

- (51) International Patent Classification:

A61K 45/06 (2006.01) A61K 31/352 (2006.01)
- (21) International Application Number:

PCT/US2024/034614
- (22) International Filing Date:

19 June 2024 (19.06.2024)
- (25) Filing Language:

English
- (26) Publication Language:

English
- (30) Priority Data:

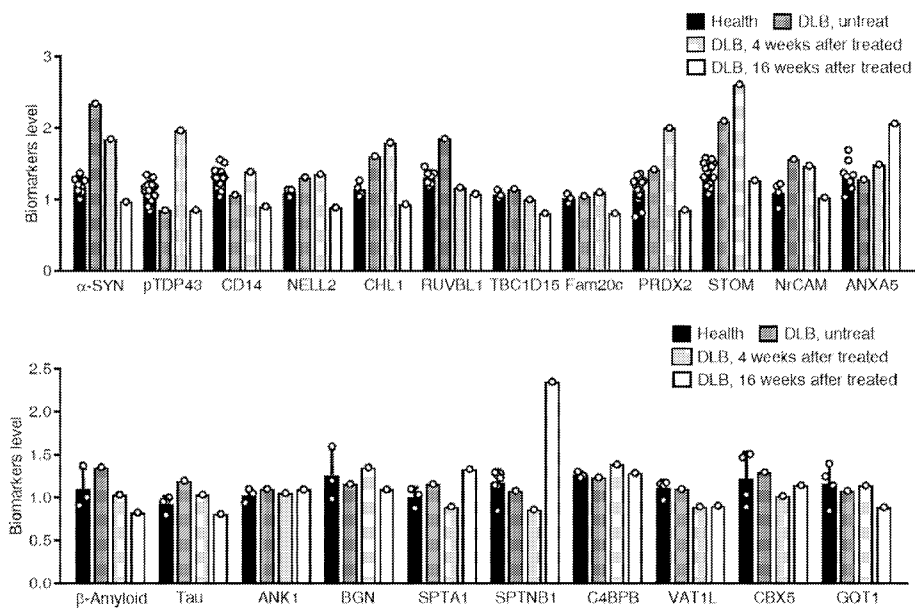
63/521,954 20 June 2023 (20.06.2023) US
- (71) Applicant: YEEFAN MED INC [US/US]; 4989 Santa Anita Avenue, Temple City, California 91780 (US).
- (72) Inventors: WANG, I-Fan; No. 1, Ln. 52, Fuchun Rd., Nantou County, Caotun Township, 542020 (TW). CHANG, Hsiang-Yu; No. 7, Aly. 2, Ln. 166, Shuren Rd., Wufeng Dist., Taichung City, 413015 (TW). TING, Chen-Hung; Rm. 3, 3F., No. 5, Aly. 38, Ln. 56, Jinmei Rd., Yilan County, Yilan City, 260023 (TW).
- (74) Agent: PASSINO, Sean A. et al.; Pilloff Passino & Cosenza LLP, 2000 Duke Street, Suite 300, Alexandria, Virginia 22314 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available):

AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

(54) Title: PHARMACEUTICAL COMPOSITION AND USES THEREOF

Fig. 1



(57) Abstract: Described herein are pharmaceutical compositions and methods for treating, detecting, characterizing, and reducing misfolding proteins and preventing related diseases such as neurodegenerative diseases and normal aging.

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- *without international search report and to be republished
upon receipt of that report (Rule 48.2(g))*

PHARMACEUTICAL COMPOSITION AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 63/521,954, filed June 20, 2023, which application is incorporated by reference herein in its entirety.

TECHNOLOGY FIELD

[0002] The present invention relates to pharmaceutical compositions and methods for the treatment of normal aging and proteinopathies including neurodegenerative diseases by reducing misfolded proteins of disease associated proteins.

BACKGROUND OF THE INVENTION

[0003] Neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) and Dementia Lewy Bodies (DLB) cause major public health problems worldwide and profoundly affect patients and their families.

[0004] Accumulations of misfolded proteins are a major cause of neurodegeneration. Multiple misfolded proteins frequently co-occur in patients with dementia. Considerable efforts have been made to alleviate aggregation pathology in neurodegeneration therapies, but the effective drug is not yet available.

