ g/mL Hygromycin B, and 2.5 μ g/mL Blasticidin S. The cells were seeded in a 96-well plate at the density of 25,000 cells/well and incubated overnight at 37°C in a humidified gas chamber
15 containing 5% CO₂. On the next day, H4-hAPP cells were treated with 22 μ g/mL CFF. In the CFF treatment, CFF was added to the medium of H4-hAPP cells at 22 μ g/mL, and the cells were incubated. Another H4-hAPP cells were treated with 400 nM DAPT instead of CFF as the positive control.
20 After treatment for 24 hours, the cell culture supernatants were collected for amyloid- β (A β) analysis. H4-hAPP cells without CFF treatment and DAPT treatment were used as vehicle control, and the cell supernatant was collected.

[0065]

25 C. Amyloid- β ELISA assay

The collected cell culture supernatants were diluted 1:10 and analysed for human amyloid- β ₁₋₃₈ (A β ₁₋₃₈), amyloid- β ₁₋₄₀ (A β ₁₋₄₀), and amyloid- β ₁₋₄₂ (A β ₁₋₄₂) with 6E10 A β Triplex Assay kit. Multiplex assay was carried out according to
30 manufacturer's instructions, and the plate was read on Sector Imager 2400 (Meso Scale Discovery). Amyloid- β concentrations were calculated by reference to the standard curve. The sensitivity of the multiplex kit was <5 pg/mL.

[0066]

35 D. Statistical analyses

Statistical analysis was conducted using GraphPad Prism version 5.0. All results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed by analysis of variance (ANOVA) followed by posthoc Tukey's multiple comparison test. Data was considered significant when P-value, $p < 0.05$.

[0067]

Results

FIG. 5, FIG. 6, and FIG. 7 show the results. FIG. 5 is a graph showing the concentration of amyloid- β_{1-38} in the cell culture supernatant of H4-hAPP cells. FIG. 6 is a graph showing the concentration of amyloid- β_{1-40} in the cell culture supernatant of H4-hAPP cells. FIG. 7 is a graph showing the concentration of amyloid- β_{1-42} in the cell culture supernatant of H4-hAPP cells.

In FIG. 5, FIG. 6, and FIG. 7, "CFF" is CFF-treated cells. "Control" is the vehicle control, and "DAPT" is the positive control. Values represent the mean $\pm$ SD ($n = 6$). "****" indicates significant difference with $p < 0.001$ relative to Vehicle Control.

[0068]

CFF was able to reduce accumulation of amyloid- β_{1-38} , amyloid- β_{1-40} , and amyloid- β_{1-42} in H4-hAPP cells. CFF has an effect of reducing accumulation of amyloid- β_{1-38} , amyloid- β_{1-40} , and amyloid- β_{1-42} in H4-hAPP cells.

CLAIMS

Claim 1. A composition for reducing at least one of amyloid- β production or amyloid- β accumulation, comprising:

5 Cyclo(Phe-Phe) or a salt thereof as an active ingredient.

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

Claim 3. The composition according to claim 1 or 2,
wherein the composition reduces amyloid- β
15 accumulation by promoting microglial phagocytosis.

Claim 4. The composition according to claim 1 or 2,
wherein the composition is for oral use.

20 Claim 5. The composition according to claim 1 or 2,
wherein the composition is a food or beverage or a pharmaceutical product.

Claim 6. The composition according to claim 1 or 2,
25 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",
30 "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
35 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
5 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".

10 Claim 7. Use of Cyclo(Phe-Phe) or a salt thereof in production of a composition for reducing at least one of amyloid- β production or amyloid- β accumulation.

15 Claim 8. Use of Cyclo(Phe-Phe) or a salt thereof for reducing at least one of amyloid- β production or amyloid- β accumulation.