[0005] Parkinson disease (PD) is a progressive neurodegenerative disorder primarily affected motor system, usually occurred of rigidity, hypokinesia and tremor. Non-motor symptoms also developed as the disease worsens, including cognitive changes. PD is caused predominantly by the loss of dopaminergic neurons in the substantia nigra (SN), a basal ganglia structure located in the midbrain. The signature pathology of the PD is lewy bodies (LBs). Misfolded alpha-synuclein (α -Syn) as the major component of the LBs observed in sporadic PD; and that mutations in the α -Syn are associated with some rare familial PD. The second risk factor for PD is the Leucine-rich repeat kinase 2 (LRRK2). *LRRK2* gene mutation accounts for 5% of familial PD as well as 3% of sporadic cases. *LRRK2* harbored G2019 mutation is more susceptible to forming α -Syn inclusions that links *LRRK2* with protein-misfolding pathology. Currently, the diagnostic process often takes over a year to complete, and currently no methods is available for early diagnostic and monitoring disease progression.

[0006] Despite advances made in the diagnosis and treatment of neurodegenerative disease over the last 50 years, the medical community is still faced with the challenge of treating numerous types of neurodegenerative diseases. Accordingly, there is still a need for a more effective and safe treatment for neurodegenerative diseases. The present invention addresses this need.

BRIEF SUMMARY OF THE INVENTION

[0007] Pharmaceutical compositions are provided herein: a composition comprising an antithrombosis drug; a composition comprising a flavonoid; a composition comprising a blood pressure medicine; a composition comprising a combination of an antithrombosis drug and a flavonoid; a composition comprising a combination of an antithrombosis drug and a blood pressure medicine; a composition comprising a combination of a blood pressure medicine and a flavonoid; or a composition comprising a combination of an antithrombosis drug, a flavonoid and a blood pressure medicine. Advantageously, each of these combinations improves synucleinopathy-related symptoms and cognition decline. It has been discovered that these combinations achieve reduction of α -Syn-misfolded proteins by synergistic effects.

[0008] Methods for reducing misfolded aggregates by administering a therapeutic agent to a cell, a tissue or a subject, are also provided herein.

[0009] Methods for improving synucleinopathies by administering a therapeutic agent to a cell, a tissue or a subject, are also provided herein. Example of synucleinopathies includes, but not limited to AD, Parkinson disease dementia (PDD), Dementia with Lewy body (DLB), Parkinson disease without dementia, and multiple system atrophy (MSA).

[0010] The terms “invention,” “the invention,” “this invention” and “the present invention” used in this patent are intended to refer broadly to all of the subject matter of this patent and the patent claims below. Statements containing these terms should be understood not to limit the subject matter described herein or to limit the meaning or scope of the patent claims below. Embodiments of the invention covered by this patent are defined by the claims below, not this summary. This summary is a high-level overview of various aspects of the invention and introduces some of the concepts that are further described in the Detailed Description section below. This summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used in isolation to determine the scope of the claimed

subject matter. The subject matter should be understood by reference to appropriate portions of the entire specification, any or all drawings and each claim.

[0011] The invention will become more apparent when read with the accompanying figures and detailed description which follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Illustrative embodiments of the present invention are described in detail below with reference to the following Figures:

[0013] Fig.1 is an assembly of images illustrating an analysis of longitudinal tracking of α -Syn, p-TDP-43, tau, beta-amyloid, and novel identified biomarkers of AD and PD from a single patient with synucleinopathies by b-isox based ELISA.

DETAILED DESCRIPTION

[0014] Pharmaceutical compositions and methods for treating neurodegenerative diseases, such as synucleinopathies, are described herein. The compositions can be selected from a composition comprising a composition comprising a antithrombosis drug; a composition comprising a flavonoid; a composition comprising a blood pressure medicine; a composition comprising a combination of a antithrombosis drug and a flavonoid; a composition comprising a combination of a antithrombosis drug and a blood pressure medicine; a composition comprising a combination of a blood pressure medicine and a flavonoid; or a composition comprising a combination of a antithrombosis drug, a flavonoid and a blood pressure medicine. When administered to cells, tissues or subjects, each of these various combinations synergistically reduces plasma α -Syn level.

[0015] In addition, methods are described herein for reducing plasma α -Syn in a cell, a tissue or a subject by administering an effective amount of a therapeutic agent in the cell, the tissue or the subject to reduce the level of α -Syn misfolded proteins. In one embodiment, the therapeutic agent is a flavonoid. In another embodiment, the therapeutic agent is the pharmaceutical composition described herein.

Definitions

[0016] As employed above and throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

[0017] As used herein, the singular forms “a,” “an,” and “the” include the plural reference unless the context clearly indicates otherwise.

[0018] The term “treating,” “treated,” or “treatment” as used herein includes preventative (e.g. prophylactic), palliative, and curative uses or results.

[0019] The term “reducing” or “reduce” includes slowing or stopping the formation of α -Syn aggregates, or disassembling the α -Syn misfolded aggregates that have already been formed.

[0020] The term “therapeutic intervention” as used herein refers broadly to actions taken that are expected to yield healing results, symptoms improvement or health restoration.

[0021] The term “amount” is used in this document to denote the quantity or distribution of something. In some embodiments, the present invention can utilize any of the foregoing information falling within the meaning of the term “amount” in relation to one or more proteins, as well as classes and subclasses of such proteins. Combination of such information on the amount of proteins can be referred to as “pattern.”

[0022] The term “subject” as used herein typically refers to a human or an animal having conformational disease or suspected of having conformational disease. It is to be understood that a subject can be subjects without known or suspected conformation disease, such as research subjects, are also included within the scope of the term “subject.”

[0023] The term “flavonoid” includes a flavone, which includes baicalein originally isolated from the roots of *Scutellaria baicalensis*. Baicalein is an inhibitor of CYP2C9, an enzyme of the cytochrome P450 system that metabolizes drugs in the body. The flavonoid includes baicalein and its derivatives.

[0024] The term “synucleinopathies” as used herein typically refers to a group of neurodegenerative disorders characterized by the accumulation of misfolded α -Syn proteins in cell bodies of neurons and glia. Disorders with synucleinopathies include Parkinson's disease (PD), dementia with Lewy bodies (DLB), pure autonomic failure (PAF), and multiple system atrophy (MSA).

Methods for Treating Synucleinopathies by a Combination of a Flavonoid, an Antithrombosis drug, and a Blood Pressure Medication.

[0025] Applicants observed by oral administration of a combination of baicalein, Norvasc and PLAVIX film-coated tablets, a patient with synucleinopathy saw a 50%

reduction in plasma α -Syn and novel identified biomarkers of PD after 4 weeks and a 100% reduction after 16 weeks (Fig. 1).

[0026] The single patient with synucleinopathy demonstrated significant cognitive gain on behavior over the course of treatment and no longer showed forward leaning of the whole body, smaller steps, or delirium and seldom exhibited visual hallucinations.

[0027] Rather than slowing the cognitive decline of patients, as seen in trials with other agents, subjects treated with the combination of baicalein, Norvasc and PLAVIX film-coated tablets demonstrated significant cognitive improvements from baseline status over the course of treatment in terms of behavior and cognition. These results position a combination of Baicalein, Clopidogrel Sulphate and Amlodipine as the first oral agent that can stop and prevent synucleinopathies in patients and healthy individuals at risk for the disease.

[0028] Pharmaceutical compositions for treating symptoms of synucleinopathies are provided herein. The pharmaceutical compositions provided herein are useful for reducing misfolded proteins.

[0029] The pharmaceutical composition includes a flavonoid, such as baicalein, a derivative thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof in combination with an antithrombosis drug, such as clopidogrel sulphate, a derivative thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof. Optionally, the pharmaceutical composition further includes a blood pressure medication, such as amlodipine, a derivative thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0030] In some embodiments, the flavonoid, such as baicalein or its derivative, is dosed in an amount ranging from about 100 mg to about 2000 mg per day, or 100 mg to 2000 mg per day.

Description of Materials and Methods Used in the Examples

[0031] The following materials and methods were used in the Examples described below.

[0032] **Reagents and Antibodies:** b-isox was purchased from Sigma and Dalton dissolved in dimethyl sulfoxide (DMSO). The primary antibodies against CA1 (#MBS1492724), FAM20C (#MBS769211), VAT1L (#MBS1492099), SPTA1 (#MBS9417174), C4BPB (#MBS9125430) and PRDX2 (#MBS7046127) were purchased from Mybiosource. The primary antibodies against alpha-synuclein (#10842-1-AP),

phospho-TDP-43 (Ser 409/410) (#22309-1-AP), BGN (#16409-1-AP), CBX5 (#11831-1-AP), CD14 (#17000-1-AP), NELL2 (#11268-1-AP), CHL1 (#25250-1-AP), GOT1 (#14886-1-AP), RUVBL1 (#10210-2-AP), STOM (#12046-1-AP), N-CAM (#14255-A-AP), and ANXA5 (#11060-A-AP) were purchased from Proteintech. Beta-amyloid CT695 (#51-2700) and ANK1 (PA5-42203) were purchased from ThermoFisher. TBC1D15 (#ab121396) antibody was from Abcam.

[0033] b-isox-ELISA procedures: Blood samples were firstly collected through Blood Collection Tubes. Centrifugation of the tubes for 15 min at 2,200×g. The resulting supernatant (upper layer) as the plasma sample. Before immunoassay, gently mixed 100μL plasma (or CSF) with 1μL b-isox through pipetting and rotated for 1h at 4°C. Wash each streptavidin-coated microwell 3 times by 200μL wash buffer (WB/(25mM Tris, 150mM NaCl; pH 7.2), 0.1% BSA, 0.05% Tween®-20) (do not allow wells to dry). Add 100μL of the reaction mixtures to each well and incubate for 2h with shaking (~60 rpm) at room temperature (RT). Wash 3 times with 200μL WB when reaction finished. Add 100μL primary antibody diluent (appropriate primary antibody dilution in WB), incubating for 1h at RT with shaking. At this step, a no primary antibody control should be included (Add antibody diluent alone in a sample well). Wash 3 times with 200μL WB. Add 100μL antibody diluent with appropriately diluted HRP-conjugated secondary antibody, incubating for 1h at RT with shaking. Equilibrate the TMB substrate solution to RT at this step. Wash 3 times with 200μL WB. Add 100μL of the TMB Substrate Solution to each microplate well, incubating for 15-30 min until the color develops. Stop the reaction by adding 100μL 2M sulfuric acid (or 2N HCl). Measure the optical density of at 450 nm by a microplate reader.

EXAMPLES

Example 1: A pilot clinical trial in synucleinopathy: A combination of baicalein, clopidogrel sulphate and amlodipine

[0034] In a single patient with synucleinopathy, we used b-isox based-ELISA for diagnostic, real-time pathophysiological monitoring and pharmacometric measurements of a combination of baicalein, clopidogrel sulphate and amlodipine. The patient saw a 50% reduction in plasma α -Syn and novel identified biomarkers of PD after 4 weeks and a 100% reduction after 16 weeks and demonstrated significant cognitive gain on behavior over the course of treatment.

[0035] The patient (PD CFRS=8) was 79 years old and male. He reported onset of disease on 4 Aug 2021, and his symptoms included severe delirium, constipation, visual hallucinations, forward leaning of the whole body, and smaller steps. On 15 Sep 2021, the patient was diagnosed with synucleinopathy by clinical features and b-isox -based ELISA based on the patient having only a high level of α -Syn in the plasma (Fig 1.).

[0036] The patient received 500 mg of baicalein orally twice a day for 5 days and 500 mg of baicalein orally once a day for 5 days and was assessed for the primary outcome of change from baseline of plasma α -Syn levels and PD related biomarkers by b-isox -based ELISA. Meanwhile, the patient takes Norvasc (2.5 mg) and PLAVIX film-coated tablets (75 mg) daily.

[0037] The patient soon showed significant improved cognition. The third diagnosis was performed on 21 Jan 2022 and showed that the patient's plasma α -Syn and p-TDP-43 levels returned to normal. As of Feb 2022, he could visit a sick friend by himself, ride a bicycle, calculation and discuss national affairs with family and friends. He no longer showed forward leaning of the whole body, smaller steps, or delirium and seldom exhibited visual hallucinations.

[0038] Baicalein, Clopidogrel Sulphate and Amlodipine can be given as an easy-to-administer oral tablet that has shown potential for robust efficacy with a favorable safety profile.

CLAIMS

What is claimed is:

1. A method for preventing or treating synucleinopathy in a subject, comprising administering to the subject in need thereof an effective amount of a therapeutic agent to reduce the symptom or sign of synucleinopathy.
2. The method of Claim 1, wherein the therapeutic agent is flavonoid.
3. The method of Claim 1, wherein the therapeutic agent is a combination of antithrombosis drug and a flavonoid.
4. The method of Claim 2, wherein the flavonoid is baicalein or its derivative.
5. The method of claim 4, wherein the flavonoid is dosed in an amount ranging from 100 mg to 2000 mg per day.
6. The method of Claim 3, wherein the antithrombosis is clopidogrel sulphate or its derivative.
7. The method of claim 1, wherein the synuclein related disorder is PD, DLB, AD and MSA.
8. A pharmaceutical composition, comprising
 - (a) a antithrombosis drug
 - (b) a blood pressure medicine; and
 - (c) a flavanoid.
9. The pharmaceutical composition of claim 7, wherein the antithrombosis drug is clopidogrel sulphate or its derivative.
10. The pharmaceutical composition of claim 7, wherein the blood pressure medicine is amlodipine or its derivative.
11. The pharmaceutical composition of claim 7, wherein the flavonoid is baicalein or its derivative.

Fig. 1

